Summary

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Summary

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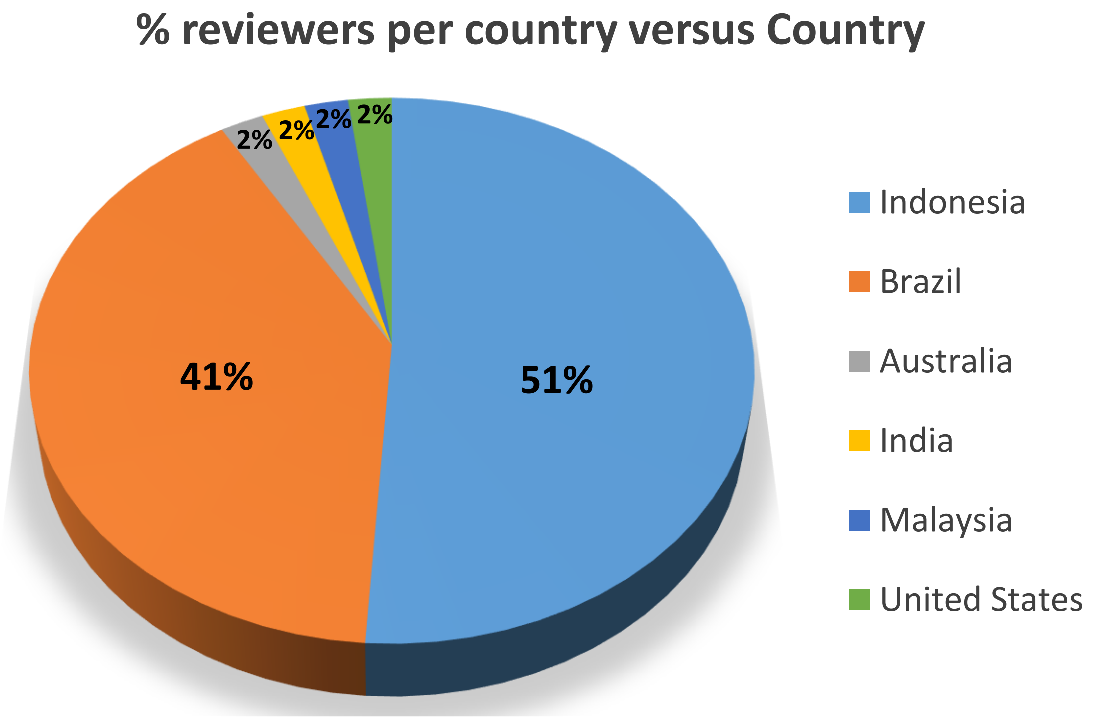

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Critical thinking skills of prospective chemistry teachers in chemistry learning with Ethno-Socio-Scientific issues integrated inquiry

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Abstract

This study aims to develop a valid and practical Ethno-SSI integrated inquiry strategy to improve the critical thinking skills of prospective chemistry teachers. The research method used is Plomp's research and development model, with a convenience sampling technique obtained from a sample of 52 prospective chemistry education teachers at one of the universities in Indonesia. Data was collected through observation, a critical thinking test, and a questionnaire. Data analysis was carried out qualitatively and quantitatively. The results of strategy development obtained a new syntax called OETACA (orientation, exploration, transformation, argumentation, conclusion, and application) with a content validity score of 0.83 and construct 0.93. The results of improving critical thinking skills on a small scale get an N-Gain score of 0.7 in the high category supported by the perceptions of prospective chemistry teachers who strongly agree (43%) and agree (41%) to implement the Ethno-SSI integrated inquiry strategy in chemistry learning in the classroom.

Article History

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1. critical thinking skills;
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Highlights

- Ethno-SSI is a combination of ethnoscience and Socioscientific Issues.
- Religion, environment, culture, health, and society.
- Orientation, exploration, transformation, argumentation, conclusion, application.
- Thermochemical material in the context of burning snail satay.
- Controversial, dilemmatic, and complex issues.

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1. Introduction

The development of education in the 21st century requires students to have a strong understanding of content areas such as math, language, and science (Boholano, 2017; Council, 2012), and also to various skills such as critical thinking, problem-solving, communication, collaboration, and curiosity (Tan *et al.*, 2017; Wahono *et al*., 2021). These skills are needed to face future global challenges (Lee *et al*., 2012). Innovation in education is required so that the educational process is in line with the circumstances and needs of prospective chemistry teachers in the 21st century. Therefore, educational practices that were initially only in the form of transferring knowledge from lecturers to students are no longer effectively used to prepare young people who will be competent in the 21st century: student-centered learning that will provide opportunities for students to develop their interests and talents (Boholano, 2017); learning that develops the ability to gather information from all sources so that there is a long-life learning process (Lee *et al*., 2012); the use of Information and Communication Technology (ICT) and various virtual devices provides flexibility to find quality learning resources (Dewi *et al*., 2019); emphasizing hands-on learning (Fuad *et al*., 2017); developing soft skills of critical thinking, creativity, problem-solving, collaboration, and social interaction (Rahayu, 2017); and providing flexibility in the learning process (Hussin, 2018).

Critical thinking skills are part of High Order Thinking Skills (HOTS), one of the skills needed and needs to be continuously trained so prospective chemistry teachers can survive in the 21st century (Barak and Dori, 2009; Tajudin and Chinnappan, 2016). Choi *et al*., (2011) research results show that one of the aspects developed in the dimensions of science literacy in the habitual thinking domain is critical thinking skills. This skill is the basis for solving challenging problems in the 21st century that occur in a global society (Boholano, 2017). Ennis (1990) mentions that critical thinking aims to decide what to believe or do reasonably, using reasoning and logic in thinking and learning the truth based on specific thinking patterns (Ennis, 2011a). A different definition is given by Facione (2011), who states that critical thinking is a mind that aims to prove a point/opinion, interpret something meaningful, and solve problems (Facione, 2011). Although several theories and educators have developed definitions and conceptualizations of critical thinking, studies on factors influencing critical thinking skills still need to be completed (Kwan and Wong, 2015), especially in chemistry learning.

Prospective chemistry teachers must be able to understand the characteristics of chemistry, namely being able to explain phenomena macroscopically, submicroscopically, and symbolically; investigate the dynamics of processes and energy changes during chemical reactions by conducting investigations; and explain chemical processes in life (Suwahyu and Rahayu, 2023). Prospective chemistry teachers instil in themselves that chemistry is essential, that chemistry exists in life, and that all life processes have chemistry so that when they graduate, they can provide understanding directly to the community (Putri *et al*., 2022). In addition, chemistry is full of sometimes confusing language terminology, so it is necessary to introduce scientific language to prospective chemistry teachers so that they can use chemical languages correctly (Bechtel, 2016).

Chemistry learning has rarely been given issues that prospective teachers can debate, such as socioscientific issues (Rahayu *et al*., 2020). The case of socioscientific problems is characterized by controversial contemporary issues; ethical, moral, and dilemmatic elements raise debate and the need to make decisions in solving SSI problems (Sadler and Zeidler, 2005; Zeidler *et al*., 2019). SSI cases can be used to dialogue, discuss, and debate, among the characteristics of science-literate people (Barrue and Albe, 2013; Herman *et al*., 2019; Leung, 2022). These SSI cases are essential in chemistry education because they can be used as more relevant science learning in life, improving learning outcomes, argumentation skills, scientific information, critical thinking, and other essential aspects supporting science literacy (Borgerding and Dagistan, 2018; Chen and Xiao, 2021; Fadly *et al*., 2022; Presley *et al*., 2013; Rahayu, 2019). SSI can train critical thinking skills because it involves social-scientific, moral, and ethical issues, and considers various different perspectives in decision-making (Genel and Topçu, 2016; Herman *et al*., 2021; Sadler and Donnelly, 2006; Zeidler *et al*., 2009). In the context of SSI, prospective chemistry teachers are trained to think critically by being confronted with global and contextual problems that require them to analyze data, evaluate, and draw logical conclusions based on scientific evidence (Eastwood *et al*., 2012; Herman *et al*., 2019; Rahayu, 2021).

Several research results show that the critical thinking skills of prospective chemistry teachers are still low (Hunnicutt *et al*., 2015; Mitarlis *et al*., 2020). Based on observations of early semester students in one of the universities in Indonesia, many problems need to be solved to enhance students' critical thinking and chemical literacy. This can be seen from the low understanding of student concepts; students need help based on existing evidence, facts, and theories when being given questions on chemical topics. Then, students need to be able to design scientific inquiry activities by using correct procedures before carrying out practicum activities. Furthermore, interest in chemistry and students' level of awareness in responding to current chemistry-related issues still need to be improved (Sulistina *et al*., 2021). Communication skills such as dialogue, discussion, and debate are rarely implemented in class so students become passive in learning chemistry and their critical thinking skills become low (Winarti *et al*., 2024). Students currently acquire knowledge only based on what is given by the lecturer, not looking for themselves from various other sources relevant to the material being taught, so their concept knowledge and their perception toward meaningful topic in daily life is still low (Wiyarsi *et al*., 2021).

Several studies have been done to improve the critical thinking skills of prospective chemistry teachers using various models and approaches but some of the results are not optimal (Abdurrahman *et al*., 2020; Aiman *et al*., 2020; Cahyarini *et al*., 2016; Sutiani *et al*., 2021; Zidny *et al*., 2020). There was no significant difference between the critical thinking skills of prospective chemistry teachers in the control class and the experimental class with the inquiry model (Qing *et al*., 2010). Furthermore, the Scientific Critical Thinking (SCT) of prospective chemistry teachers can be improved with the inquiry-PBL model, but the results are not optimal (Yuanita *et al*., 2019). Irwanto *et al*., (2019) used POGIL, but the results were still insignificant in improving prospective chemistry teachers' critical thinking and problem-solving skills. Prospective chemistry teachers' science process and critical thinking skills were still low due to a lack of experience in inquiry-based laboratories (Irwanto *et al*., 2019). ABI (Argument-based inquiry) affected the critical thinking skills of prospective chemistry teachers but had limitations, namely the need to conduct a more detailed analysis of indicators and trends in critical thinking skills (Sönmez *et al*., 2019). The three-level inquiry-based chemistry learning given to prospective teachers did not significantly improve critical thinking skills (Muskita *et al*., 2020). The critical thinking skills of students taught with the inquiry learning model were not strong enough, generally at the medium and low levels. Based on the results of various studies above, it can be concluded that many studies use inquiry to improve critical thinking skills, although the results are less than optimal (Arsal, 2017). This is because this inquiry model focuses on active student participation, the ability to ask scientific questions, and student involvement in investigations to find answers to scientific questions to improve their critical thinking skills (Council, 2018; Goldkuhl, 2008). Theoretically, the weaknesses of the various inquiry models above can be overcome by improvements or innovations in strategies, approaches, and steps so that the results of these improvements will be able to improve the critical thinking skills of prospective chemistry teachers by using the context of local wisdom and SSI.

Currently, the complexity of SSI in the context of local wisdom has been studied by several researchers worldwide in the fields of environment, health, socio-politics, and socio-culture. SSI problems in the environmental field include biofuels (Dauer *et al.*, 2021; Nida *et al.*, 2021a), nuclear power plants (Jho *et al.*, 2014; Wang *et al.*, 2018; Yuenyong, 2013), water pollution (Ladachart and Ladachart, 2021; Owens *et al.*, 2022), air pollution (Herman *et al.*, 2021), and global warming (Nida *et al.*, 2021b). In the health sector, they include Genetically Modified Food (GMO) (Lederman *et al.*, 2014), unslaughtered animals, synthetic food, artificial meat (Adal and Cakiroglu, 2022), uterine borrowing (Raveendran, 2021), malnutrition, toxicity (Waard *et al.*, 2020), cloning, abortion, and HIV-positive children (Bencze *et al.*, 2020). Local wisdom-based SSI in the socio-political field includes cases of discrimination, racism, colonialism, social justice power in South Africa (Diwu and Ogunniyi, 2013), waste-to-energy plants (Dawson and Carson, 2020), animal welfare issues, and whale killing methods (Grace *et al.*, 2015). Meanwhile, local wisdom-based SSI in the socio-cultural field includes traditional rituals (Mavuru and Ramnarain, 2020), myths (Canel-Çınarbaş and Yohani, 2019), gender differences, social norms, religion, and beliefs (Raveendran, 2021; Wahono *et al.*, 2021). Some of these research problems have not taken the context of controversial regional culinary specialities and traditional herbs, so researchers are interested in developing this research. Ethno-SSI is a combination of ethnoscience and Socioscientific Issues.

The SSI approach in the context of local wisdom has developed many research variables on critical thinking skills (Nida *et al.*, 2021b; Raveendran, 2021; Yacoubian, 2020; Zidny and Eilks, 2022). Critical thinking skills are one of the higher-order thinking skills (HOTS) whose activities include analyzing, evaluating, and reasoning (Ennis, 2011b; 2013). This ability is essential to develop the potential possessed by prospective chemistry teachers to analyze and solve SSI problems within the context of local wisdom. Prospective chemistry teachers with critical thinking have opened their minds to formulate problems correctly, collect information appropriately and relevantly, interpret ideas, make conclusions accompanied by reasons, communicate effectively, and find solutions to complex problems (Ennis, 2011b; Kölbel and Jentges, 2018; Masni *et al.*, 2020). Research questions:

1. How to develop a valid and practical Ethno-SSI integrated inquiry strategy?
2. What are the results of critical thinking skills of prospective chemistry teachers using the Ethno-SSI integrated inquiry strategy?
3. What is the perception of prospective chemistry teachers using the Ethno-SSI integrated inquiry strategy?

2. Method

2.1. Research design

The method used in this research is Research and Development. This research produces a product in the form of an Ethno-SSI integrated inquiry strategy to improve the critical thinking skills of prospective chemistry teachers using Plomp's model. Plomp's development model in this study consists of three stages shown in **Fig. 1**, namely (1) preliminary research (initial investigation with needs analysis and problem identification); (2) Development or Prototyping phase Initially (development and implementation of prototypes/products); (3) Assessment phase (assessment and evaluation) (Plomp *et al.*, 2013).

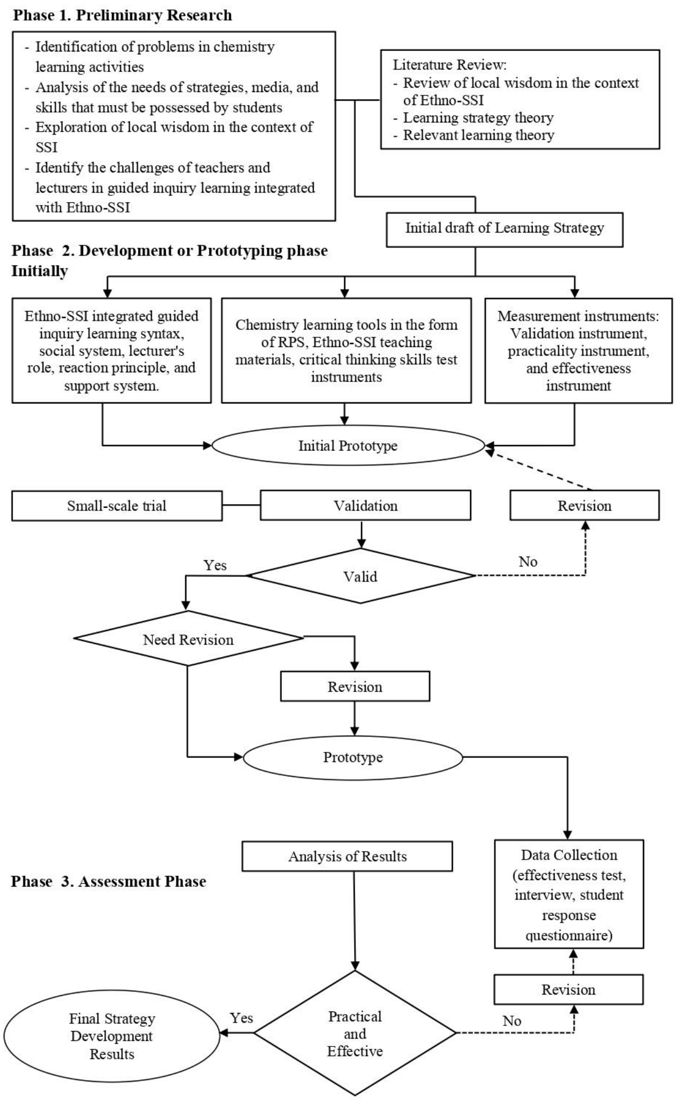


Figure 1. Flow of Research and Development learning strategy according to Plomp *et al.* (2013) model.

2.1.1. Preliminary research

Activities at this stage aim to explore initial data or information in developing learning strategies. Activities carried out in preliminary research include: (1) Identifying various problems related to the context of Ethno-Socioscientific Issues in Essential Chemistry Learning. The problems studied must contain elements of local wisdom that are controversial and dilemmatic in society regarding health, religion, culture, society, and the environment. This supports orientation activities consisting of problem identification and problem formulation used in learning strategies; (2) Reviewing learning theories that become the foundation in developing inquiry learning strategies based on Ethno-Socioscientific Issues; (3) Analyze the requirements for developing learning strategies, such as suitability to the curriculum, student conditions, number of lesson hours, learning environment, and field conditions for direct exploration activities; and (4) Formulate a rationale for the importance of developing an inquiry strategy integrated with Ethno-Socioscientific Issues to improve the critical thinking skills of prospective chemistry teachers.

2.1.2. Prototyping phase or prototype development

At this stage, activities are advanced from problem identification in preliminary research to designing problem-solving. In solving the problem, the teacher must divide the activities into coherent stages so that they become a unified whole. The solution to the problem developed in this study resulted in a prototype design that was implemented in the form of: (1) an academic manuscript or book of inquiry strategy based on Ethno-Socioscientific Issues; and (2) Learning tools or instruments consisting of RPS (Semester Lecture Plan), Ethno-SSI Context Basic Chemistry students worksheet, PPT, teaching materials and learning videos, and assessment instruments (critical thinking skills test questions). The developed product or prototype is then re-examined for the completeness of each component and then tested for validity by expert validators.

2.1.3. Assessment phase

Activities at this stage aim to assess the draft strategy that has been developed. The results of this assessment and consideration will lead to a decision on the next step. The activities at this stage are as follows: (1) Conduct validation related to the prototype designed and developed by expert validators; (2) The assessment results from the validator are then continued by making improvements based on suggestions, criticisms, and input from expert validators; and (3) Collecting, processing, and analyzing data from assessing critical thinking skills by applying the developed Ethno-Socioscientific Issues-based inquiry strategy. Phases of research and development according to Plomp *et al.* (2013) model Shown in **Fig. 2**.

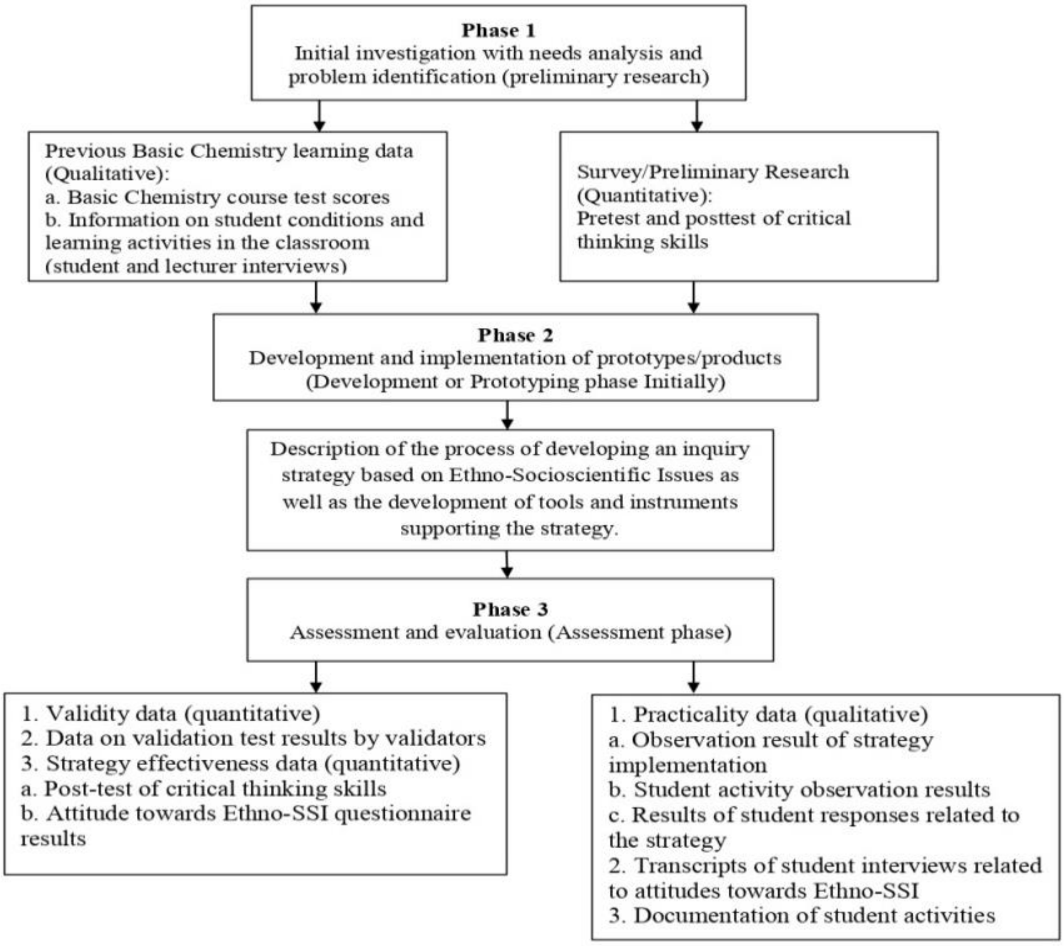


Figure 2. Phases of research and development according to Plomp *et al.* (2013) model.

2.2. Sample

This research involved one of the state universities in East Java, Indonesia, and took place from August to October 2023. The activity began with exploring Ethno-SSI in Central Java and East Java to be used as a context in chemistry learning. Sampling was conducted during the small-scale trial using the convenience method. The research sample consisted of prospective chemistry teachers (N=52) who taking basic chemistry courses in their first semester. The Basic Chemistry course in this study uses the 2023 department curriculum, learning using guided inquiry, which includes theory and practice in the laboratory. Learning was conducted for five meetings on Thermochemistry (three meetings in class learning material, one exploration activity in the community, and one practicum activity in the chemistry laboratory).

2.3. Data collection

Qualitative data collection (RQ1) was obtained from validation and observation sheets of classroom learning implementation activities and expert validation. Expert validation data in the form of criticisms, suggestions, and opinions about the developed strategy. Quantitative data collection (RQ2) was conducted with a critical thinking test. Meanwhile, qualitative data collection (RQ3) was conducted through questionnaires.

2.4. Test instrument development (RQ2)

Test instrument development uses the stages (Define, Design, Develop) developed by Thiagarajan and Ennis' critical thinking skills framework, which consists of elementary clarification, basic support, interference, advanced clarification, and strategy and tactics.

Define: First, analyse the characteristics of prospective chemistry teachers in accordance with the design of developing basic chemistry question instruments integrated with Ethno-SSI to achieve critical thinking skills. Second, develop question instruments based on a concept analysis literature review to identify material that can be integrated with ethno-SSI. Third, indicators of critical thinking skills on the questions should be identified.

Design: The second stage was designed by constructing items based on critical thinking skills indicators (elementary clarification, basic support, interference, advanced clarification, and strategy and tactics). Furthermore, we describe each indicator of essential skills of thinking, design questions, and assessment rubrics (question and answer grids), followed by writing items using Indonesian.

Develop: The third stage was to develop test instruments that experts validated. Experts were asked to review each question item based on the following criteria: (a) substance and indicators of critical thinking skills to be achieved; (b) clarity of question items in words/phrases/pictures/tables; (c) use of correct Indonesian grammar; and (d) completeness of the question instrument. The test instrument's validation results received comments/feedback and were then revised to improve the items.

Three people carried out material expert validation to evaluate the validity of the product of the Ethno-SSI context critical thinking question instrument. The validation findings produce constructive criticism and suggestions to improve the developed question instrument, which will be evaluated in small groups and field trials. During the validation process, qualitative and quantitative data will be collected. Qualitative data consists of suggestions, criticisms, responses, and input derived from validator comments. Quantitative data consists of assessment data collected through a questionnaire developed by the researcher. The completed questionnaire uses a scoring system adjusted to the criteria given on the form, using a 4-level Likert scale shown in **Table 1**.

Table 1. Criteria for validator answer levels.

Score	Description
4	good / worthy / interesting / clear / appropriate
3	quite good / quite feasible / enough
2	Interesting / clear enough / precise enough / appropriate
1	less good / less feasible / less interesting / unclear / less precise / less appropriate

The assessment results from the validators were then analysed using the CVI statistical technique. CVI is divided into two types of validation, namely (i-CVI) or the validity of the content of individual items, and (s-CVI) or the validity of the content of the items as a whole Lynn, (1986). The CVI calculation method starts with the scores obtained from each validator, which are converted into dichotomous values of 0 and 1 to be analysed with CVI. The way to convert the scale is that scores 1 and 2 are included in dichotomy 0, which means they are not feasible, while scores 3 and 4 are included in dichotomy 1 with the feasible category. Furthermore, from the three validators, the average of each item is determined or called i-CVI, and the average of i-CVI is the s-CVI value. The criteria for determining the validation results in this study refer to Guilford and Fruchter (1978) which consists of:

0.80 < Mean-CVI < 1.00 = excellent (very high validity)
0.60 < Mean-CVI < 0.80 = good (high validity)
0.40 < Mean-CVI < 0.60 = fair (medium validity)
0.20 < Mean-CVI < 0.40 = poor (very low validity)
Mean I-CVI < 0.00 = invalid

The practicality test categories of the Ethno-SSI integrated inquiry strategy in this study consist of:

81–100% = very practical
61–80% = practical
41–60% = moderate
21–40% = less practical
0–20% = not practical

Feedback, suggestions, and input from validators are used to assess the quality, relevance, and effectiveness of questions made questions that experts from chemistry education have validated. It can also increase the acceptability or acceptance of instruments because it has undergone a rigorous evaluation process. The results of the validity of the critical thinking test and questionnaire instruments assessed by three experts with the Content Validity Index (CVI) score are shown in **Table 2**.

The data in **Table 2** shows that the results of material expert validation on critical thinking skills instruments and questionnaires get high scores. The SCI-Ave score on basic chemistry questions in the Ethno-SSI context is 0.904, and S-CVI/UA receives a score of 0.714, which means the question is acceptable, relevant, and valid. Validator input and evaluation are expected to improve the instrument made. According to Dwianto *et al.* (2017), test instruments are considered feasible and suitable if there is a match between the questions and the topic, and the abilities being assessed. The test instrument is considered adequate if it fulfills all the criteria for evaluating the instrument and it can improve the critical thinking skills of prospective chemistry teachers. This study shows that the questions made have successfully fulfilled all critical thinking skills test criteria so that they can be used for small-scale trials. The results of suggestions for improvement from the material validator are shown in **Table 3**.

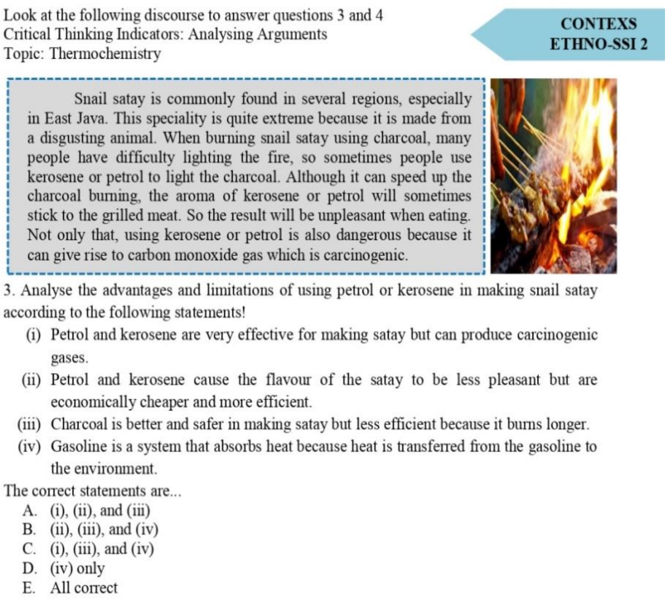
After being validated, the next step was to test critical thinking skills questions conducted on prospective chemistry teachers at one of the universities in Indonesia (N = 57) with a total of 30 multiple-choice questions. The trial of questions was carried out to check the level of difficulty of the items, construct validity, and reliability. The results of the reliability of chemical literacy items scored 0.93, indicating high reliability. An example of a critical thinking skills test instrument for the topic of Thermochemistry is shown in **Fig. 3**.

Table 2. Content validity index of research instruments.

Instrument	S-CVI/Ave	Category	S-CVI/UA	Category
Critical Thinking Test	0.904	High	0.714	Accept/relevant/valid
Questionnaire	0.890	High	0.875	Accept/relevant/valid

Table 3. Suggestions for improvement critical thinking skills test.

Category	Suggestions for Improvement
Relevance of material	- Ensure the questions are directly related to thermochemical concepts, such as enthalpy, phase change, and the laws of thermodynamics. - The questions made are relevant, but the relationship between the chemical concept and the local culture discussed needs to be strengthened.
Critical thinking skills	- Questions should encourage prospective chemistry teachers to analyze and evaluate information, not just remember the material. - The chemical concept used in the questions is correct, but it is necessary to review it again for critical thinking questions, it would be better in the form of analysis rather than calculation.
Ethno-SSI context	Include indicators of critical thinking skills in each question. the Ethno-SSI discourse discussed in the questions is quite complex, but questions can be added to compare traditional and scientific methods.
Clarity and readability	Integrate the ethno-SSI context in the questions to increase their relevance and connection to prospective chemistry teachers' daily lives.
Language, images, tables	- Ensure the language in the questions is easy to understand, avoid unfamiliar technical terms, and use EYD and appropriate chemical formulas. - The images and tables are already there, but it is necessary to clarify the illustrations in the images/tables containing the information asked in the questions.


Look at the following discourse to answer questions 3 and 4
Critical Thinking Indicators: Analysing Arguments
Topic: Thermochemistry

CONTEXS ETHNO-SSI 2

Snail satay is commonly found in several regions, especially in East Java. This speciality is quite extreme because it is made from a disgusting animal. When burning snail satay using charcoal, many people have difficulty lighting the fire, so sometimes people use kerosene or petrol to light the charcoal. Although it can speed up the charcoal burning, the aroma of kerosene or petrol will sometimes stick to the grilled meat. So the result will be unpleasant when eating. Not only that, using kerosene or petrol is also dangerous because it can give rise to carbon monoxide gas which is carcinogenic.

3. Analyse the advantages and limitations of using petrol or kerosene in making snail satay according to the following statements!
(i) Petrol and kerosene are very effective for making satay but can produce carcinogenic gases.
(ii) Petrol and kerosene cause the flavour of the satay to be less pleasant but are economically cheaper and more efficient.
(iii) Charcoal is better and safer in making satay but less efficient because it burns longer.
(iv) Gasoline is a system that absorbs heat because heat is transferred from the gasoline to the environment.

The correct statements are...
A. (i), (ii), and (iii)
B. (ii), (iii), and (iv)
C. (i), (iii), and (iv)
D. (iv) only
E. All correct

Figure 3. Critical thinking test question example based Ethno-SSI.

2.5. Data analysis

The data analysis in this study is shown in **Table 4**.

The field trial results in the first stage (small scale) conducted in this study were used to improve the Basic Chemistry strategy and instruments with the Ethno-SSI context, which aims to improve critical thinking skills. These results were then analysed and revised to improve and will be used on a wide scale or effectiveness test.

To answer RQ 1, the data were analysed descriptively and qualitatively, explaining the development of the Ethno-SSI integrated inquiry strategy and input from validators. Data analysis used to answer RQ 2 is quantitative N-Gain supported by descriptive study to describe the critical thinking skills of prospective chemistry teachers. Students' answers were grouped based on the rubric for assessing critical thinking skills indicators developed by Ennis (1993) and then processed as a percentage.

$$\text{Critical thinking skills score} = \frac{\textit{Number of correct answers}}{\textit{Maximum score}} \text{x } 100$$

According to **Table 5**, the percentage value is then interpreted as being in the very high, high, medium, low, and very low categories and Ethno-SSI critical thinking skills indicators is shown in **Table 6**.

Table 4. Research questions and tools of the research.

Research Questions	Tools	Data Analysis
RQ1: How is the development of the Ethno-SSI integrated inquiry strategy?	- Literature review - Validation sheet - Observation sheet	Descritive qualitative
RQ2: How the results of the critical thinking skills of prospective chemistry teachers using the Ethno-SSI integrated inquiry strategy in small scale?	Chemical literacy test	Quantitative, N-gain
RQ3: How the perception of prospective chemistry teachers using the Ethno-SSI integrated inquiry strategy?	Open-ended questionnaire	Descritive qualitative

Table 5. Category and criteria critical thinking skills.

Category	Score	Criteria
Very Low	0-20	Prospective chemistry teachers are only able to understand basic information without being able to make analysis and evaluation, and their arguments are very limited.
Low	21-40	Prospective chemistry teachers can make simple arguments but still have thinking errors and are less capable of deeper analysis and evaluation.
Medium	41-60	Prospective chemistry teachers can analyze and make evaluations at a sufficient level. In addition, the arguments made are also more complex, although they still show weaknesses in several aspects.
High	61-80	Prospective chemistry teachers have good critical thinking skills by recognizing, analyzing, and evaluating arguments more effectively, structured, and logically.
Very High	81-100	Prospective chemistry teachers have high critical thinking skills by being able to solve complex problems, analyze in depth, and evaluate arguments logically and cohesively.

Source: Elaborated by the author using data from Ennis (1993).

Table 6. Ethno-SSI critical thinking skills indicators.

No	Indicators	Critical thinking skills integrated Ethno-SSI
1	Elementary Clarification	Focusing on questions, analyzing questions, and asking and answering questions about a local wisdom Ethno-SSI phenomenon.
2	Basic Support	Establish the credibility of a source, scrutinize and assess the results of Ethno-SSI explorations and investigations.
3	Interference	Reduce and assess deduction, induce and assess induction, and determine the results of moral, ethical, and religious considerations in the context of Ethno-SSI.
4	Advanced Clarification	Define terms, assess definitions, and identify problematic assumptions of Ethno-SSI cases.
5	Strategy and Tactics	Decide on a course of action and interact with others to resolve Ethno-SSI cases using inquiry strategies.

As for RQ 3, students' perceptions were analysed descriptively qualitatively based on the results of a questionnaire that focused on (1) the relevance of Ethno-SSI in chemistry learning, (2) interest in the Ethno-SSI integrated inquiry strategy, and (3) improving critical thinking skills. The questionnaire in this study consisted of 10 items with scoring using a five-point Likert scale. The answers were strongly agree (score=5), agree (score=4), neutral (score=3), disagree (score=2) and strongly disagree (score=1).

2.6. Ethical clearance of research

This research obtained a research permit from the dean of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang No. 22.9.69/UN32.3.1/TU/2023 so that it can be ascertained that the data collection in this study has complied with the law and was carried out legally. This research also fulfils ethical feasibility based on the ethical feasibility certificate No. 14.6.7/UN32.14/PB/2024.

3. Results and discussion

3.1. Development of valid and practical Ethno-SSI integrated inquiry strategies

The results of content validity show the suitability of the strategy with supporting theories and learning outcomes, while the results of construct validity show the suitability of the syntax and complementary strategies developed with learning theory and empirical evidence, as shown in Table 7.

Table 7. Content validity and construct validity of Ethno-SSI integrated inquiry strategy.

Aspect	S-CVI/Ave	Category	S-CVI/UA	Category
Content validity	0.83	Medium	0.50	Accept/relevant/valid
Construct validity	0.90	High	0.71	Accept/relevant/valid

The results of content validation and construct validation conducted by experts on the academic manuscript of the Ethno-SSI integrated inquiry learning strategy resulted in an average score of 3.50 out of 4.00, which indicates that the prototype of the Ethno-SSI integrated inquiry learning strategy is declared appropriate, valid, and feasible to use with revision. Based on the results of the CVI (Content Validity Index) analysis in Table 7. content validity obtained a CVI score of 0.83 and construct validity of 0.90 with a high category so that the Ethno-SSI integrated inquiry strategy can be accepted and continued for research in improving the critical thinking skills of prospective chemistry teachers but with some improvements/revisions from the validator. Suggestions from validators include: 1) the concept of chemistry material with the Ethno-SSI context needs to be explained in more detail and contextually; 2) the rationality of the strategy needs to be considered from the novelty, supporting theory, and weaknesses of previous research; 3) improvements in content preparation starting from rationality, strategy development, and its application in chemistry learning need to be improved.

The validity results are supported by the practicality of the Ethno-SSI integrated inquiry strategy obtained from the observation sheet. The results of observations on implementing the Ethno-SSI integrated inquiry strategy were carried out through observations by two observers, as shown in Table 8.

Table 8. Learning observation.

Meeting (material)	Average Applicability		Average	Applicability (%)	Category
	Observer 1	Observer 2			
1-5 Thermochemistry	3.50	3.40	3.45	86.25	very practical

Table 8 shows that the average implementation of learning activities is 86.25%, which means that the implementation of the inquiry integrated Ethno-SSI syntax OETACA is categorized as very practical. Obstacles during learning become notes for improvement in the development of Ethno-SSI integrated inquiry strategy with OETACA syntax. The data from observing and obstacles found during the learning process using the OETACA syntax are processed and analyzed, and conclusions are drawn to complement a small-scale trial and improvements for large-scale effectiveness testing.

Based on the results of various learning models, an inquiry was one of the effective models for improving prospective chemistry teachers' critical thinking skills. Findings from the literature review results and data analysis show that the inquiry learning model can solve SSI cases (Chadwick *et al.*, 2023; Genel and Topçu, 2016; Saija *et al.*, 2022), and it is considered appropriate to apply the Ethnoscience approach (Alim *et al.*, 2020; Okechukwu *et al.*, 2014; Zidny *et al.*, 2020). The inquiry developed in this study is a modification of the 5E inquiry model (Engage, Explore, Explain, Elaborate, Evaluate) according to Bybee (2015). This model was chosen because it does not only focus on mastering material content but can also develop skills such as critical thinking, creativity, communication, and collaboration, which are the demands of 21st-century skills (Boholano, 2017; Masni *et al*, 2020; Syahrial *et al.*, 2021), so that prospective chemistry teachers are ready to face global challenges. In addition, the ethnoscience approach and socioscientific issues provide a relevant context in everyday life (Hofstein *et al.*, 2011; Ke *et al.*, 2021; Yazidi and Rijal, 2024), so that students can connect their knowledge through scientific, social, and cultural contexts according to Bybee (2015) stages. Through the exploration stage, prospective chemistry teachers can find and develop solutions to social scientific issues by analysing situations, considering various perspectives, and formulating sustainable solutions (Duschl and Bybee, 2014; Puig and Evagorou, 2020).

Bybee (2015) inquiry has weaknesses when applied to the context of Ethno-Socioscientific issues, including ethnoscience and SSI approaches involve many perspectives and complex research variables, so prospective chemistry teachers can have difficulty in understanding chemistry concepts in the Ethno-SSI context. This inquiry also has not placed the aspect of reconstruction or transformation, which is an important aspect of the ethnoscientific approach. Modifying the 5E inquiry steps (Engage, Explore, Explain, Elaborate, Evaluate) Bybee, (2015) The theoretical review and needs analysis results formed a new syntax with the term OETACA (Orientation, Exploration, Transformation, Argumentation, Conclution, Applications). The learning process with OETACA syntax, as shown in Table 9, aims to improve the critical thinking skills of prospective chemistry teachers. The conceptual framework of inquiry integrated Ethno-SSI syntax OETACA with critical thinking skills is shown in Fig. 4.

Table 9. The learning process of Ethno-SSI integrated inquiry syntax OETACA with critical thinking skills indicators.

Phase (modification)	Learning Objectives	Learning Process	Critical thinking skills indicators
Orientation **(Engage phase)**	Chemistry teacher candidates actively engage in the process of discovery and scientific inquiry by analyzing ethno-SSI cases involving health, religious, cultural, environmental, and social aspects.	- Listen to the lecturer's explanation regarding the topic and learning objectives of thermochemistry, which will be discussed in the context of ethno-SSI burning snail satay. - Listen to the apperception of ethno-SSI integrated chemistry material, both from the discourse in the student worksheets and also the video of the ethno-SSI problem of burning snail satay in the news. - Divide the discussion group. - Identify ethno-SSI problems with burning snail satay from the perspectives of health, religion, culture, environment, and society. - Formulate problems appropriately on the thermochemical material discussed according to Ethno-SSI of burning snail satay.	Basic clarification
Exploration **(Exploration phase)**	Solve problems by interacting with their environment so prospective chemistry teachers can gain experience and find new knowledge from Ethno-SSI exploration activities.	- Explore the Ethno-SSI case of burning snail skewers on producers. - Make data collection instruments by making interview guidelines and observation sheets. - Collect data by conducting observations, interviews, and documentation related to the making and burning of snail satay. - Record the results of the exploration on the student worksheets provided. - Provide a checklist (√) for interview data that can be transformed into scientific knowledge. - Search for references that support the Ethno-SSI of burning snail satay from several sources, including books, scientific journal articles, news in mass media/online, or other sources relevant to the topic as a reference in scientific transformation activities.	Basic support
Transformation **(Reconstruction)**	Can connect indigenous knowledge to scientific knowledge.	- Reconstruct the original knowledge of the community into scientific understanding from the references obtained. - Design Ethno-SSI practicum/project activities for charcoal making and thermochemical tests from exploratory studies.	Advanced clarification

		– Record the Ethno-SSI practicum/project data results on the student worksheets provided. – Paste evidence of exploration/observation/interview results on the student worksheets provided. – Transfer the transformable interview data into the transformation table.	
Argumentation **(Explain and elaborate)**	Knowledge does not only exist in an individual's mind but also through a reconstruction process by considering relevant sources, laboratory activities, and the surrounding physical, cultural, and social environment.	– Discuss in groups to solve/answer questions about the Ethno-SSI context of burning snail skewers in scientific arguments. – Answer scientific arguments about burning snail skewers with claims, evidence, and explanations. – Direct students in arguing through dialogue, discussion, and debate, and then making the right decision. – Determine whether the issue is true/false, good/no, agree/disagree. – Provide supporting evidence for claims in the form of data obtained during experiments/practicums and data obtained from the results of scientific transformations. – Make explanations in statements to support evidence, namely the reasons for claims based on applicable theories, laws, or principles.	Advanced clarification
Conclusion **(Evaluate)**	Train opinion, dialogue, discussion, debate, and make decisions by finding data and facts to solve problems based on claims, evidence, and explanations.	– Make conclusions based on the results of exploration, transformation/experimentation, data analysis, and scientific argumentation. – Make decisions about chemical problems in the Ethno-SSI context of burning snail satay. – Listen to the lecturer clarify the correct answers and conclusions based on the chemical material of the ethno-SSI context of burning snail satay discussed.	Inference
Application **(Evaluate)**	Understand chemistry concepts in the context of ethno-SSI herbs, solve problems, and find solutions by drawing conclusions based on moral reasoning, data interpretation, and scientific evidence so that chemistry learning becomes more contextualized.	- Apply the Ethno-SSI context of burning snail satay in everyday life on exercise/evaluation questions. - Work on questions in the application stage using the correct method. - Relate the questions to health, culture, environment, religion, social, and chemical processes in the Ethno-SSI context of burning snail satay. - Communicate the results of working on thermochemical Ethno-SSI application questions. - Listen to the lecturer clarify the correct answer. - Reconstruct students' knowledge after the learning process by mentioning what knowledge they have gained, difficulties experienced in the learning process, and alternative solutions to overcome problems. - Make a bibliography and attach evidence of the results of the Ethno-SSI practicum/project activities.	Strategy and tactics

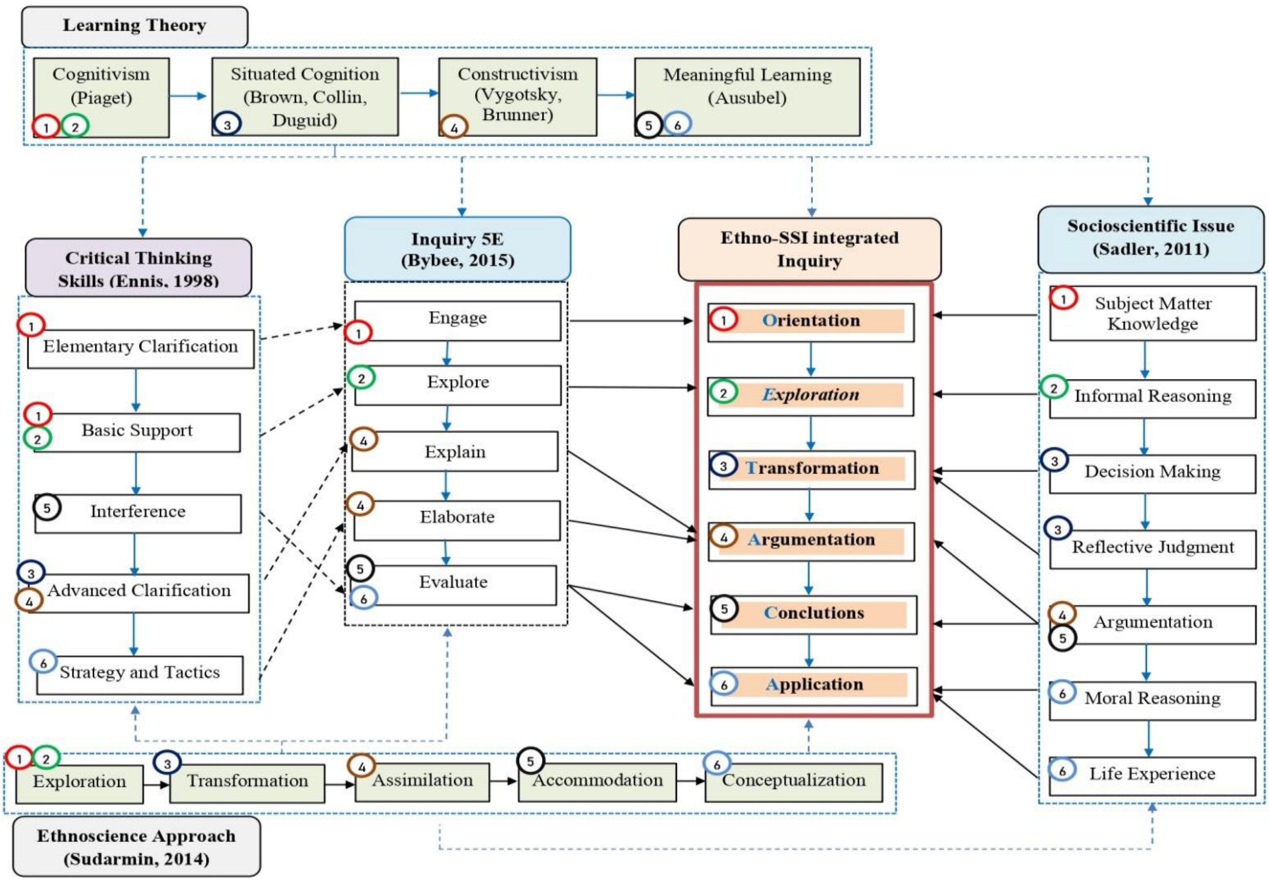


Figure 4. The conceptual framework of inquiry integrated Ethno-SSI syntax OETACA with critical thinking skills.
Source: Elaborated by the author using data from Ennis (1998), Bybee (2015), Sadler (2011) and Sudarmin (2014).

3.1.1. Orientation

The first phase is Orientation, which is a modification of Engage in Bybee (2015) inquiry. It aims to increase the elementary clarification indicator in critical thinking skills. Orientation is a step to foster a responsive atmosphere or climate at the beginning of learning (Cavagnetto, 2010; Chang and Park, 2020; Duschl and Bybee, 2014). In Phase 1, "Orientation", students in groups read discourse, watch videos, analyze existing problems related to thermochemical material in the context of burning snail satay, and then formulate the problem. On the student worksheet, video links, article links, and material links that students can access have been provided so that group activities carried out by students are watching videos, reading articles, and then recording interesting findings and problems from videos and articles that have been accessed. Students are asked to identify the concept of Ethno-SSI problems of burning snail satay in terms of scientific, environmental, health, religious, cultural, and social aspects and then formulate problems related to the context of Thermochemistry. The following identifies the Ethno-SSI context of burning snail satay, shown in **Table 10**.

Table 10. Orientation phase of the process of burning snail satay context.

Ethno-SSI Aspects	Explanation
Health	- Snail slime is considered to have antibacterial and anticancer properties. - Snail mucus can treat tuberculosis, asthma, and toothache. It is also believed to have cosmetic properties that improve the appearance and smoothness of the skin. - Charcoal-grilled meat can be a carcinogen. - Snails are animals that can host various microorganisms, including pathogens, germs, bacteria, and parasites.
Religion	- There is a debate among scholars and religious experts regarding the halal or haram of eating snails. - Certain foods may be considered haram if they are deemed unclean or impure. However, if they are found to have beneficial properties for human health, they may be considered halal or permissible for consumption.
Culture	Snail satay is a local wisdom regional specialty food maintained today because it is believed to benefit the community.
Environment	- Snail processing waste, such as shells and dung, can be used as fertilizer. - Some people consider snails pests because of their rapidly growing population, which lays eggs and damages crops. - The smoke from burning snail satay contains CO and CO2 gas, can cause shortness of breath/asthma.
Social	The processing of snail satay can be a livelihood for the community and increase income.

3.1.2. Exploration

The second phase is Exploration, which is the same as Bybee (2015) exploration phase, which aims to improve the Basic Support indicators in critical thinking skills. In Phase 2 "Exploration," activities are designed by making observation sheets, interviews, and documentation at the place where the snail satay Ethno-SSI product is produced. Students are asked to explore where the Ethno-SSI context is made, then write down the interview results in a student worksheet and attach evidence of the observations and interviews. This interview and documentation activities are conducted to obtain data on the community's original knowledge regarding manufacturing procedures, the benefits of the products, and controversial issues. In addition to direct observation and interviews with Ethno-SSI producers of snail satay, students are asked to find supporting references from several sources, including books, scientific journal articles, news in the mass media/online, or other sources relevant to thermochemistry. These references will later become references in scientific science transformation activities.

3.1.3. Transformation

The third phase is Transformation, an additional modification of the Bybee (2015) phase that aims to improve the Advanced Clarification indicators in critical thinking skills. In Phase 3 "Transformation", after data collection activities related to the original science knowledge of the community are fulfilled, the next step is to provide a scientific explanation for the findings. Scientific explanation in the ethnoscience approach is included in the transformation stage (Diliarosta *et al.*, 2021). At this stage, scientific explanations of evidence and claims from indigenous knowledge form scientific or ethnoscientific knowledge as a product of the synergy of culture and science (Sumarni *et al.*, 2022; Zidny *et al.*, 2020). Students are asked to provide a checklist and then fill in the table provided to transform indigenous knowledge into scientific knowledge. In the transformation phase, the student worksheet also contains Ethno-SSI practicum activities to prove the Ethno-SSI context scientifically, and students are asked to write independently the practicum procedures to be carried out. Examples of discussions from the Indigenous Knowledge interview results that can be transformed into Scientific Knowledge are shown in **Table 11**.

Table 11. Ethno-SSI transformation of snail satay burning.

No.	Indigenous Knowledge	Scientific Knowledge
1	The fuel for burning snail satay uses wood-type charcoal because wood is relatively cheaper and easier to obtain.	Wood burning requires a specific activation energy to start a chemical reaction. After burning, an exothermic reaction that releases energy occurs, but this process is not instant and occurs gradually. Wood charcoal is good for cooking because it contains high carbon produced from carbonizing wood with a high cellulose content.
2	Cooking oil/kerosene added to charcoal can accelerate the flame in the combustion process.	The heat decomposes the hydrocarbon chain from the cooking oil/kerosene into a gas from its generation, creating a fire to burn charcoal faster. This is because as the hydrocarbon bonds in the combustion reaction release the energy stored in the bonds, a hotter flame will be created.
3	Burning using charcoal takes longer.	For charcoal to burn, oxygen in the air must enter the solid structure, meet up with the surface of the carbon, and undergo a chemical reaction with the carbon to produce heat and, after some seconds, fire. This process is slower because of the restricted access to air and the chemical reactions that must take place within the solid structure of the charcoal.
4	When burning a snail satay, the heat from the burning will be felt around it.	The system and environment are related to the context of burning snail satay and charcoal. When burning snails stay, heat is transferred from the system to the environment, causing people around it to feel warm.
5	Burning snail satay meat is not suitable for health.	If it contains lots of carbon, high consumption of burnt satay can be carcinogenic because it forms carcinogenic compounds like polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines (HCA), which can enhance cancer risk. If the snail satay is too burned, the ingredients are burned, and the nutrients in the satay will also be destroyed.

In addition to scientific transformation, the activities in this phase are also carried out by investigating directly through practicum activities to make charcoal from organic waste. Charcoal is a black residue containing impure carbon substances produced by removing the existing water content and derived from volatile components in animals or plants. Charcoal is generally made by heating or burning other organic or inorganic objects. This black-colored, easily crushed, lightweight, coal-like charcoal has a substance consisting of 85% to 98% carbon, and the rest is ash or other chemicals. Several types of charcoal are distinguished based on the origin of the raw materials, such as wood charcoal, rice husks, coconut husks, coconut shells, corn stalks, litter/leaves, bamboo, and fruit skins such as durian, mahogany, cocoa, and cotton/and skins. Using charcoal to utilize household waste can save fuel and be more environmentally friendly. **Table 12** below briefly investigates charcoal's calorific value and moisture content.

Table 12. Determination of calorific value and moisture content of charcoal.

No.	Charcoal Type	Temperature after charcoal (0C)	Mass before heating (gr)	Mass after heating	Moisture content (%)
1	Wood charcoal	56.8	81.206	80.505	4.1
2	Wood dust charcoal	62.8	69.240	64.629	48.8
3	Rice husk charcoal	43	95.200	95.15	54
4	Coconut fiber charcoal	80.5	9.425	6.809	27.75
5	Coconut shell charcoal	89.3	95.492	94.800	10.28
6	Corncob charcoal	39.5	19.837	10.240	48.4

The results of making charcoal from organic waste by a prospective chemistry teacher are shown in **Fig. 5**.

Figure 5. Practicum for making charcoal from organic waste in thermochemical concept.

Based on investigations conducted by prospective chemistry teachers and data from practicum results, the better organic waste used as charcoal is the type of coconut shell. This is because coconut shell charcoal has a higher density than charcoal from other materials, so it is more durable and provides more efficient combustion results because the water content is less.

3.1.4. Argumentation

The fourth phase is argumentation, which modifies the explanation and elaboration in Bybee (2015) inquiry and aims to increase the advanced clarification indicator in critical thinking skills. In Phase 4 "Argumentation", students focus on sharing content/material in the interaction between lecturers and students. In this process, students can discuss responding to the Ethno-SSI case of burning snail satay in groups through dialogue, discussion, and debate activities. Students conduct group discussions to analyze the data collected to be able to answer questions at the argumentation stage. Lecturers guide students in conveying the results of activities that have been carried out using their ideas or words so the process of constructing knowledge will be well developed. This argumentation activity aims for students to argue to solve Ethno-SSI problems and to determine the right decision.

This argumentation stage is expected to help students clarify their conceptual understanding and communicate their knowledge in various aspects such as health, religion, environment, culture, and society. In this activity, students record their discussion activities to see the achievement of their ability to argue and decide on problem-solving. Debating activities are used in class discussions to train students' critical thinking about the relationship between science, technology, and society, and to make decisions based on the questions asked (Albe, 2008).

Students develop quality scientific arguments from their investigations using the components of claims, evidence, and explanations (Berland and McNeill, 2010).

- Claim: the answer or conclusion when encountering a problem/question.
- Evidence: support for claims in the form of data obtained during experiments/practicum or from scientific transformation results.
- Explanation: a statement to support the evidence, which is why (rationale) of the evidence you use to support the claim is based on the theory, law, and theories.

3.1.5. Conclusion

The fifth phase is the Conclusion, which modifies the evaluation in Bybee (2015) inquiry to improve the Inference indicator in critical thinking skills. The conclusions are formulated by describing the results of findings obtained based on discussions and scientific transformation in the context of Ethno-SSI of burning snail satay. To arrive at the correct conclusion, the lecturers must show students what information they need to describe in the right way to get important information from the Ethno-SSI problem of burning snail satay. At this stage, the lecturer guides students in formulating conclusions based on the data collected from the results of experiments/practicums and exploratory studies. Based on the practicum results, it can be concluded that the higher the water content in charcoal, the smaller the calorific value; on the contrary, if the water content is low, the calorific value is more significant.

3.1.6. Application

The sixth phase is the Conclusion, a modification of the evaluation in Bybee (2015) inquiry, which aims to improve the strategy and tactics indicator in critical thinking skills. Application is the final stage of this phase. The aim is to evaluate students' critical thinking skills by applying thermochemical problems. After that, students can communicate the results of group discussions in front of the class. This application provides meaningful and contextualized learning for students. Lecturers can reflect on all the activities that have been carried out to see the success of each phase. Giving Ethno-SSI-based practice questions to students positively impacts students in finding more information about the subject matter so that it can improve critical thinking skills. In addition, practice questions allow students to deepen their understanding of learning concepts by applying theory in the context of basic chemistry questions.

3.2. Critical thinkings results using Ethno-SSI integrated inquiry strategy

The results of critical thinking skills obtained through the application of the Ethno-SSI integrated inquiry strategy reflect the ability of prospective chemistry teachers to analyze, evaluate, and apply local wisdom-based chemical knowledge. Prospective chemistry teachers show a deep understanding of thermochemical concepts, such as enthalpy, phase change, and the laws of thermodynamics. They can relate theory to daily practice, especially in local culture, such as how the burning process in snail satay affects energy and temperature changes and tell it to the principles of thermochemistry. Significant improvement in students' critical thinking skills can be seen using the Normalized Gain (N-gain) test shown in Table 13.

Table 13. Improvement of students' critical thinking skills.

Class	Pretest Average	Posttest Average	N-Gain <g>	Category
Offering A and B	43.6538	83.7179	0.70132	High

The increase in critical thinking skills is expressed by the normalized gain test based on the pretest and posttest scores on offering A and B to as many as 52 students. The normalized gain test results show that improving critical thinking skills in offering A and B is included in the high category (N-Gain = 0.7). The results of the statistical calculations are shown in Table 14.

Table 14. Statistical calculation results analysis.

Category	Pretest	Posttest
∑X	2270	4353.333
χ	43.6538	83.7179
Max	80	96.6667
Min	20	66.6667
s^2	97375,7	357492
s	312.05	597.905
Range	60	30

The data in Table 14 show that the average posttest (83.71) increased from the pretest (43.65). These results indicate that the ethno-SSI integrated inquiry strategy teaches prospective chemistry teachers to produce scientific knowledge and skills to address ethno-SSI issues related to chemistry learning. This strategy also helps prospective chemistry teachers build relationships and correlations between culture, science, and the environment and encourages critical and systematic thinking in addressing various problems. The critical thinking skills test score data was also analyzed per indicator to obtain an overview of each indicator of critical thinking skills, according to Ennis, as shown in Table 15.

Table 15. Student critical thinking skills test results per indicator.

No.	Critical Thinking Skills Indicator	Average score per indicator	Category
1	Elementary Clarification	85	Very High
2	Basic Support	75	Medium
3	Interference	80	High
4	Advanced Clarification	78	Medium
5	Strategy and Tactics	75	Medium

3.2.1. Elementary clarification

Critical thinking skills on the indicator of providing simple explanations in this study, including obtaining the highest skill level among other indicators with an average score of 85. This finding shows prospective chemistry teachers can formulate and answer questions by reading and understanding the content. Prospective chemistry teachers can also correctly write chemical symbols or formulas in question questions and their equations. This is because, during the learning process, prospective chemistry teachers are continuously trained to work on chemistry questions on Thermochemistry that are integrated with the Ethno-SSI context. In this indicator, prospective chemistry teachers are expected to be able to identify images, diagrams, and symbols based on the information contained in the problem. According to Ennis, (1990), students can think critically by making habits that are continuously carried out, such as formulating questions and answering questions that require explanation.

Indicators of critical thinking skills in the Elementary Clarification aspect include questions that focus on research activities, assessing a statement, and answering questions relevant to the explanation according to the Ethno-SSI context. This indicator can evaluate the possibility of prospective chemistry teachers in formulating appropriate problem specifications by recognizing important components and using images, diagrams, and symbols to describe scenarios based on data and facts (Masni *et al.*, 2020). The findings in the study showed that prospective chemistry teachers made few mistakes when solving problems, such as a lack of reading and understanding of Ethno-SSI content. Students who answered incorrectly on this indicator indicated that they could not understand the significance of the Ethno-SSI problem due to failure to remember or understand the content of the material discussed. Students can also not transcribe known facts from the problem in pictures, tables, or diagrams, so prospective chemistry teachers need to be trained in formulating problems and answering based on data and their knowledge related to material content (Fijar *et al.*, 2019).

3.2.2. Basic support

Students' critical thinking skills on the Basic Support indicator obtained an average score of 75 in the medium category. The results of this indicator show that prospective chemistry teachers can determine the proper steps to analyze problems based on concepts and phenomena that exist in Ethno-SSI questions, even though the results are not optimal, and not all answers are correct. This shows that many prospective chemistry teachers still have not been able to analyze the phenomena and symptoms that

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arise from the statements in the problem, which are essential information related to the Ethno-SSI context, so it is not easy to analyze. In Ethno-SSI questions, some prospective chemistry teachers experience errors when identifying the relationship between concepts and statements or phenomena. This can happen because so far, prospective chemistry teachers tend to memorize material and less at the time of application with the local context or SSI, so even though students know the concept but do not know how to apply it in everyday life (Kasi and Widodo, 2022).

In this indicator, students are asked to establish the credibility of a source and research and assess the results of research on the topic of explaining enthalpy changes and types of enthalpy and explaining energy and energy changes in chemical reactions. This study's basic support indicator measures prospective chemistry teachers' skills in analyzing assumptions, using procedures, and existing investigations to solve problems (Maknun, 2020). This indicator considers whether the source of information presented in the issue is reliable or not (Nurdin *et al.*, 2018).

3.2.3. Interference

Students' critical thinking skills on the Interference indicator obtained an average score of 80 in the high category. This indicator was identified by the element needed to conclude report data, principles, judgments, beliefs or opinions, concepts, and descriptions with the Ethno-SSI context. The question on this indicator discusses drawing findings related to thermochemistry in burning snail satay. Shows the attitude and ability to understand and analyze Ethno-SSI problems from various factors so prospective chemistry teachers can draw the correct conclusions. By developing skills in this indicator, prospective chemistry teachers will become more competent in understanding scientific concepts and more sensitive to the social and environmental context in which they are located. This indicator can also prepare prospective chemistry teachers to become individuals who think critically and responsibly when facing the challenges of a complex society.

3.2.4. Advanced clarification

Critical thinking ability on the indicator of providing a further explanation has the lowest value compared to other indicators. Based on the analysis of student answers, a score of 78 was obtained in the moderate category; this was due to the low initial knowledge of prospective chemistry teachers about the concept of thermochemistry, making it difficult to relate to the Ethno-SSI context. The indicator of providing a further explanation in this study was carried out by constructing arguments by analyzing and providing explanations in the form of defining terms, considering definitions using appropriate criteria, and identifying assumptions in the Ethno-SSI context. In this indicator, many prospective chemistry teachers still have not been able to identify assumptions. Answering these questions requires a sufficient understanding of the material; if students do not master the material, it will affect them in identifying assumptions and solving problems, especially in describing equations from several related equations or finding relationships from several thermochemical equations.

3.2.5. Strategy and tactics

Critical thinking skills on indicators of organizing strategies and tactics score 75 with a low category. Based on the analysis results, most prospective chemistry teachers have not been able to decide the proper action to solve problems in the problem, especially when using thermochemical formulas or equations. Indicators Organising strategies and tactics is done by considering possible solutions to solve Ethno-SSI context problems. Prospective chemistry teachers can think with their knowledge to formulate an alternative solution by solving problems that occur, especially using formulas by the thermochemistry concept. If prospective chemistry teachers are not trained to think critically and analytically, they will have difficulty designing effective strategies and tactics to address real-world Ethno-SSI problems.

3.3. Perception of prospective chemistry teachers using the Ethno-SSI integrated inquiry strategy

Implementation of the inquiry-integrated Ethno-SSI syntax OETACA has positively responded to almost all components. This indicates that the implementation of the inquiry-integrated Ethno-SSI syntax OETACA prototype is very good, where students are very interested when being given a case/problem so that they are motivated to analyze Ethno-SSI problems and can explore independently and in groups. Furthermore, students are motivated to determine the steps of problem-solving and design Ethno-SSI experimental procedures. Experimental procedures designed by students can make it easier to identify chemical concepts and principles so that students can solve Ethno-SSI cases given by lecturers. Learning activities through the Ethno-SSI integrated inquiry strategy directly impact students, especially in getting a pleasant learning experience, having the courage to argue, being helped in formulating problems, more easily understanding and comprehending the material studied, and having a high willingness to attend lectures. Furthermore, the graph of student responses to the Ethno-SSI integrated inquiry strategy is shown in **Fig. 6**.

Based on the responses to the Ethno-SSI integrated inquiry strategy, as shown in **Fig. 2**, most (92%) agreed and strongly agreed to implement this strategy in chemistry learning in the classroom. This strategy proved to be practical and effective in facilitating understanding of chemical concepts, primarily related to Ethno-SSI problems encountered, such as in the context of Thermochemistry, namely burning snail satay. Chemistry teacher candidates (78%) agreed and strongly agreed that the Ethno-SSI inquiry steps (orientation, exploration, transformation, argumentation, conclusion, and application) improve critical thinking skills. These results align with research by Zidny and Eilks (2022) that ethnoscience and SSI will be actively involved through scientific inquiry activities such as exploration, practical activities in the laboratory, data analysis, and scientific argumentation that require logical reasoning.

Another finding is that the % of chemistry teacher candidates 75% agreed that this strategy teaches how to reconstruct indigenous knowledge into scientific knowledge in the context of Ethno-SSI. Ethnoscience is a learning approach that contains activities to reconstruct indigenous knowledge into scientific knowledge that can be accounted for related to local wisdom (Dewi *et al.*, 2022; Diliarosta *et al.*, 2021; Sudarmin *et al.*, 2020). This strategy can improve critical thinking skills in solving the Ethno-SSI context 84% of prospective chemistry teachers agreed. The results of many previous studies mention that ethnoscience-based learning approaches (Prayogi *et al.*, 2023; Sudarmin *et al.*, 2019; Zidny *et al.*, 2020) and SSI (Barrue and Albe, 2013; Borgerding and Dagistan, 2018; Yacoubian and Khishfe, 2018) can improve critical thinking skills.

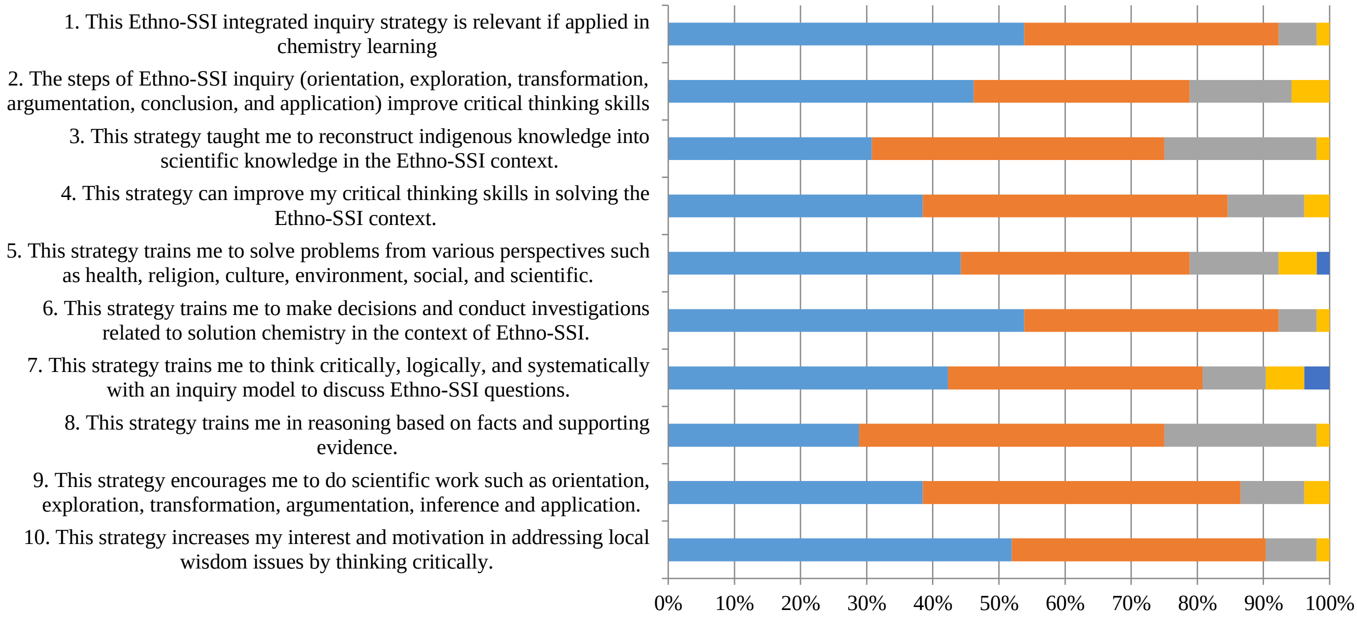

Figure 6. Responses of prospective chemistry teachers (N=52) to the Ethno-SSI integrated inquiry strategy.

This strategy trains in solving problems from various perspectives such as health, religion, culture, environment, social, and scientific 78% of prospective chemistry teachers answered in the affirmative. Learning with the SSI strategy trains problem-solving from various perspectives, including social, environmental, economic, and political, based on applicable morals and ethics (Herman *et al.*, 2021; 2019; Holbrook *et al.*, 2020; Raveendran, 2021; Songer and Ibarrola Recalde, 2021). This strategy trains in making decisions and conducting investigations related to solution chemistry materials in the Ethno-SSI context, and 92% agree. This is by the results of research by Gupta *et al.* (2015) which states that learning chemistry with guided inquiry instruction can improve critical thinking skills through investigation and decision-making activities. Another study mentioned that the inquiry learning model with the 5E cycle (engagement, exploration, explanation, elaboration, evaluation) using the SSI context effectively improves critical thinking skills (Cahyarini *et al.*, 2016).

The statement that this strategy trains me to think critically, logically, and systematically with the inquiry model to discuss Ethno-SSI questions received a response of 82% agree. The inquiry learning model can help prospective chemistry teachers think critically, logically, and systematically by searching, finding, and analyzing information independently to make learning more active (Capps and Crawford, 2013; Wenning, 2011). This inquiry-learning model can improve critical thinking skills by creating an engaging and interactive learning environment through experimentation and proof based on relevant evidence (Adnan *et al.*, 2021; Cheung *et al.*, 2020). This strategy trains in expressing reasons based on facts and supporting evidence 75% of prospective chemistry teachers agreed. This finding shows prospective chemistry teachers need alternative learning strategies to help them understand, analyze data, and make decisions based on facts and evidence. In Thermochemistry, prospective chemistry teachers are involved in experimental activities such as thermochemical practicum, energy calculation, and basic theory of thermochemical concepts. Activities linking scientific facts with socioscientific issues can improve students' critical thinking skills.

This strategy encourages prospective chemistry teachers to do scientific work such as orientation, exploration, transformation, and argumentation, and 85% agree. Ethno-SSI integrated inquiry learning strategy is an innovative learning strategy constructed to train the critical thinking skills of prospective chemistry teachers. This strategy combines inquiry learning models with ethnoscience approaches and socioscientific issues related to local wisdom. Thus, prospective chemistry teachers not only learn about chemistry concepts but also understand how these concepts can relate to everyday life and the challenges faced by controversial, dilemmatic, and complex issues (Rahayu, 2019; Saija *et al.*, 2022). This strategy increases the interest and motivation of prospective chemistry teachers in addressing local wisdom issues with critical thinking 91% agree. Chemistry learning associated with the context of ethnoscience and socioscientific issues can increase awareness of the importance of social and environmental impacts so that the interest of prospective chemistry teachers can increase to contribute to addressing these issues (Zidny and Eilks, 2022).

4. Conclusions

The successfully developed Ethno-SSI integrated inquiry strategy is a learning strategy that incorporates local wisdom to improve the critical thinking skills of prospective chemistry teachers. The syntax of this learning strategy includes six phases, namely: (1) orientation, (2) exploration, (3) transformation, (4) argumentation, (5) conclusion, and (6) application. The Ethno-SSI integrated inquiry learning strategy has fulfilled the valid and practical requirements. The validation results with CVI of the Ethno-SSI integrated inquiry strategy in the content aspect received a score of 0.83 and a construct aspect of 0.93 in the high category. The results of the critical thinking skills test instrument validation received a CVI score of 0.90, while the questionnaire received a score of 0.89, both of which were in the high category. The practicality of the Ethno-SSI integrated inquiry strategy is based on the assessment of learning implementation, which obtained an average learning implementation of 86.25% with a convenient category. The results of the critical thinking skills test on a small scale show an N-gain of 0.7 in the high category, but the criteria for each indicator are in the moderate category. The results of prospective chemistry teachers' responses to the Ethno-

SSI integrated inquiry strategy, on average, gave a positive response to each component with the categories strongly agree (43%), agree (41%), undecided (12%), disagree (3%), strongly disagree (1%).

5. Recommendation

Further research is recommended to implement the inquiry-Ethno-SSI strategy with OETAKA syntax on a large scale by conducting an effectiveness test on the strategy developed. The context of local wisdom studied is more relevant to chemistry learning. This research can also be conducted at several universities to obtain many data sources. The instruments and teaching materials developed can be adjusted to the Ethno-SSI context to make chemistry learning more exciting and relevant.

6. Limitation

This research only focuses on the topic of Thermochemistry. The local wisdom raised is only related to the burning snail staying in an area prospective chemistry teachers may not know. The data collected in this study is only limited to small-scale trials, so it is less specific and quantitatively in-depth. This research requires a long time in field exploration activities because students must still be trained in observation and interview activities.

Authors' contribution

Conceptualization: Ratna Kumala Dewi; Sri Rahayu; **Data curation**: Muntholib Muntholib; Ratna Kumala Dewi; **Formal Analysis**: Woro Sumarni; **Funding acquisition**: Not applicable; **Investigation**: Ratna Kumala Dewi; **Methodology**: Ratna Kumala Dewi; Sri Rahayu; **Project administration**: Ratna Kumala Dewi; **Resources**: Muntholib Muntholib; **Software**: Not applicable; **Supervision**: Sri Rahayu; **Validation**: Woro Sumarni; **Visualization**: Ratna Kumala Dewi; **Writing – original draft**: Ratna Kumala Dewi; Sri Rahayu; Muntholib Muntholib; Woro Sumarni; **Writing – review & editing**: Ratna Kumala Dewi; Sri Rahayu; Muntholib Muntholib; Woro Sumarni.

Data availability statement

The data will be available upon request.

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Conflict of interest

The authors declare that there is no conflict of interest.

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The effectiveness of guided inquiry learning based on Anderson's sketch analysis on students' higher order thinking skills in reaction rate

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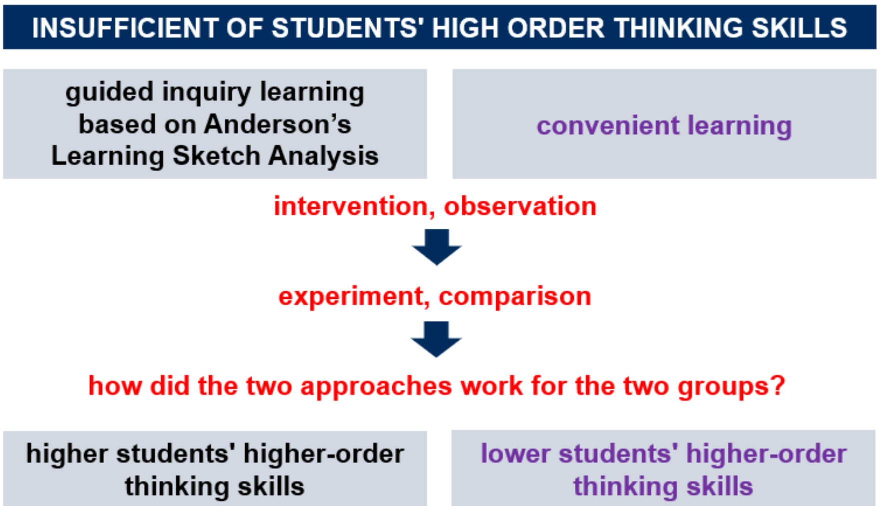
Abstract

The 21st century learning process focuses on enhancing higher-order thinking skills (HOTs). In Indonesian schools, students' HOTs in the reaction rate topic need improvement. This study investigates the effectiveness of guided inquiry learning based on Anderson's learning sketch analysis in enhancing HOTs. Involving 60 of 11th grade science students from a public high school in Malang, the study used a quasi-experimental design with an experimental class (Anderson's learning sketch) and a control class (conventional learning). The research instrument was a HOTs assessment with 10 essay questions. Data analysis using an independent sample t-test showed a significant difference ($p = 0.002$), with the experimental class scoring higher (69.3) than the control class (49.9). The findings indicate that Anderson's Learning Sketch Analysis is effective in improving students' HOTs, with the experimental class outperforming the control class in skills such as analysis (63% vs. 39%), evaluation (71% vs. 55%), and creation (78% vs. 70%). These results highlight the importance of guided inquiry in enhancing HOTs.



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Highlights

- Guided inquiry learning for promoting higher order thinking skills.
- Anderson's sketch analysis platform for measuring higher order thinking skills.
- Employing reaction rate teaching, a tool for higher order thinking skills.

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1. Introduction

The Indonesian Curriculum is designed to equip students with the competencies necessary for the demands of the 21st century, focusing on the development of 4C skills: (a) critical thinking and problem-solving, (b) communication, (c) creativity and innovation, and (d) collaboration. These skills are essential for preparing students to navigate complex, rapidly changing global environments. To achieve this, the curriculum emphasizes cultivating higher-order thinking skills (HOTs) throughout the learning process, enabling students to engage in deeper, more analytical thinking and become effective problem solvers (Kemdikbud, 2018).

Previous research has extensively explored strategies and teaching models aimed at enhancing students' higher-order thinking skills (HOTs) in chemistry education. One effective strategy for fostering HOTs is the use of guided inquiry-based learning. For instance, Mawardi *et al.* (2020) demonstrated that guided inquiry-based student worksheets significantly promote the development of HOTs. Similarly, research by Prahani *et al.* (2016) showed that the guided inquiry model is particularly effective in improving students' problem-solving abilities. This approach enables students to actively engage in the learning process through independent investigation and problem-solving, while still receiving guidance from the teacher. As a result, students are better able to grasp complex concepts and develop critical thinking skills more efficiently.

Anderson *et al.* (2001) developed a taxonomy-based learning outline consisting of knowledge and cognitive dimensions to serve as a foundational framework in the teaching process. The learning components, such as learning objectives, learning activities, and assessment, are categorized in the taxonomy table based on their knowledge and cognitive dimensions. The management of these learning components within the outline developed by Anderson is referred to as Anderson's Sketch Learning.

Anderson's Learning Sketch Analysis can be an effective tool for developing students' HOTs by focusing on the cognitive processes of analyzing (C4), evaluating (C5), and creating (C6), as outlined in the revised Bloom's Taxonomy. This approach is designed to help teachers promote HOTs during instruction by organizing learning components within the higher-order thinking categories. This structured classification enhances the learning process, ensuring that it is both targeted and effective in fostering critical thinking.

As a teaching method, Anderson's learning sketch analysis can be seamlessly integrated into various instructional models, including the guided inquiry model, to achieve more optimal learning outcomes (Anderson *et al.*, 2001). For example, Net *et al.* (2024) developed science worksheets oriented toward HOTs through inquiry-based learning, demonstrating the value of this approach. Additionally, Nzomo *et al.* (2023) used inquiry-based learning to build students' self-efficacy in chemistry, further improving their HOTs. These studies highlight the potential of combining Anderson's Learning Sketch Analysis with inquiry-based methods to support and enhance HOTs development.

The core competencies outlined in Minister of Education and Culture Regulation No. 37 of 2018 highlight that the topic of reaction rates in chemistry is one that demands HOTs. According to Habiddin and Page (2019), Indonesian students' HOTs in chemical kinetics, including reaction rates, remain underdeveloped and require improvement. However, the nature of the reaction rate topic—characterized by reasoning, laboratory work, and problem-solving—presents a significant opportunity to effectively cultivate HOTs within this area of learning.

The study titled "Development of Teaching Materials for the Reaction Rate Subject Oriented to HOTs based on Anderson's sketch analysis" by Herunata *et al.* (2021) categorizes the reaction rate topic into four subtopics: 1) the concept of reaction rate, 2) reaction order, 3) theories of reaction rate, and 4) factors affecting reaction rate. These components of reaction rate learning are then systematically organized within Anderson's learning sketch analysis framework, as detailed in **Table 1**.

Table 1. Anderson's sketch analysis for the reaction rate topic.

Knowledge dimension	Cognitive dimension					
	Remember	Understand	Apply	Analyze	Evaluate	Create
Factual knowledge						
Conceptual knowledge				Objective 1 Assessment 1 Objective 3 Activity 3 Assessment 3	Objective 2 Assessment 2	
Procedural knowledge				Activity 1	Activity 2	Objective 4 Activity 4 Assessment 4
Metacognitive knowledge						

Given the background outlined above, it is essential to investigate the effectiveness of guided inquiry learning based on Anderson's Learning Sketch Analysis in enhancing students' HOTs, specifically in the context of reaction rates. This research builds upon previous studies that assessed students' abilities after learning about reaction rates without any specific interventions. The findings from those studies identified the key aspects of HOTs that need further strengthening in the learning process.

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2. Method

This research was conducted at a public senior high school in Malang City during the first semester of the 2022/2023 academic year. The study population comprised of 11th grade science students from the school. A cluster random sampling technique was employed to select the study sample. The sample consisted of two classes: 11th Grade Science 3, designated as the experimental group, and 11th Grade Science 2, designated as the control group.

This study utilized a quasi-experimental design. The experiment aimed to compare the HOTs of students in the experimental group, who received guided-inquiry learning based on Anderson's sketch learning analysis on the topic of reaction rates, with the control group, who underwent convenient teaching of reaction rates topic. The purpose was to assess the impact of these different teaching approaches on students' HOTs abilities. The design is:

O1 (first observation)	*X*	O2 (last observation)
O1 (first observation)		O2 (last observation)

X: Treatment: guided inquiry learning based on Anderson's sketch analysis in reaction rate topic.

Before experimenting, we performed a statistical analysis to confirm the equivalence of abilities between the students in the experimental and control groups, allowing for a direct comparison of their final capabilities. The data for this analysis were drawn from the students' report card grades from the previous semester. To assess normality, we employed the Mann-Whitney U test.

In the experimental class, we implemented a guided inquiry model integrated with Anderson's Learning Sketch Analysis approach to enhance the learning experience. In contrast, the control class utilized more conventional teaching methods. In the experimental group, learning activities focused on providing illustrations and teacher-guided questions to deepen students' understanding and foster higher-order thinking skills (HOTS), in alignment with the framework outlined in Anderson's learning sketch analysis. Meanwhile, the control class was taught without any specific interventions, relying primarily on lectures and discussions. The conventional teaching methods employed in the control group aimed to develop HOTS through a scientific approach, which is a form of guided inquiry-based learning.

The impact of the treatment in this study was evaluated using statistical tests on the assessment results of students' higher-order thinking skills (HOTs) in both classes after they completed their learning on reaction rates. The independent sample t-test was employed for this analysis, following prerequisite tests for normality and homogeneity. Additionally, the data on students' HOTs assessment results were examined both quantitatively and descriptively to provide a comprehensive overview of their abilities across each HOTs category.

For this study, we utilized an assessment instrument specifically designed to measure students' HOTs, which comprised 10 open-ended questions focused on the HOTs relevant to reaction rates. The validity of this instrument was established through evaluation by two expert validators: a chemistry lecturer and a high school chemistry teacher. The average validity percentage, as shown in **Table 2**, was an impressive 89% from both validators, confirming the instrument's appropriateness for the study following minor revisions based on their feedback.

Furthermore, the HOTs assessment instrument underwent empirical testing with grade XI science students at a senior high school in Batu City to further assess its validity and reliability. Statistical analysis of the pilot test data revealed that all items in the HOTs assessment instrument were valid, with calculated r values ranging from 0.466 to 0.804, all exceeding the critical value of 0.433. The reliability analysis produced a Cronbach's Alpha value of 0.830, indicating strong reliability for the instrument. According to established standards by George and Mallery (2016), an alpha above 0.70 is considered acceptable for social science research. Thus, the instrument exhibits a robust level of internal consistency, making it suitable for assessing HOTs in this context.

Table 2. Category of validity levels.

Percentage	Validity levels
81–100%	Very high
61–80%	High
41–60%	Moderate
21–40%	Low
0–20%	Very low

The implementation of Anderson's learning sketch analysis in this study utilized a set of teaching materials that were validated by the same experts who assessed the HOTs assessment instrument. The materials included lesson plans, student worksheets, and learning media (such as PowerPoint presentations), with four distinct sets created for each type of resource. The validation results for these teaching materials are presented in **Table 3**.

These validation results indicated a very high level of validity for the prepared teaching materials, allowing them to be used in the teaching process for this study after incorporating revisions based on the validators' feedback.

Table 3. Results of teaching materials validation.

Teaching material	Validator 1 score	Validator 2 score	Average score	Validity levels
Lesson plans	95%	90%	92%	Very high
Student worksheets	100%	95%	98%	Very high
Learning media	92%	90%	91%	Very high

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3. Results and discussion

3.1. Analysis of students' initial abilities

The students' initial abilities were assessed using data from their previous semester's reports. This data was subjected to statistical analysis to evaluate whether there were significant differences between the initial abilities of the control and experimental groups, with a significance level set at 0.05. A homogeneity test was conducted to confirm that both groups had the same variance, and the results are presented in **Table 4**. Following this, a normality test was performed as a prerequisite for the parametric comparison test, with the findings detailed in **Table 5**.

The normality test of the students' initial abilities indicated a significance value of less than 0.05, suggesting that the data is not normally distributed. Consequently, the hypothesis test to assess the significant difference in initial abilities between the two classes was conducted using a non-parametric statistical test, specifically the Mann-Whitney test. The null hypothesis for this comparison posits that there is no significant difference in the initial abilities of the experimental and control classes, while the alternative hypothesis proposes that a substantial difference exists. The results of the Mann-Whitney test are summarized in **Table 6**.

The Mann-Whitney test yielded a significance value greater than 0.05, leading to the acceptance of the null hypothesis and the rejection of the alternative hypothesis. This indicates that there is no significant difference between the initial abilities of the experimental and control classes. Consequently, the two classes can be directly compared in this study, as their initial skills are considered equivalent.

3.2. Analysis of students' higher-order thinking skills

The higher-order thinking skills (HOTS) of students were assessed based on the results from the HOTS assessments conducted after the completion of the reaction rate learning module in both classes. This data underwent statistical analysis to determine if there was a significant difference in HOTS abilities between the control and experimental groups, with a significance level set at 0.05. The homogeneity test for the HOTS ability data is presented in **Table 7**, confirming that both sample groups exhibit the same variance. Following this, a normality test was performed as a prerequisite for the parametric comparison, and the results are detailed in **Table 8**.

A normality test on the students' HOTs ability data yielded a significance value greater than 0.05, indicating that the data is normally distributed. As a result, a parametric statistical test, specifically the independent samples t-test, can be used to assess whether there is a significant difference in HOTs abilities between the two classes. The null hypothesis (H_0) for this test posits that there is no significant difference in HOTs abilities between the experimental and control groups. Conversely, the alternative hypothesis (H_1) suggests that a significant difference exists between these two groups. The outcomes of the independent samples t-test are displayed in **Table 9**.

The independent samples t-test produced a significance value below 0.05, leading to the rejection of the null hypothesis and acceptance of the alternative hypothesis. This result indicates a significant difference in HOTs abilities between the experimental and control classes, with the experimental class achieving higher average HOTs scores. Therefore, it can be concluded that Anderson's Learning Sketch Analysis effectively enhances students' higher-order thinking skills in the context of reaction rates. Additionally, students' HOTs assessment data were analyzed both quantitatively and descriptively to provide insights into their performance in each higher-order thinking category. The average percentage of correct answers for the experimental and control classes in each HOTs category is presented in **Table 10**.

Table 4. Homogeneity test results of student's initial abilities data (Levene test).

Class	Average score	Sig. value	Homogeneity
Experimental	84	0.566	Homogeneous
Control	83		

Table 5. Normality test results of student's initial abilities data (Mann-Whitney U test).

Class	Average score	Sig. value	Normality
Experimental	84	0.015	Not normal
Control	83	0.000	Not normal

Table 6. Mann-Whitney test results of student's initial abilities data.

Class	Average score	Sig. value	Conclusion
Experimental	84	0.634	H_0 is accepted
Control	83		

Table 7. Homogeneity test results of students' HOTs abilities data.

Class	Average score	Sig. value	Homogeneity
Experimental	68.3	0.730	Homogeneous
Control	49.9		

Table 8. Normality test results of students' HOTs abilities data.

Class	Average score	Sig. value	Normality
Experimental	68.3	0.126	Normal
Control	49.9	0.117	Normal

Table 9. Independent sample t-test results of students' HOTs abilities data.

Class	Average score	Sig. value	Conclusion
Experimental	68.3	0.002	H_0 is rejected
Control	49.9		

Table 10. The average percentage of correct student responses in each HOTs skill.

Class	HOTs category		
	Analyzing (C4)	Evaluating (C5)	Creating (C6)
Experimental	63%	71%	78%
Control	39%	55%	70%

3.3. Students' higher-order thinking skills in the analyzing skill

In the students' HOTs assessment instrument, five questions were categorized as involving the ability to analyze. The percentage of correct answers from the control class students and the experimental class students for the questions tagged as studying (C4) are presented in **Table 11**.

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Table 11. Percentage of correct answers in the analyzing skill (C4).

Question number	Main topic	Question indicator	%Correct answers	
			Control class	Experimental class
1	Concept of reaction rate	Given data on time and moles of reactants in a chemical reaction, students can calculate the time needed to obtain a certain amount of reaction products by analyzing the rate comparison between substances in the given response.	17	45
2	Theories of reaction rate	Given illustrations of two different experiments based on submicroscopic representation, students can analyze the factors affecting the occurrence of a reaction.	52	75
5	Factors affecting reaction rate	Given experimental data for the reaction between $Na_2S_2O_3$ and HCl under various conditions, students can predict the reaction rate order of several provided responses.	62	78
9	Reaction order and rate equation	Given various information about the effect of changing reactant concentrations on reaction rate and the rate constant value at a specific reactant concentration, students can determine the rate constant by analyzing the provided information.	31	47
10	Reaction order and rate equation	Given submicroscopic illustrations of several experiments with different numbers of reactant molecules along with their rate equations, students can identify correct statements about the reaction rate.	34	68
		AVERAGE	**39**	**63**

The results of the HOTs assessment show that students in the experimental class, who learned the reaction rate material through Anderson's learning sketch analysis, demonstrated stronger analytical skills than those in the control class, who were taught using traditional methods. This is evident from the higher percentage of correct answers in the experimental class compared to the control class across the five questions in the "analyzing" category. Specifically, the experimental class achieved an average of 63% correct answers, while the control class averaged 39%. An example question used to assess students' analytical HOTs abilities is provided below.

Question 9. The reaction experiment A B C produces the following data:

- When the concentration of B remains constant, and the concentration of A has increased two times the original rate, the reaction rate becomes four times faster
- When the concentration of A remains the same, and the concentration of BA is increased two times the original rate, the reaction rate becomes two times faster
- When [A]=2M and [B]=3M the reaction rate is 0.24 Ms^{-1}

Based on these data, the reaction rate constant is...

Question 9 serves as an example of an "analyzing" category question, specifically under the subcategory of attributing. In this question, students are asked to analyze the purpose and meaning of given data or statements and apply their analysis to solve a problem. Statements 1 and 2 provide clues about the order values of each reactant, requiring students to understand how changes in reactant order values affect the reaction rate. By attributing the information in these statements, students can determine the reactant order values and use them to infer the reaction's rate equation. Statement 3 then guides students to calculate the rate constant using the provided data and the derived rate equation. Sample student responses to Question 9 from both the experimental and control classes are shown in Figs. 1 and 2.

The student response from the experimental class demonstrates strong analytical skills. The student effectively interpreted the meaning of each provided data point and statement to solve the problem accurately. In analyzing statements 1 and 2, the student correctly identified the order values of each reactant and supported their conclusions with logical reasoning. For statement 3, the student skillfully applied mathematical calculations to determine the rate constant, using the given concentration and rate data, along with the reactant order values derived from the earlier analysis.

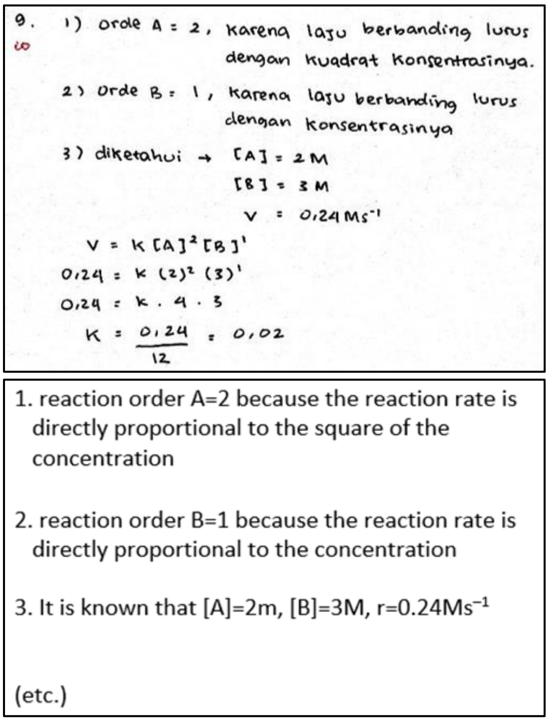


Figure 1. An example answer from the experimental class student for analyzing category questions.

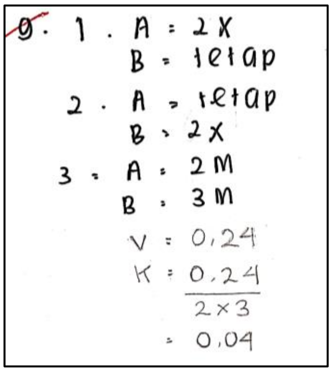


Figure 2. An example answer from a control class student for analyzing category questions.

The student from the control class demonstrates a need for stronger analytical skills. He struggled to accurately analyze statements 1 and 2, which discussed the importance of order values concerning reaction rates, preventing him from determining the correct order of each reactant. When addressing statement 3, he attempted to calculate the rate constant using the provided concentration and rate data. However, his calculations were inaccurate due to insufficient information about the order values of the reactants, which should have been derived from the previous statements.

3.4. Student's higher-order thinking skills in the evaluating category

Three questions involve evaluating abilities in the instrument used to measure students' HOTs. The percentage of correct answers from the control class students and the experimental class students for the questions in the evaluating category (C5) is presented in Table 12.

Table 12. Percentage of correct answers in the evaluating category (C5).

Question Number	Main Topic	Question Indicator	%Correct Answers	
			Control Class	Experimental Class
4	Factors affecting reaction rate	Given an illustration of a reaction experiment between $CaCO_3$ and HCl, students can examine the correctness of the steps taken to increase the reaction rate.	63	81
6	Factors affecting reaction rate	Given an illustration of a reaction with a catalyst, students can examine the correctness of several statements regarding the influence of adding a catalyst on reaction products.	46	70
8	Reaction order and rate equation	Given various data on reactant concentrations and reaction rates of a chemical reaction, along with statements about the effect of changing reactant concentrations on the reaction rate, students can examine the correctness of the given words.	55	63
		AVERAGE	**39**	**55**

The results of the HOTs assessment indicate that students in the experimental class, taught reaction rate material using Anderson's Learning Sketch Analysis, demonstrated stronger evaluation skills than those in the control class, who were taught using traditional methods. This is evident from the higher percentage of correct answers in the experimental class across all three evaluation-focused questions. The experimental class achieved an average of 71% correct answers in this category, compared to 55% in the control class. Below is an example question used to assess students' evaluation abilities within HOTs:

> **Question 8.** The reaction of nitrogen monoxide and hydrogen gas at 1280 °C which produces products in the form of nitrogen gas and water can be written as the reaction:
>
> $2NO_{(g)} + 2H_{2(g)} \rightarrow N_{(2)g)} + 2H_2O_{(g)}$
>
> The experimental data obtained is as follows
>
> "Changing the concentration of nitrogen monoxide gas to 2 times the original with a constant concentration of hydrogen gas causes the reaction rate to increase 4 times the original".
>
> Is this statement true? Explain your reasons and prove it with calculations!

Question 8 is an example of an evaluation question under the subcategory of "checking." This question asks students to assess the accuracy of a conclusion or statement based on the given data. Students have two approaches to evaluate the validity of the information. The first approach requires calculating the order of NO gas from the data and determining its impact on the reaction rate to assess the accuracy of the statement. The second approach involves analyzing how changes in NO gas concentration, while keeping H_2 concentration constant, affect the reaction rate, then concluding the accuracy of the statement. Sample student responses for Question 8 from both classes are shown in Figs. 3 and 4.

Based on the answer provided (Figs. 3), a student from the experimental class demonstrated strong evaluation skills. The student accurately assessed the given statement by first calculating the order of the NO reactant through careful data analysis. By comparing two data points with the same H_2 concentration, the student was able to deduce the effect of varying NO concentrations on the reaction rate. Finally, the student evaluated the accuracy of the statement and provided a well-reasoned explanation for their conclusion

Based on the provided answer (Fig. 4), a student from the control class still needs to develop strong evaluation skills. While the students correctly calculated the order of NO from the data, they struggled to interpret its significance about the effect of concentration changes on the reaction rate. As a result, their evaluation of the given statement was inaccurate. Additionally, the student unnecessarily calculated the order of H_2, which was not required to answer the question.

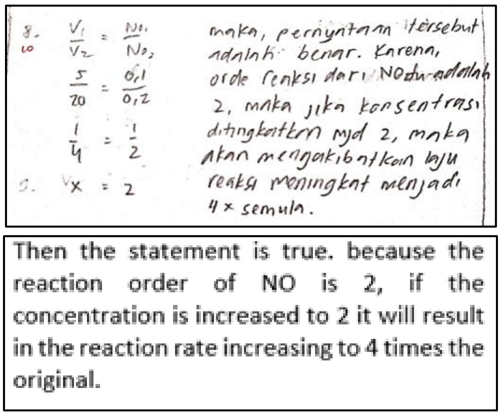


Figure 3. An example answer from the experimental class student for evaluating category questions.

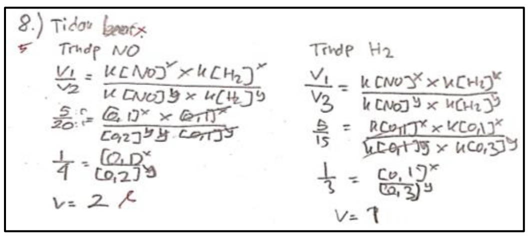


Figure 4. An example answer from a control class student for evaluating category questions.

3.5. Students' higher-order thinking skills in the creating category

In the instrument used to measure students' higher-order thinking skills (HOTs), two questions involve the ability to create. The percentage of correct answers from the control class students and the experimental class students for questions in the creating category (C6) is presented in **Table 13**.

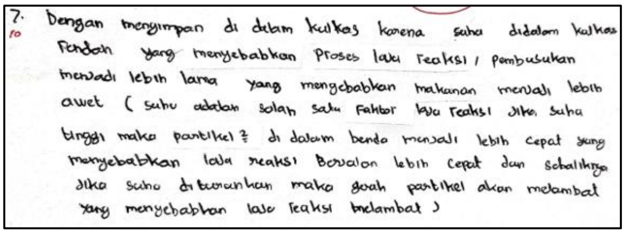
The results of the HOTs assessment show that students in the experimental class taught reaction rate concepts using Anderson's learning sketch analysis, demonstrated stronger creative abilities compared to those in the control class, who were taught using traditional methods. This is evident from the higher percentage of correct answers in the experimental class on the two questions in the "creating" category. The experimental class achieved an average of 78% correct answers, while the control class scored 70%. Below is an example of a question used to assess students' HOTs in the creating category.

Question 7. According to alodokter.com, in general, food can become rotten due to the activity of putrefactive bacteria, which can release chemicals and damage the structure of the food, resulting in changes in the aroma, appearance, and taste of the food. If it is related to factors that can influence the reaction rate, provide suggestions on how to store food so that it does not spoil quickly and the reasons.

Question 7 is an example of a task within the "creating" category, specifically under the subcategory of "planning." This question asks students to devise a strategy or method for solving a problem using the information provided. The informational text explains that a chemical change occurs as food decays. Therefore, to address the problem of food decay, the proposed strategy should focus on ways to reduce reaction rates. Students can apply their understanding of factors that influence reaction rates to suggest methods for slowing food decay. Examples of student responses to Question 7 from different classes are shown in **Figs. 5** and **6**.

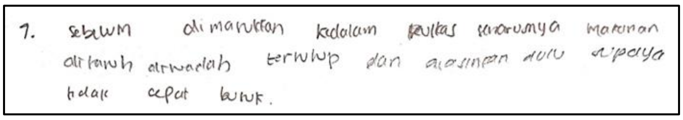
Table 13. Percentage of correct answers in the creating category (C6).

Question Number	Main Topic	Question Indicator	%Correct Answers	
			Control Class	Experimental Class
3	Factors affecting reaction rate	Given illustrations of various experiments with information on the form of substances, solution concentrations, and temperatures, students can determine experimental procedures that yield conclusions about the influence of temperature on reaction rate.	55	63
7	Factors affecting reaction rate	Given information about food spoilage causes, students can suggest ideas for quickly preserving certain foods from spoiling by utilizing factors affecting reaction rate.	85	93
		AVERAGE	**56.5**	**76**



Storing it in the refrigerator because the temperature in the refrigerator is low causes the decay reaction rate to take longer so that the food lasts longer (the reason is that temperature is one of the reaction rate factors. If the temperature is high, then the particles in the substance move faster, and conversely, if the temperature is low/lowered, then the particle movement will slow down, which causes the reaction rate to be slower)

Figure 5. Example answers from the experimental class student for creating category questions.



Before putting the food in the refrigerator, the food should be put in a closed container and stored first so that it doesn't rot quickly.

Figure 6. Example answer from control class student for creating category questions.

Based on the provided answer (**Fig. 5**), a student from the experimental class has demonstrated good creative abilities. This student suggested a solution focused on the temperature factor's influence on reaction rates. The student proposed a strategy to prevent food decay, which involves storing food in the refrigerator. Additionally, the student provided reasons for the proposed method by explaining the effect of temperature changes on reaction rates.

Based on the response provided (**Fig. 6**), a student from the control class demonstrates a need for further development of creative problem-solving skills. The student proposed storing food in a covered container and acidifying it before refrigeration as a way to prevent decay. While this strategy is not entirely incorrect, it does not directly address the core of the question, which asked students to focus on factors affecting reaction rates. The student's answer lacks alignment with these factors and does not provide sufficient reasoning or explanation for the proposed method.

4. Conclusions

This study concludes that there is a significant difference in higher-order thinking skills (HOTs) between experimental and control groups. The experimental group consistently outperformed the control group, with average scores of 69.3 compared to 49.9. Across various categories of HOTs, students in the experimental group gave more accurate responses than those in the control group. The findings suggest that Anderson's learning sketch analysis is effective in enhancing students' higher-order thinking abilities in the context of reaction rates. Specifically, the experimental group demonstrated 63% proficiency in analysis skills compared to 39% in the control group, 71% proficiency in evaluation skills compared to 55%, and 78% proficiency in creation skills compared to 70%.

Authors' contribution

Conceptualization: Herunata Herunata; **Data curation:** Hayuni Retno Widarti; **Formal analysis:** Herunata Herunata; **Funding acquisition:** Not applicable; **Investigation:** Ibnatullatiefah Ibnatullatiefah; Putri Nanda Fauziah; **Methodology:** Habiddin Habiddin; **Project administration:** Ibnatullatiefah Ibnatullatiefah; Putri Nanda Fauziah; **Resources:** Not applicable; **Software:** Ibnatullatiefah Ibnatullatiefah; **Supervision:** Habiddin Habiddin; Munzil Munzil; **Validation:** Hayuni Retno Widarti; **Visualization:** Not applicable; **Writing – original draft:** Herunata Herunata; Ibnatullatiefah Ibnatullatiefah; **Writing – review & editing:** Habiddin Habiddin; Munzil Munzil.

Data availability statement

All data were acquired and analyzed in the present investigation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Determining design thinking elements in chemistry education: A Fuzzy Delphi method

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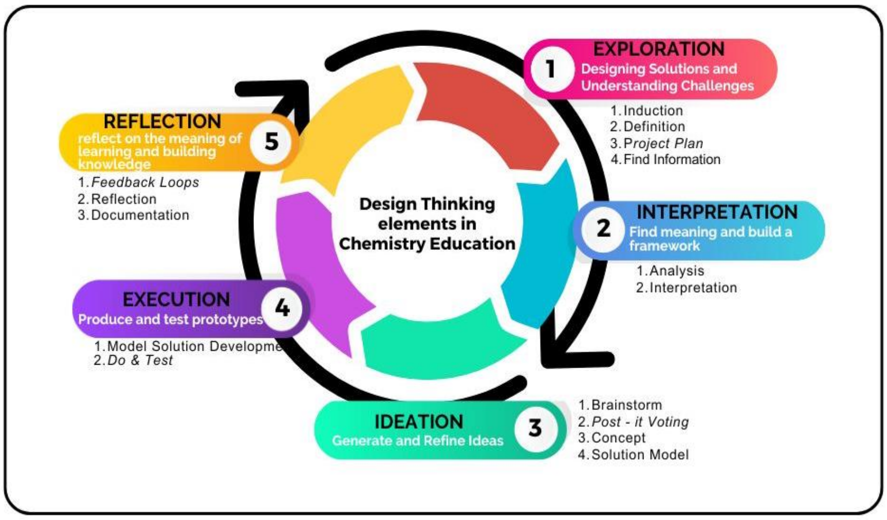
Abstract

Creating a high-quality learning environment where students can solve real-world problems and be receptive is essential for fostering students' innovation competencies. Using appropriate pedagogical strategies and classroom activities is a crucial aspect of Malaysian education. This article uses the Fuzzy Delphi Method (FDM) to design chemistry classroom teaching strategies based on the design thinking paradigm. This research involves 12 experts in purposive sampling to form a diverse panel encompassing expertise in Chemistry Education, Curriculum, Module Development, Research, and Innovation. Using the Fuzzy Delphi method (FDM), the data were analyzed. Four elements for exploratory constructs, two elements for construct interpretation, four elements for ideation, two elements for execution, and three elements for construct evolution met the FDM requirements, according to the findings. Its threshold value is less than 0.2, the expert consensus is less than 75%, and the average score of the fuzzy number is over 0.5. Encouraging design thinking in chemistry classes and thereby enhancing students' innovation skills, this research unquestionably induces a paradigm shift in teaching practice.



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Natany Dayani de Souza Assai

Highlights

- Designed innovative chemistry teaching strategies using the Fuzzy Delphi method.
- Promoted design thinking to boost students' innovation competencies in chemistry.
- Emphasizes real-world problem-solving and inquisitive learning in chemistry.
- Integration of design thinking to foster student innovation skills.
- Verified expert consensus on the design thinking framework in chemistry education.

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1. Introduction

Innovation competency emphasizes the need for an educational strategy promoting active learning and real-world problem-solving (Hero *et al.*, 2017) and highlights the seamless technological integration of teaching and learning transitions (Falloon, 2020). Academic scholars propose the inclusion of innovation competence and its various dimensions in the curriculum to cultivate innovation competence through educational practices (Bascopé *et al.*, 2019; Durall *et al.*, 2022). There is consensus in the research that education positively impacts students' creativity and innovation (Hu *et al.*, 2016; Ovbiagbonhia *et al.*, 2020). In contemporary days, it is imperative for academics and educational authorities to actively promote the development of students' inventive competency (Ojeda *et al.*, 2021). To foster the development of students' innovation competence, educators require clear principles for designing instructional approaches and curricula that effectively enhance expected competence (Franco *et al.*, 2019; Herodotou *et al.*, 2019). Nevertheless, there is a massive gap between the curricular aspirations in facing real-world needs and the actual learning outcomes and competencies of students in chemistry education (Hero, Pitkäjärvi, and Matinheikki-Kokko 2021; OCDE 2018). Additionally, the current educational setting may not offer a conducive environment for fostering students' competency in innovation (Keinänen and Kairisto-Mertanen, 2019). Studies have shown that chemistry students struggle to explain phenomena based on knowledge (Kanapathy et al. 2019) and solve problems in real-world contexts or generate original ideas from learned concepts (Broman *et al.*, 2018).

The importance of innovation skills in addressing global challenges, particularly in chemistry, has been well recognized (Garcia-Martinez, 2021). Chemistry significantly advances many Sustainable Development Goals (SDGs) outlined by the United Nations to foster a more sustainable future by 2030. These goals include nanotechnology, sustainable energy transition, smart cities, innovative industries, and addressing social and environmental concerns (Anastas and Zimmerman, 2018). The emphasis on innovation within the National STEM Action Plan for 2017–2025 underscores the importance of cultivating expertise in innovation. The collaboration between the Federal Ministry of Science, Technology, and Innovation (MOSTI) and the Malaysian Ministries of Education (KPM) and Higher Education (KPT) encompasses various areas such as innovation, cultural research, and the enhancement of teaching and learning quality. The discipline of chemistry education equips students with the necessary skills and knowledge to foster innovation in several domains as the demand for creativity grows across multiple industries (Droescher, 2018; García-Pérez *et al.*, 2021; Gomollón-Bel, 2020). Previous research shows that chemistry educators' instructional techniques and classroom practices have still to cultivate innovation and problem-solving skills (Keinänen and Kairisto-Mertanen, 2019) effectively. Additionally, restricted resources and teachers' heavy workloads pose obstacles to enhancing innovation competence in the classroom (Lo *et al.*, 2019). Given the critical role that chemistry educators must play, this study uses a Fuzzy Delphi approach, which gathers views from a diverse panel of experts, synthesizes their knowledge, and coordinates different perspectives to create a strategic framework. This framework aims to integrate elements of design thinking into the chemistry curriculum, thereby increasing students' innovation efficiency in a dynamic educational environment.

2. The potential of design thinking in stimulating innovation competencies

The key to developing this innovation competency is creating a quality learning environment that allows students to solve real-world problems and be curious and open-minded (Keinänen *et al.*, 2018). The question here is how the development of innovation competence and maximizing digital technology through one method can impact the development of students' innovation competence. Scholars, among them, have proposed several solutions to apply the design thinking approach as a modern learning paradigm in the classroom (Hsiao *et al.*, 2017; Koh *et al.*, 2015; Zupan *et al.*, 2018) support this viewpoint, states that when teachers use a design thinking approach to create learning materials and lectures for students, they improve student learning. The quality of the classroom improves. Design thinking should be one of the solution methods to provide students with the ability to solve problems innovatively (Pruneau *et al.*, 2021; Scott *et al.*, 2021). The development of innovation competence can be encouraged by creating a learning environment that promotes student engagement with real-world challenges and encourages curiosity and creativity (IDEO, 2012; Keinänen *et al.*, 2018).

Ultimately, design thinking effectively develops students' innovation competencies (Androutsos and Brinia, 2019; Raber *et al.*, 2018). Design thinking gives students a structured framework for developing innovation skills (Garcia-Vaquero, 2021; Lynch *et al.*, 2021). By embracing design thinking, students are equipped with a structured yet inventive problem-solving methodology, enabling them to approach challenges creatively and systematically. Within this structured guidance, educators play a crucial role, instilling in students the art of methodical problem-solving that fosters innovation (Jan *et al.*, 2017). However, in the context of Malaysia, elements of the design thinking approach are still not disclosed to Science and Mathematics teachers (Adam and Halim, 2019) and teachers are still unclear about the design thinking approach and how it can be applied in the classroom (Noh and Karim, 2021; Noh, 2020) to encourage the development of students' innovation competencies. Therefore, this study aimed to provide concrete solutions to stimulate and enhance student innovation competencies and employ the Fuzzy Delphi Method to determine the elements of Design Thinking in Chemistry Classroom Teaching Strategies. The research questions that need to be answered are:

1. What are the elements of design thinking implementation in chemistry classroom education through expert consensus?
2. What are this item's values and rankings for each element based on an expert consensus?

3. Research design

This research adopts the Fuzzy Delphi technique to gain expert approval. The Fuzzy Delphi technique, or the Fuzzy Delphi method (FDM), is a measurement and tool developed or modified from the Delphi method. As a result, FDM is not a new method because it is based on the classic Delphi method, which has been widely used and accepted in many studies (Cone and Unni, 2020; Jamil and Noh, 2020). The selection of experts is significant in meeting the consensus of experts in this FDM as it involves a process of agreement from a group of experts to verify, evaluate, reject, or add elements to the framework. Thus, selecting experts is critical to meet the context of expert consensus in this FDM method. The FDM can be a more effective measurement tool in placing the strength of element selection for design thinking elements in chemistry classroom teaching strategies based on expert consensus. Studies also prove that this method can solve problems with inaccuracy and uncertainty for a study (Bui *et al.*, 2020; Lim *et al.*, 2021; Zhu *et al.*, 2023).

Berliner (2004) emphasizes the significance of selecting experts with a minimum of five years of consistent expertise to guarantee a comprehensive understanding of the issues under study. This study recruited a purposeful sample of 12 diverse experts (**Table 1**) consistent with the recommendation of Adler and Ziglio (1996), who suggest involving 10 to 15 experts in the Delphi method to obtain a high consensus. The criteria for the experts were as follows: **1.** Experts in chemistry or STEM education; **2.** Expert in curriculum and instruction development; **3.** Experts in module development; **4.** Experts in development, research, and innovation.

Table 1. Fuzzy Delphi method expert demography.

Expert	Institution	Position	Experience	Field Expertise
1	Public University	Senior Lecturer	More than 21 years	- Chemistry education - Chemistry curriculum development
2	Public University	Associates Professor	More than 21 years	- STEM education - STEM culture study center
3	Public University	Associates Professor	More than 21 years	- STEM Education
4	Public University	Senior Lecturer	6–10 years	- Chemistry education - Module development
5	Public University	Senior Lecturer	11–15 years	- Curriculum and instruction development - Module development
6	Public University	Senior Lecturer	More than 20 years	- Module development - Development, research, and innovation
7	Public University	Senior Lecturer/ Professional Chemists	15–20 years	- Chemistry education
8	Public University	Senior Lecturer	11–15 years	- Chemistry education - Module development
9	Institute of Teacher Education	Senior Lecturer	More than 21 years	- Curriculum and instruction development - Development, research, and innovation
10	Institute of Teacher Education	Lecturer	6–10 years	- Development, research, and innovation - Module development
11	Institute of Teacher Education	Senior Lecturer	15–20 years	- Development, research, and innovation
12	Education Resources and Technology Division of the Malaysian Ministry of Education	Assistant director	More than 21 years	- Chemistry education

4. Data collection and analysis

To ensure the empirical nature of this study, the researcher implemented the Fuzzy Delphi method following **Fig. 1**.

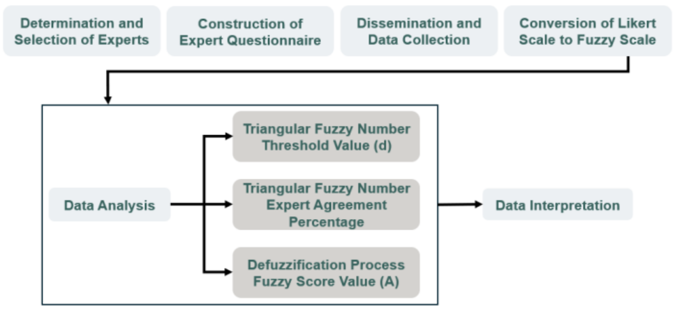

Figure 1. Fuzzy Delphi method (FDM) approach.
Source: Retrieved from Jamil *et al.* (2015).

Step 1: ***Determination and selection of experts***

The researcher sought out specialists who met specific criteria. A letter of appointment and approval as an expert panel was sent to experts willing to participate in the research.

Step 2: ***The construction of the experts' questionnaire***

This study created a questionnaire using a standards-based literature review and expert consultations (Sekaran and Bougi, 2016). The fuzzy questionnaire presented is used to obtain expert agreement on the elements in the required activity design components, as shown in **Table 2**.

The questionnaire employed the seven-point Likert scale to reduce ambiguity and increase expert consensus. Noh, Halili and Siraj (2020) and Jamil *et al.* (2015), revealed that 7-point Likert scales are more accurate than 5-point ones. This scale is transformed into a fuzzy set with unit intervals between 0 and 1. The Triangular Fuzzy Number represents the lowest, reasonable, and maximum m1, m2, and m3 (**Fig. 2**).

Table 2. Number of items for each element in the questionnaire.

Implementation of the IDEO Design Thinking Model in Chemistry Learning	
Design thinking phase	**Number of item**
Exploration	4
Interpretation	4
Ideation	4
Execution	3
Reflection	4

Source: Elaborated by the authors using the literature review: IDEO (2012); Kalkbrenner and Horton-Parker (2016); Ovbiagbonhia *et al.*, (2023); Auernhammer and Roth (2021); Pande and Bharathi (2020); Abidin (2020).

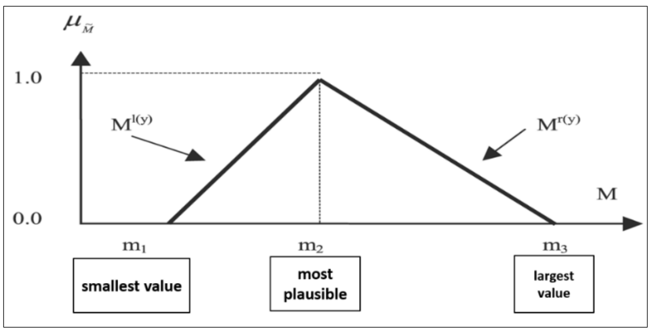

Figure 2. Triangular Fuzzy Number.
Source: Retrieved from (Siraj *et al.*, 2021).

The field experts rated their agreement with the assertions, facilitating content validation through this process. This fuzzy approach allows for a more refined analysis, accommodating the uncertainty inherent in human judgment. For example, when an expert rates "Students cooperate effectively during group assignments" with a score of 5 on a 7-point Likert scale, indicating agreement, the Triangular Fuzzy Number (TFN) translates it into a fuzzy scale range (0.50, 0.70, 0.90) representing a 50% agreement value for m1, 70% for m2 and 90% for m3. This range captures the potential variation in expert opinion, reflecting a single point of agreement and a spectrum that can be slightly skewed toward a percentage of agreement. Ambiguity at this scale improves data accuracy, makes analysis more robust, and reflects the real-world complexity inherent in expert judgment.

Step 3: ***Dissemination and data collection***

This phase involved distributing surveys to recognized experts using one of two methods: There are two main ways to engage experts: Meeting with each expert in person or using email for conversation and information sharing.

Step 4: ***Conversion of Likert scale to fuzzy scale***

The linguistic variables are converted into fuzzy triangular numbers, and each criterion is allocated a fuzzy rij number to indicate the expert's competence. Data Average Value was determined using a Delphi Fuzzy analysis template intended for Microsoft Excel (**Eq. 1**).

$$I = 1 \ldots m, j = 1, \ldots n, \quad K = 1 \ldots., k$$
$$and \tag{1}$$
$$rij = 1/K\ (r^1 ij \pm r^2 ij \pm r^K ij)$$

Step 5: ***Data analysis***

To obtain the agreement and consensus of the expert panel, three main conditions must be met, which rely on the triangular fuzzy number and the defuzzification process. The condition for *triangular fuzzy numbers* is to involve *the threshold* value (d) and the percentage of expert agreement. For the defuzzification *process*, there is only one condition: the *fuzzy* score value (A). These three conditions will be analyzed using *Microsoft Excel*. **Table 3** shows the interpretation of the Triangular score values of the Fuzzy Number and Defuzzification Process to measure the consensus of the expert group.

Table 3. Interpretation of score values for acceptance conditions based on expert agreement Fuzzy Delphi method data analysis (FDM).

Condition	Process	Criterion	Value	Interpretation
1	*Triangular fuzzy number*	*Threshold* value (d)	*Threshold* (d) ≤ 0.2 (equal or less than 0.2)	Accepted (Chen, 2000; Cheng and Lin, 2002)
2	*Triangular fuzzy number*	The percentage of expert agreement	Percentage of expert agreement ≥ 75% (equal to or greater than 75%)	Accepted (Chu and Hwang, 2008; Murry and Hammons, 1995)
3	*Defuzzification process*	*The fuzzy* score value (A)	*The fuzzy* score value (A) ≥ 0.5 (α– cut value equal to or greater than 0.5)	Accepted (*Ranking*) (Bodjanova, 2006; Tang and Wu, 2010)

a: Determining Threshold Value (d)

Each item's threshold value (d) must be less than or equal to 0.2 to reflect experts' consensus (Cheng and Lin, 2002). The **Eq. 2** calculated the distances between two fuzzy numbers, m = (m1, m2, m3) and n = (n1, n2, n3).

$$d(\tilde{m}, \tilde{n}) = \sqrt{\frac{1}{3}[(m_1 - n_1)^2 + (m_2 - n_2)^2 + (m_3 - n_3)^2]} \tag{2}$$

b: Expert Consensus Percentage

Expert consensus must exceed 75% to indicate agreement. Non-agreement items were eliminated (Garriga *et al.*, 2016).

c: Defuzzification Process

The defuzzification method determined item scores and rankings. The symbol for defuzzification is Amax. The fuzzy score (A) must be greater than the median value (α-cut value) of 0.5 (Tang and Wu, 2010) to indicate expert agreement and item acceptance. If (A) exceeds 0.5, it signifies expert consensus to accept the item in the question (Bodjanova, 2006). This α-cut-based decision-making process is a critical determinant in accepting or rejecting items within the study (**Eq. 3**).

$$Amax = \frac{1}{3} \times (m_1 + m_2 + m_3)$$
$$Amax = \frac{1}{4} \times (m_1 + 2m_2 + m_3) \tag{3}$$
$$Amax = \frac{1}{6} \times (m_1 + 4m_2 + m_3)$$

5. Findings and results

5.1. What are the elements of design thinking implementation in chemistry classroom education?

Dewey's Experiential Learning Theory (Roberts, 2003) and IDEO's design thinking model (IDEO, 2012) have been implemented in developing activities based on design thinking in chemistry classes. The activity design is guided by the Curriculum Standard Document and Chemistry Assessment in the Secondary School Standard Curriculum (BPK 2018). Area 6.0 on the topic of acids, bases, and salts is mapped to content standards (SK) with design steps that students will undertake. For activity 1: Design of environmentally friendly washing soap, there are three content standards involved, namely SK 6.1, SK 6.2, and SK 6.3. For activity 2, the smoke filter, Eco involves SK 6.4 and SK 6.7. Based on the findings, there are five elements of design thinking implementation in Chemistry Classroom Teaching Strategies based on the experts' consensus. The elements are exploration, interpretation, ideation, execution, and reflection. These five phases are used as a structured phase framework to improve the quality of the teaching and learning process through the integration of design thinking.

Students learn to approach problems structured and systematically and develop the problem-solving approach required to enhance innovation competencies. Teaching and learning activities are systematically mapped to the design components corresponding with a phase of the IDEO Design Thinking Model (IDEO, 2012): Exploration, Interpretation, Ideation, Execution, and Reflection. The parameters of each phase from the IDEO model are integrated into elements of our activity design, which results in a holistic learning experience. Practice examples in Table 4 adapt the activity design types proposed and refined via expert consensus to show how these phases of design thinking can be applied.

Table 4. Design thinking elements in chemistry classroom teaching strategies.

Item	Elements
Exploration Phase	
E1.1	*Project Plan:* Students build structured plans, assign distributions in groups, and outline plans to follow during their design thinking project assignment. Students make strategies, plan action plans to overcome environmental issues and distribute tasks.
E1.2	*Induction*: The teacher raises the issue of the problem and introduces students to the project's objectives, methods, and expectations. Students answer KNOWLEDGE practice questions, and students explore "Acids and Alkalies: How do they affect the Environment".
E1.3	*Definition:* Students actively define problem issues, clearly outlining the problem statements and objectives to be addressed. Students define related issues and list pollutants that affect the quality of the environment (like sulfur dioxide (SO_2) and nitrogen oxides (NO_x), which lead to acid rain, alter the pH of water bodies, and harm aquatic life).
E1.4	*Find Information:* Students search for information from various sources and research and collect relevant data on the problem. Students explore and find information on the concept of pH for acid, base, and salt in (laboratory station activity), test the pH levels of different substances, and analyze how acidic or basic solutions can neutralize to mitigate environmental damage.
Interpretation Phase	
E2.1	*Interpretation:* Students evaluate and interpret information to draw meaningful conclusions. Students connect the concept of pH value and concentration of a solution and suggest possible solutions.
E2.2	*Synthesis:* Students synthesize the information obtained, integrate information, and generate creative solutions to address assigned problems. Combine their understanding of chemical reactions, such as neutralization, with real-world applications to propose methods to reduce the acidity of affected water bodies.
E2.3	*Analysis:* Students analyze the exploration results, breaking down complex information to identify key patterns, trends, or relationships related to a defined problem. Students present ideas, analyze, and make connections about how the pH value of a cleaning solution affects the effectiveness of washing.
E2.4	*Concept mapping:* Students visually organize and connect key concepts and ideas from the information gathered. Map the relationship between different types of acids and bases, their reactions with salts, and their effects on the environment.
Ideation Phase	
E3.1	*Concept:* Students associate the solution idea and the chemical concepts involved. Students link the chemical concepts of acids, bases, and salts with the solution concept. Students state how the properties of acids, bases, and salts allow solutions that are suggested.
E3.2	*Post-it Voting:* A collaborative decision-making process in which students use Post-it notes to vote for the most appropriate ideas. Evaluate ideas based on feasibility, functionality, and alignment with problems foster collaboration.
E3.3	*Brainstorm:* Collaborative and open sessions where students generate ideas for solving defined problems. Students consider the properties and characteristics of acids, bases, and salts during brainstorming.
E3.4	*Solution Model:* Students suggest a solution model for implementing the selected solution. Visualize and sketch a model of their solution for a smoke filter – eco. Label the main components and processes involved in their solution.
Execution Phase	
E4.1	*Solution Model Development*: Students develop prototypes and detail the selected solution model.
E4.2	*Do & Test:* Students test a prototype to solve a pre-defined problem. Students test the functionality and ability of prototypes to solve air or air pollution problems (acid, base, and agar concepts). List the apparatus and materials needed in the testing experiment. Students must propose their investigative activity procedures. List the apparatus and materials needed for the testing experiment. Students are required to propose their investigative activity procedures.
E4.3	*Re-Test:* Revising and re-evaluating the solution model implemented based on feedback and results.
Reflection Phase	
E5.1	*Documentation:* Students make project documentation systematically recording and presenting learning outcomes and modifications made throughout the design process carried out.
E5.2	*Feedback loops:* continuous feedback, allowing for repeated improvements based on input from peers, teachers, or self-reflection.
E5.3	*Sustainability:* Students consider developed solutions' long-term viability and impact on environmental, social, and economic effects.
E5.4	*Reflection:* Students reflect on the entire design process, including challenges, successes, and areas for improvement. Reflect on how design projects in creating environmentally friendly cleaning solutions challenge an understanding of chemical concepts such as pH, chemical reactions, and the properties of acids and bases.

Source: Elaborated by the authors from expert views.

Some elements did not meet the requirements of the acceptance of elements in the FDM analysis, such as elements in the design of the activities of the interpretation phases. These elements meet the first requirement but do not meet the second requirement; the percentage requirement of the expert agreement should be more than or equal to 75%.

The synthesis elements (67%) and *concept mapping* (50%) in the interpretation phase, as well as *Re-test* elements (67%) in the implementation phase, meet the first requirement *of the threshold* value (d) less than 0.2 but have less than 75% of expert agreement. Meanwhile, the sustainability element ($d = 0.204$) in the reflection phase meets the second requirement but does not meet the first requirement *of the threshold* value (d) less than 0.2.

Therefore, as formulated in **Table 5**, these four elements have been rejected. The results also show the expert's consensus on the elements in the design thinking component of the activity with the *threshold value* (d) of the exploration phase between (0.098 to 0.126) and the ideation phase (0.057 to 0.092) and has met the first FDM requirement in the *Triangular fuzzy number* which is (d) smaller than 0.2. The testing of the percentage of agreement for the second FDM conditions also showed that the design thinking element of the activity for these two phases received a high rate of agreement of 96% and 94% for the percentage of the entire component.

Table 5 also presents the consensus reached among experts regarding 15 out of 19 elements in design thinking elements. In total, all four elements in the exploration phase and the ideation phase, two of the four elements in the interpretation phase, two of the three elements in the experimental phase as well as three of the four proposed elements were accepted by consensus for the design component of the activity in the design thinking implementation in chemistry classroom education.

Table 5. Expert consensus on design thinking elements.

	Triangular Fuzzy Numbers		*Defuzzification Process*					
Item	Threshold Value (d)	Percentage Expert Consensus (%)	m1	m2	m3	*Fuzzy* Score (A)	Ranking	Results
Exploration Phase								
E1.1	0.098	92%	0.800	0.942	0.992	0.911	3	Accepted
E1.2	0.023	100%	0.883	0.992	1.000	0.958	1	Accepted
E1.3	0.042	100%	0.867	0.983	1.000	0.950	2	Accepted
E1.4	0.126	92%	0.767	0.917	0.975	0.886	4	Accepted
Interpretation Phase								
E2.1	0.097	92%	0.783	0.933	0.992	0.903	2	Accepted
E2.2	0.201	67%	0.617	0.800	0.925	0.781	-	Rejected
E2.3	0.068	100%	0.833	0.967	1.000	0.933	1	Accepted
E2.4	0.147	50%	0.533	0.733	0.900	0.722	-	Rejected
Ideation Phase								
E3.1	0.082	83%	0.700	0.883	0.983	0.856	4	Accepted
E3.2	0.076	100%	0.800	0.950	1.000	0.917	2	Accepted
E3.3	0.057	100%	0.850	0.975	1.000	0.942	1	Accepted
E3.4	0.092	92%	0.767	0.925	0.992	0.894	3	Accepted
Execution Phase								
E4.1	0.068	100%	0.833	0.967	1.000	0.933	1	Accepted
E4.2	0.068	100%	0.767	0.933	1.000	0.900	2	Accepted
E4.3	0.187	67%	0.633	0.808	0.933	0.792	-	Rejected
Reflection Phase								
E5.1	0.091	83%	0.717	0.892	0.983	0.864	3	Accepted
E5.2	0.095	92%	0.817	0.950	0.992	0.919	1	Accepted
E5.3	0.204	83%	0.400	0.600	0.800	0.600	-	Rejected
E5.4	0.102	83%	0.733	0.900	0.983	0.872	2	Accepted

Requirement: *Triangular Fuzzy Numbers* (1) *Threshold Value* (d) ≤ 0.2, (2) Percentage of Expert Consensus $\geq 75.0\%$; *Defuzzification Process* (3) *Fuzzy* Score (A) $\geq \alpha - \text{cut} = 0.5$.

5.2. What are this item's values and rankings for each element based on an expert consensus?

After successfully addressing the initial two requirements, the analysis proceeds to ascertain the fulfillment of the third FDM requirement through defuzzification analysis. Notably, the highest fuzzy score value secures the top rank within each design thinking element. In the defuzzification analysis, a higher fuzzy score means to be the most likely or agreed upon sub-element within each phase of design thinking. Presenting these higher scores first highlights the most critical items, making the key findings more prominent and impactful. The final resulting value (A) is compared against α-cut values; any score below 0.5 reflects expert consensus to reject the item, while scores exceeding 0.5 indicate sufficient literature support to adopt it (Bodjanova, 2006). The high fuzzy scores (ranging from 0.958 to 0.856) in **Table 6** confirm broad consensus (Roldan Lopez *et al.*, 2021), which is crucial for applying the design thinking framework to the chemistry education model.

Overall, the analysis results indicate a logical structure within the Design Thinking process, with specific priorities for each phase. In the Exploration Phase, the Induction element, which introduces the process, holds the highest importance (A=0.958), followed closely by Definition (problem definition) at 0.950. However, Finding Information (gathering information) ranks the lowest at 0.886, suggesting that a clear understanding of the problem is paramount before collecting data. In the Interpretation Phase, the Analysis element (A=0.933) is considered more critical than Interpretation (A=0.903), emphasizing the necessity of thorough data analysis prior to drawing conclusions. Moving to the Ideation Phase, Brainstorming (creative idea generation) is the highest-ranked element (A=0.942), followed by *Post-it Voting* (selection of the best ideas) at 0.917, and Solution Model Development (A=0.894). The Concept (basic concept) element ranks the lowest at 0.856, implying that generating and selecting ideas should precede the development of a solution model. In the Execution Phase, Solution Model Development (A=0.933) is prioritized over Do & Test (A=0.900), underlining the importance of having a well-defined solution model before testing. Lastly, in the Reflection Phase, Feedback Loops (continuous feedback) are deemed the most crucial (A=0.919), followed by Reflection (self-reflection) at 0.872, with Documentation being the least important (A=0.864). This suggests that feedback exchanges should take precedence over mere documentation of reflections. In conclusion, this ranking emphasizes the sequential and structured approach in Design Thinking, where each phase—understanding the problem, conducting analysis, generating and selecting ideas, modeling solutions, implementing them, and reflecting—must follow a clear order of priority to effectively foster innovative and systematic solutions.

Figure 3 displays a visual picture of the ranking formulation of each element of the design of the activity in all phases of design thinking according to the priority position.

Table 6. Ranking of elements in the design thinking phase activity to the value of Fuzzy evaluation.

Item	Design Thinking Activity Components	Fuzzy Score (A)	Ranking
Design Thinking (Exploration) Elements			
E1.1	Project Plan	0.911	3
E1.2	Induction	0.958	1
E1.3	Definition	0.950	2
E1.4	Find Information	0.886	4
Design Thinking (Interpretation) Elements			
E2.1	Interpretation	0.903	2
E2.3	Analysis	0.933	1
Design Thinking (Ideation) Elements			
E3.1	Concept	0.856	4
E3.2	Post-it Voting	0.917	2
E3.3	Brainstorm	0.942	1
E3.4	Solution Model	0.894	3
Design Thinking (Execution) Elements			
E4.1	Solution Model Development	0.933	1
E4.2	Do & Test	0.900	2
Design Thinking (Reflection) Elements			
E5.1	Documentation	0.864	3
E5.2	Feedback loops	0.919	1
E5.4	Reflection	0.872	2

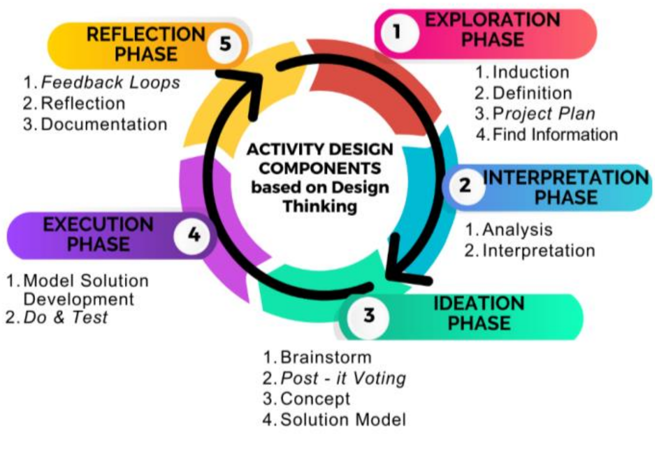

Figure 3. Activity Design Thinking Component through Fuzzy Delphi method approach.

6. Discussion

The results of this study demonstrate the effective utilization of the FDM in developing teaching techniques for chemistry classrooms that align with the design thinking paradigm. The consensus levels among the expert panel, which ranged from 83 to 100%, validate the appropriateness and comprehensiveness of the proposed design thinking principles in the context of chemistry education. The elements classified as exploration, interpretation, ideation, execution, and reflection jointly play a significant role in nurturing students' innovation competencies. The consensus reached, as indicated by the low threshold values (d ≤ 0.2), demonstrates that experts have agreed on the fundamental elements required for a learning environment to be effective. The value of supporting inquiry-driven learning and problem-solving in the Malaysian school setting is highlighted by this consensus, which is consistent with earlier research that emphasizes the importance of active learning approaches (Halim *et al.*, 2022; Maneeratana *et al.*, 2019).

In addition, the examination of defuzzification provides valuable insights into the relative importance of each part of design thinking. The Fuzzy score values, which regularly range from 0.856 to 0.958, emphasize the importance of each element in effectively implementing design thinking ideas in chemistry teaching. The prominence of the exploration element in the exploration portion is particularly noteworthy, as it emphasizes the importance of enabling students to engage with real-world situations to frame challenges appropriately. Likewise, prioritizing analysis and interpretation components highlights the significance of employing critical thinking during the ideation process. The above findings align with prior research that underscores the crucial significance of design thinking in cultivating innovation skills and aptitude for resolving problems (Buhl *et al.*, 2019; Ellah, *et al.*, 2019)

7. Conclusions

In conclusion, this research makes a valuable contribution to the advancement of pedagogical approaches in the field of chemistry education in Malaysia. It achieves this by offering empirically supported design thinking components that align with the requirements for fostering students' innovation competencies. The efficacy of employing the Fuzzy Delphi method to establish consensus among experts serves to enhance the validity of the offered plans. The results emphasize the necessity for educational establishments to integrate design thinking principles, thereby fostering an environment that motivates students to engage in exploration, ideation, and implementation to address authentic challenges. By emphasizing these components, educators can cultivate a dynamic educational setting that encourages creativity, critical thinking, and innovation within the student body. The findings derived from this research can provide a basis for educators, curriculum creators, and policymakers to formulate and execute efficacious approaches for fostering student innovation competencies.

Authors' contribution

Conceptualization: Norliyana Binti Md. Aris; Nor Hasniza Binti Ibrahim; Noor Dayana Binti Abd Halim; **Data curation:** Norliyana Binti Md. Aris; Muhammad Nidzam bin Yaakob; **Formal Analysis:** Norliyana Binti Md. Aris; Nor Hasniza Binti Ibrahim; Noor Dayana Binti Abd Halim; **Funding acquisition:** Norliyana Binti Md. Aris; **Investigation:** Norliyana Binti Md. Aris; **Methodology:** Norliyana Binti Md. Aris; Muhammad Nidzam bin Yaakob; **Project administration:** Norliyana Binti Md. Aris; Nurul Hanani Binti Rusli; **Resources:** Norliyana Binti Md. Aris; Nor Hasniza Binti Ibrahim; Noor Dayana Binti Abd Halim; **Software:** Not applicable. **Supervision:** Nor Hasniza Binti Ibrahim; Noor Dayana Binti Abd Halim; **Validation:** Nor Hasniza Binti Ibrahim; Noor Dayana Binti Abd Halim; **Visualization:** Norliyana Binti Md. Aris; Nurul Hanani Binti Rusli; **Writing – original draft:** Norliyana Binti Md. Aris; Nor Hasniza Binti Ibrahim; Noor Dayana Binti Abd Halim; Nurul Hanani Binti Rusli; **Writing – review & editing:** Norliyana Binti Md. Aris; Nor Hasniza Binti Ibrahim; Noor Dayana Binti Abd Halim.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Scientific creativity of secondary school students on colloid system

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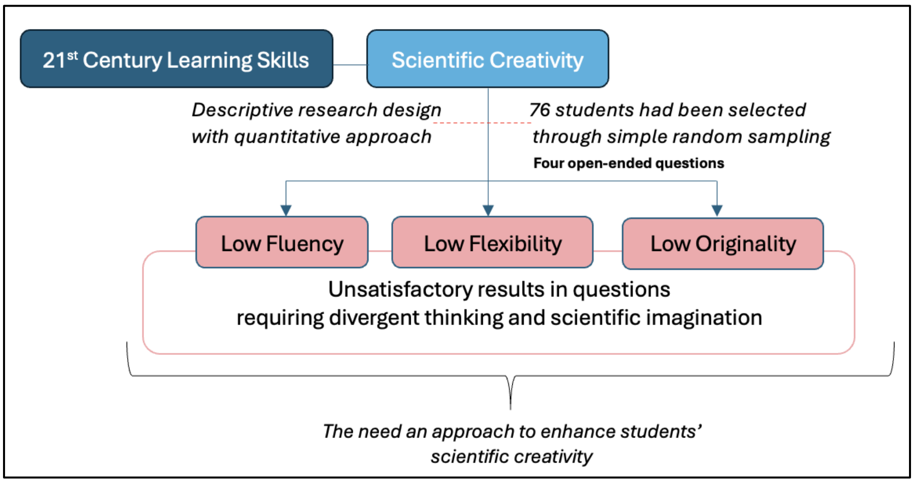
Abstract

The significance of scientific creativity in science has been highlighted for nearly two decades. In chemistry education, this involves students' ability to generate concepts related to chemical problems and phenomena, such as the colloid system found in daily life. Understanding the colloid system requires students to produce scientific ideas for problem-solving. Therefore, assessing students' scientific creativity is crucial. This research aimed to determine students' scientific creativity concerning the colloid system using a descriptive, quantitative approach. Seventy-six students were selected through simple random sampling. Data collection involved four open-ended questions, analyzed using a scoring rubric and percentage scores. The study revealed that students' fluency, flexibility, and originality were low, leading to unsatisfactory results in questions requiring divergent thinking and scientific imagination. These findings highlight the need to enhance students' ability to generate scientific ideas, emphasizing the importance of fostering scientific creativity in education.



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Highlights

- Fluency, flexibility and originality low levels need to focus on creativity.
- Focusing on the colloid system as a key topic in chemistry education.
- Assessing students' scientific creativity using a descriptive/quantitative approach.

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1. Introduction

The skills necessary in the 21st century encompass fundamental capabilities like creativity, problem-solving, effective communication, critical thinking, innovation, logical reasoning, adaptability, managing complexity, and self-direction (Beers, 2011; Soland *et al.*, 2013). These skills are intended to equip individuals for the distinct challenges of the contemporary era, setting them apart from the previous century (Tirri *et al.*, 2017). Within this skill set, creativity plays a prominent role, being recognized as a vital competency across various contexts within the 21st century (Chan and Yuen, 2014; Nakano and Wechsler, 2018; Soland *et al.*, 2013; Tirri *et al.*, 2017; Zulkarnaen *et al.*, 2018).

The significance of creativity lies in its engagement with high order thinking skills. It proves invaluable in presenting solutions to diverse issues through the generation of creative ideas. In the educational context, creativity is defined as students' ability to participate in learning activities aimed at discovering and implementing novel and unconventional ideas while maintaining a foundation of logical and rational thinking (Gunawan *et al.*, 2018). Therefore, the educational field places substantial importance on the process of uncovering and nurturing creative potential (Kanematsu and Barry, 2016).

Additionally, Liang (2002) determined that creative individuals cannot be generally creative across all fields, as someone might exhibit creativity in art but not necessarily in scientific subjects. In the domain of science, creativity is referred to as scientific creativity. This includes the ability to tackle problems through the formulation of ideas and hypotheses. The difference between scientific creativity and general creativity lies in the involvement of innovative experiments, discoveries, problem-solving activities, and the associated characteristics (Obote, 2016). Over the past six years within Indonesia's education system, student creativity concerning the colloid system has exhibited a low level in terms of creative thinking (Sulastri *et al.*, 2019; Ulfah *et al.*, 2020; Wahyu *et al.*, 2017; Wahyuliani *et al.*, 2022). The colloid system is a chemical concept that explains natural phenomena and has numerous applications in daily life (Arini *et al.*, 2021). However, many people are still unaware of the presence of colloids in daily life. For instance, soy milk (Sumarni and Kadarwati, 2020) and air fresheners (Isbullah *et al.*, 2019) are examples, and processes like bleaching, deodorizing, tanning, dyeing, and refining involve adsorption on the surface of colloidal particles, as do the production of fish oil, capsule medicine, and injectable penicillin. Hence, it is essential to study this topic in depth (Hayati *et al.*, 2014).

The low creative thinking is attributed to students' inability to generate their original ideas or novel insights during learning, resulting in insufficient development of their creative thinking skills (Wahyuliani *et al.*, 2022). Moreover, students lack practice in generating new ideas or multiple viewpoints (Ulfah *et al.*, 2020). In essence, there are lack of teachers who employ learning methods aimed at nurturing creative thinking skills and enhancing students' grasp of colloids (Sari and Hidayat, 2017), even in sub-topics like various types of colloids. Students often struggle to differentiate between different types of colloids, and their ability to provide diverse opinions and creative ideas is limited (Wahyu *et al.*, 2017).

The capability of students to think and generate ideas to address forthcoming chemical problems associated with the colloid system is imperative. This ability to think and understand the value of studying colloidal materials is closely tied to students' competence in chemistry (Arma and Supriadi, 2022; Hairida, 2017; Hasanah *et al.*, 2020). However, the involvement of creative thinking in creativity exploration within the realm of chemistry education remains incomplete. It necessitates scientific creativity because, as per Ikiao (2019), scientific creativity influences students' sensitivity to chemical problems, scientific observations, and scientific concepts, thereby fostering flexible thinking in solving chemical problems.

Furthermore, Hu and Adey (2002) contend that creativity in science extends beyond creative thinking to encompass other dimensions such as creative products, processes, and traits. Creative products encompass technical products, scientific knowledge, scientific phenomena, and problems. The creative process entails thinking & imagination, and creative traits encompass fluency, flexibility, and originality. Moreover, scientific inquiry skills, scientific knowledge, creative experimentation, scientific investigation, creative problem-solving, and scientific creative activities constitute elements of scientific creativity (Kirimi *et al.*, 2017; Park, 2004; Suyidno *et al.*, 2018; Zulkarnaen *et al.*, 2018). Consequently, investigations into students' creative thinking within the field of chemistry have yet to comprehensively portray the entire creativity in chemistry. Hence, there is limited information regarding scientific creativity that specifically focuses on the colloid system. Recognizing the importance of scientific creativity in chemistry, this study aims to discern students' scientific creativity concerning the topic of the colloid system.

2. Methodology

This study employs a descriptive research design with a quantitative approach. A total of 76 secondary school students in grade XI (science class), aged 17-18 years, were selected randomly using a simple random sampling technique from the same school. The selected secondary school holds an 'A' accreditation, and its teachers have previously taught the colloid system, ensuring that the students possess the basic knowledge to generate their ideas.

The scientific creativity test was conducted over the course of one hour to assess the overall scientific creativity of the students. The test took place in a classroom setting, with the teacher present and the researchers providing initial instructions. The research instrument utilized was a chemistry scientific creativity test, consisting of four open-ended questions developed by the researchers. The instrument was validated by professors in chemistry education. The validation process begins with sending instruments to professors, and then the feedback is the foundation for researching instrument improvement. The experts provided feedback in terms of quantitative (score) and qualitative (comments). Once the experts agree to accept the research instrument, the validation score is analyzed and confirmed by referring to the validation criteria and the experts' decision. The valid instrument was further tested on 30 students to test reliability and found Cronbach's alpha of 0.62 (reliable).

The scientific creativity model used in this study is the *Scientific Structure Creativity Model* (SSCM) by Hu and Adey (2002). The SSCM encompasses three main components: trait, process, and product. In this study, the traits assessed include originality, fluency, and flexibility. The creative product evaluated is the students' chemical knowledge when faced with scientific problems related to the colloid system. The process in this study involves divergent thinking and scientific imagination, both of which are integrated into the test items. For example, items 1 and 3 involve divergent thinking, while items 2 and 4 involve students' scientific imagination. To determine the level of scientific creativity, the scores for traits and products are combined, then calculated using a specific formula and compared against the levels of scientific creativity to identify the students' overall creativity level.

Table 1 below details each dimension of scientific creativity assessed in this study, with each product representing four questions that include both process and trait dimensions.

The result of the chemistry scientific creativity test was analyzed using a rubric of scoring. Students' score was calculated in the form of a percentage of the score and compared to the criteria level. Table 2 shows the rubric of scoring for creative traits. Table 3 shows the scoring criteria for chemical knowledge (creative product).

Table 1. Detail of chemistry scientific creativity test.

Item	Product (chemical knowledge)	Process	Trait
1	Type & Properties of colloid	Divergent thinking	Fluency, originality
2	Colloid manufacture	Scientific imagination	Flexibility, originality
3	Principle of colloid	Divergent thinking	Fluency, originality
4	Colloids in daily life and industry	Scientific imagination	Flexibility, originality

Source: Elaborated by the authors.

Table 2. Creative trait scoring criteria.

Creative trait	Indicator	Score
Fluency	Student cannot provide ideas	0
	Student can come up with one idea/answer	1
	Student can come up with two ideas/answers	2
	Student can come up with three ideas/answers	3
	Student can come up with more than three ideas/answers	4
Flexibility	Students are not able to provide category of ideas/Ideas are at same category	0
	Students can come up with one category of ideas	1
	Students can come up with two categories of ideas	2
	Students can come up with three categories of ideas	3
	Students can come up with more than three categories of ideas	4
Originality	Student do not answer/ideas are wrong	0
	If the ideas produced by students are general/common ideas/no originality (9 and above)	1
	If the ideas produced are moderately unique (ideas produced by students are 5 to 10% off like each other) (4-8 students)	2
	If the ideas produced are moderately unique (the ideas produced are smaller than 5% like each other) (2-3 students)	3
	If the ideas produced are unique (the ideas produced are only one student)	4

Source: Elaborated by the authors.

Table 3. Creative product scoring criteria.

Creative product	Indicator	Aspect	Score
Chemical knowledge	Unanswered / Misunderstanding / Misconception	If students do not answer/Ideas produced are wrong concept/Ideas produced are misconception	0
	Partial understanding	If the students' ideas are correct and ideas cover one chemical representation	1
		If the students' ideas are correct and ideas cover two chemical representations	2
	Understanding	If the students' ideas are correct and ideas cover all chemical representations namely symbolic, macroscopic, and sub-microscopic	3

Source: Elaborated by the authors.

The score obtained was further calculated in the percentage form of the score using (Eq. 1) and compared to level criteria as shown in Table 4.

$$\text{Percentage Score} = \frac{Total\ Score}{Score\ Maximal}\ x\ 100 \quad (1)$$

Table 4. Level criteria.

Percentage of score	Level criteria
68–100	High
34–67.9	Moderate
0–33.9	Low

Source: Elaborated by the authors.

3. Results and discussion

Scientific creativity holds significance as an important skill because of its connection with the ability of students to problem-solve from a scientific standpoint, and showcasing a mastery of scientific reasoning (Demir, 2014; O'Donoghue *et al.*, 2014). Moreover, it enhances students' capacity to grasp scientific knowledge and engage in scientific issue resolution (Wang and Yu, 2011). Furthermore, it provides insight into an individual's cognitive ability to tackle challenges by generating unique concepts (Antink-Meyer and Lederman, 2015). Unfortunately, the result identifying scientific creativity shows that most students are at a low level. Table 5 below shows the level of scientific creativity of most students.

Table 5. Level of scientific creativity of students.

Level of scientific creativity	Frequency	Percentage (%)
High (68–100)	0	0
Moderate (34–67.9)	17	22.4
Low (0–33.9)	59	77.6

Source: Elaborated by the authors.

Based on Table 5, 77.6% of students are at a low level of scientific creativity and only 22.4% of students are at a moderate level. The low level of scientific creativity shows that most students are not capable of generating large numbers of ideas with different categories in solving colloid system problems. Also, the ideas produced are common / not unique. In addition, 22.4% of students with moderate levels of scientific creativity show students can produce ideas, however, the ideas are still limited either in terms of the total of ideas or various ideas. The finding in this study corresponds with Ikiao (2019) and Kamonjo (2019), that there is a low level of scientific creativity among students in Kenya. Moreover, similar findings also have emerged in Malaysia, indicating a low level of scientific creativity among chemistry students (Jamal *et al.*, 2020; Omar *et al.,* 2017). In conclusion, the scientific creativity of students in chemistry still needs to improve.

According to Hu and Adey (2002), there are three dimensions of scientific creativity: creative trait, creative product, and process. The level of scientific creativity is measured by calculating the score from creative trait and product. In this case, the creative process (scientific imagination and divergent thinking) is integrated into two creative traits and product. It is because to produce the ideas with scientific knowledge, the students involved their scientific imagination and divergent thinking in which the items specifically are developed based on those skills. Table 6 below shows the level of creative traits found in students (fluency, flexibility and originality).

Table 6. Level of creative trait.

Aspect	Percentage of score	Level criteria
Fluency	27	Low
Flexibility	23	Low
Originality	21	Low

Source: Elaborated by the authors.

Based on Table 6, the level of creative traits (fluency, flexibility, originality) of students is low. The lowest score of creative traits is originality with a percentage of score is 21. It means that the ideas produced by students are common or the ability of students to produce unique/original ideas is still low. Besides, the flexibility of students is also low (percentage of score = 23), meaning that students cannot generate a variety of ideas. Besides originality and flexibility, the fluency of students is also at a low level. It indicates that students' ability to produce a large of number ideas is low. To conclude, the student's ability in this study to solve problems related to the colloid system is low.

Furthermore, the quality of ideas relates to the scientific knowledge of students. It is proved by Hu and Adey (2002) and Park (2004) that scientific knowledge is a prerequisite of scientific creativity. Consequently, to exhibit scientific creativity, students must possess a firm grasp of scientific knowledge through a deep comprehension of scientific concepts. In this case, students' ideas need to contain the correct concept of the colloid system. Table 7 shows the result of creative product (scientific knowledge) integrated with divergent thinking ability.

Table 7. The result of creative product in divergent thinking question.

Aspect	Score	Divergent Thinking			
		Item 1		Item 3	
		Frequency	Percentage (%)	Frequency	Percentage (%)
Unanswered / Misunderstanding / Misconception	0	32	42.10	42	55.26
Partial understanding	1	41	53.95	34	44.74
	2	3	3.95	0	0
Understanding	3	0	0	0	0
Total		76	100	76	100

Source: Elaborated by the authors.

Table 7 shows students' creative product ability (scientific knowledge related to the colloid system) in item 1 and item 3. Item 1 and item 3 contain scientific knowledge related to the type and properties of colloids and the use of the principle of colloids in daily life. Both items involve a creative process, namely divergent thinking. Item 1 requires students' ability to produce ideas related to air pollution as a chemical phenomenon in colloid systems. Based on Table 7, 42.10% of students obtained a score of 0. It means students cannot give the correct answer or the ideas produced are wrong concepts or misconceptions. Furthermore, 53.95% of students obtained a score of 1, meaning that the students' ideas are correct, however, it only covers one chemical representation. Besides, only 3.95% of students can afford to obtain a score of 2 which means the ideas generated are correct and cover two chemical representations. In conclusion, most students' ideas range from wrong conception to partial understanding.

Item 3 requires a question emphasizing colloid principle in solving water pollution. According to Table 7, 55.26% of students obtained a score of 0, meaning that the ideas produced are wrong concepts or students cannot produce ideas for solving water pollution. Besides, 44.74% of students obtained a score of 1 which means the ideas produced are correct, however, it only covers one chemical representation. To sum up, the students have a partial understanding of the colloid principle in solving water pollution.

Both items involve divergent thinking, according to Runco and Acar (2012), divergent thinking is the main contributor to creativity and a manifestation of creative potential. It includes individuals associating situations that occurred with the colloid system concept, describing the situation and the applicable colloid system principles, and combining components from situations, objects, and colloid system concepts into a new conclusion, it is adopted from divergent thinking (Sun *et al.*, 2020). According to the findings, 42.1% of students were unable to correctly relate and describe the issues in the questions regarding the colloid system and draw accurate conclusions about air pollution as a chemical phenomenon (item 1). Similarly, 55.26% of students struggled to connect and explain the issues in the questions related to the colloid system, leading to incorrect conclusions about the colloid principle in addressing water pollution (item 3). This indicates that students' divergent thinking skills still need improvement.

Furthermore, the creative product is also discussed related to colloid manufacture and the colloid principle in waste treatment which is integrated with the scientific imagination aspect as a creative process. Table 8 discusses in detail.

Table 8. The result of creative product in scientific imagination question.

Aspect	Score	Scientific imagination			
		Item 2		Item 4	
		Frequency	Percentage (%)	Frequency	Percentage (%)
Unanswered / Misunderstanding / Misconception	0	35	46.05	42	55.26
Partial understanding	1	41	53.95	34	44.74
	2	0	0	0	0
Understanding	3	0	0	0	0
Total		76	100	76	100

Source: Elaborated by the authors.

In terms of scientific imagination, items 2 and 4 are considered. Item 2 requires students' ability to answer questions regarding design experiments on colloid manufacturing. Based on Table 8, as many as 46.05% of students cannot give ideas to design experiments of colloid manufacture or the answers are not correct. In addition, a total of 53.95% of students could design experiments, however, it only covers chemical representation namely macroscopic. This means most students have a partial understanding of design experiments about colloid manufacturing. Furthermore, the ability of students to use the colloid principle in waste treatment is still lacking. This is because 55.26% of students obtained a score of 0, meaning that the students cannot generate ideas to use the colloid system to solve waste treatment. Besides, this is only 44.74% of students can produce ideas for good treatment of waste. However, all ideas only cover one chemical representation. Therefore, it can be concluded that students' abilities either in designing experiments of colloid manufacture or using the colloid principle in waste treatment are lacking.

4. Study limitations

This study was limited to identifying students' scientific creativity regarding their scientific knowledge of the colloid system. In addition, students' scientific creativity refers to the scientific structure creativity model by Hu and Adey (2002). Thus, students' scientific creativity would be different from other scientific knowledge and the use of another scientific creativity model could influence the result. In terms of traits, this study only involved fluency, flexibility and originality, so further research still needs to be conducted.

5. Implication and future studies

This study provides valuable insights into the scientific creativity of students specifically related to their understanding of the colloid system. However, given that the study focused on this particular area of scientific knowledge, the implications of these findings may not fully extend to other scientific domains. Future research should explore students' scientific creativity in other areas of science to determine if similar patterns emerge or if different knowledge areas elicit distinct creative responses.

Moreover, the study's reliance on the scientific structure creativity model by Hu and Adey (2002) suggests that different models of scientific creativity could yield varying results. Future studies should consider utilizing alternative models to provide a more comprehensive understanding of how different approaches might influence the assessment of scientific creativity.

Finally, this study focused on three traits of scientific creativity: fluency, flexibility, and originality. Future research could expand on this by including additional traits or dimensions of creativity, such as elaboration or curiosity, to offer a more holistic view of students' creative potential in science. This would help to identify a broader range of creative abilities and contribute to the development of more effective educational strategies that foster scientific creativity across various contexts.

6. Conclusions

The study revealed that students' fluency, flexibility, and originality were at a low level, resulting in unsatisfactory outcomes when addressing questions that necessitated divergent thinking and scientific imagination. This result implies a need to enhance students' ability to generate scientific ideas. Hence, the result of this study could be an overview of students' scientific creativity and the need for heightened attention to students' scientific creativity.

Authors' contribution

Conceptualization: Wimbi Apriwanda Nursiwan; **Data curation**: Wimbi Apriwanda Nursiwan; **Formal Analysis**: Nor Hasniza Ibrahim; **Funding acquisition**: Not applicable; **Investigation**: Wimbi Apriwanda Nursiwan; Chuzairy Hanri; **Methodology**: Wimbi Apriwanda Nursiwan; Chuzairy Hanri; **Project administration**: Wimbi Apriwanda Nursiwan; Chuzairy Hanri; **Resources**: Nor Hasniza Ibrahim; **Software**: Wimbi Apriwanda Nursiwan; Nor Hasniza Ibrahim; **Supervision**: Chuzairy Hanri; Nor Hasniza Ibrahim; **Validation**: Wimbi Apriwanda Nursiwan; Chuzairy Hanri; **Visualization**: Wimbi Apriwanda Nursiwan; Chuzairy Hanri; **Writing – original draft**: Wimbi Apriwanda Nursiwan; Nor Hasniza Ibrahim; **Writing – review & editing**: Wimbi Apriwanda Nursiwan; Chuzairy Hanri.

Data availability statement

Data sharing is not applicable.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Short Review

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Phenolic compounds and biological potential of *Eugenia uniflora* L.: A short review

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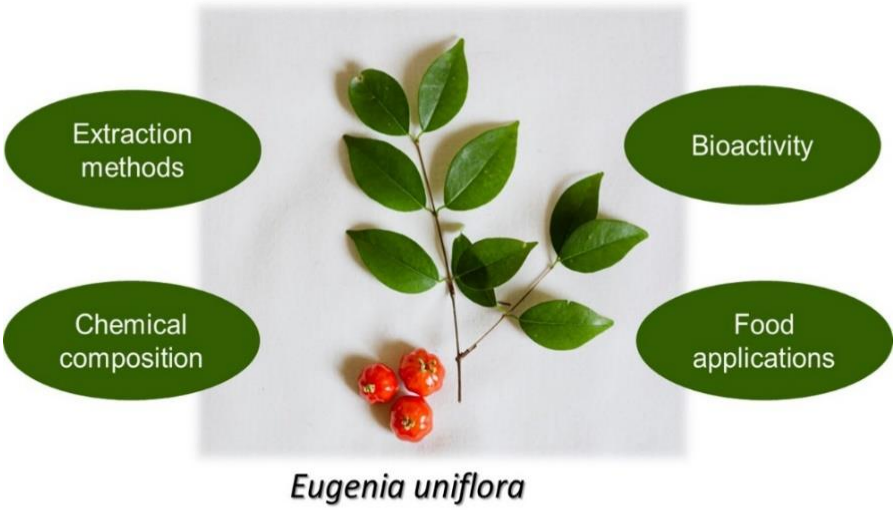
Abstract

Eugenia uniflora L. (Myrtaceae) is native to Brazil and it is known as pitanga. In traditional medicine, this species is used to treat cough, skin allergies and asthma. Different parts of this plant displayed insecticidal, antimicrobial, and antioxidant activities. The main phenolic compounds found in the extracts of this species are flavonoids and tannins, which display relevant biological activities. This review shows recent phytochemical studies on *E. uniflora*, emphasizing the phenolic compounds, including a description of methods of extraction of these metabolites. Besides, the diversified biological activities and the potential of this plant for the food industry are reported. The pharmacological and nutraceutical potential attributed to *E. uniflora* justify the growing scientific interest in this species.



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1. *Eugenia uniflora*;
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Highlights

- More demand for natural antioxidants motivates research about phenolic compounds.
- *Eugenia uniflora* can be used as a functional ingredient by the food industry.
- Distinct applications of *E. uniflora* spire us to continue the studies on this plant.

CONTENTS

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1. Introduction

The genus *Eugenia* is one of the most important within the Myrtaceae family, which has about 2000 species widespread from Southern Mexico to Argentina, and a small number of species in Africa. Many of these species present highlighted nutritional values and commercial applications, due to the occurrence of bioactive compounds (Saval *et al.*, 2023; Santoso *et al.*, 2021).

Eugenia uniflora L. is native to Brazil and it is known as pitanga. It is the most studied species from the *Eugenia* genus, regarding the essential oil composition and bioactivity. Considering the economic context, pitanga is a promising fruit to be exploited by agroindustry due to its diversified use, exemplified by juices, jellies, ice creams, and fruit compotes, besides its fresh consumption (Luciano *et al.*, 2021a; Santoso *et al.*, 2021; Vargas *et al.*, 2019). In addition, *E. uniflora* leaf essential oil is extensively used to produce a range of personal care products due to its astringent characteristics, added to the peculiar and pleasant aroma (Tobal and Rodrigues, 2019).

In Brazilian folk medicine, the leaves of the pitanga are used in the form of teas prepared as an infusion or decoction. Traditional applications include the treatment of hypercholesterolemia, as a digestive, hepatoprotective, diuretic, antihypertensive, anti-inflammatory, and antimicrobial (Bagatini *et al.*, 2023; Silva *et al.*, 2023). The occurrence of new or rare *E. uniflora* secondary metabolites in other plants, especially polyphenolic compounds and volatile terpenoids, is an awakening for research into new activities and applications.

Due to the importance of *E. uniflora* in different areas, this work aimed to present a short review of the published studies on methods of extraction and isolation of compounds present in extracts of the leaves and fruits of *E. uniflora*, as well as some biological activities of this species reported between 2018 and 2024.

2. Methodology

Data relating to *E. uniflora* L. and bioactive potential were obtained through PubMed and Google Scholar and published in the last 6 years. The following exclusion criteria were adopted: 1) article whose full text was not accessible in the database; 2) publications that did not include the term "*Eugenia uniflora*" and the specific bioactivity in the abstract or title; 3) articles which are not written in English; and 4) articles in which the phytochemicals used in the biological activity assays were not isolated from that species but were acquired from industries. In this short review, 48 publications were included.

3. Extraction and isolation techniques

The extraction of phenolic compounds, glycosylated flavonoids, and tannins from the fruits and leaves of *E. uniflora* has been carried out using conventional and non-conventional extraction techniques, the first being the most used. Generally, for the extraction of phenolic compounds, water and organic solvents (ethanol, methanol, acetone, and n-butanol) are used. It is often necessary to mix the solvents to increase efficiency (Sobeh *et al.*, 2019).

In fruits, the percolation technique with ethanol/water was used to isolate the sesquiterpenoids Eugenilone A– N (Chen *et al.*, 2023), while hydrolysable tannins, carotenoids, iridoids, anthocyanins, and flavonoids were isolated by maceration with acetone, methanol/water and HCl/methanol (Biazotto *et al.*, 2019; Rodrigues *et al.*, 2020). Combined techniques showed promise in obtaining phenolic compounds in *E. uniflora* fruits. An example is the maceration with sonication using pure or mixtures of solvents such as methanol, ethanol, water (Migues *et al.*, 2018; Ramalho *et al.*, 2019; Santos *et al.*, 2021), hexane, and ethyl acetate (Rashmi and Negi, 2022) in obtaining anthocyanins, tannins, flavonoids and phenolic acids.

Conventional extraction is the most used due to ease of use and low cost. However, unconventional extraction techniques have environmentally important advantages, such as shorter extraction time and amount of solvent, high yield and better reproducibility. Unconventional extraction techniques such as supercritical CO_2 have been used to extract different metabolites from leaves (Bezerra *et al.*, 2020; Canabarro *et al.*, 2020). Souza *et al.* (2022) described a green method combining the extraction assisted by microwave using natural deep eutectic solvent (NADES) composed of choline and lactic acid to isolate bioactive phenolic compounds from the leaves of *E. uniflora*. The low energy consumption associated with the method's reproducibility was highlighted in that study.

Different chromatographic techniques are used for the isolation and purification of phenolic compounds present in the leaves and fruits of *E. uniflora*. Thin-layer chromatography (TLC) analysis using silica gel (Rashmi *et al.*, 2023), column chromatography (CC) performed on silica gel, Sephadex LH-20 and Diaion HP-20 (Chen *et al.*, 2023; Sobeh *et al.*, 2019; Tenório *et al.*, 2024), flash chromatography system Sepacore® X50 with RP-18 column (Sobeh *et al.*, 2019), high-performance liquid chromatography (HPLC) performed on a reversed-phase octadecylsilanized silica gel (ODS) column and with refractive index detector (Biazotto *et al.*, 2019; Rodrigues *et al.*, 2020), and UV/VIS photodiode array detector (Ramalho *et al.*, 2019; Santos *et al.*, 2021).

For the structural elucidation of the isolated substances from *E. uniflora,* the comparison with standard samples of phenolic compounds (gallic acid, vanillic acid, ellagic acid, *p*-coumaric acid, ferulic acid) and flavonoids (kaempferol, resveratrol, quercetin, catechin, epicatechin and rutin), for example, is a conventional method. (Bagatini *et al.*, 2023). Afterwards, the structural elucidation of the isolated metabolites is generally performed using hyphenated techniques. In these cases, the equipment (Gas or liquid chromatograph) used for the isolation of the constituents is coupled to a mass spectrometer that is operated by distinct ionization types (Bagatini *et al.*, 2023; Souza *et al.*, 2022; Tenório *et al.*, 2024). Additionally, FTIR and one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopies are essential techniques also used in the structural elucidation of the isolated metabolites (Rashmi and Negi, 2022).

4. Chemical composition

E. uniflora is a source of secondary metabolites from distinct classes, such as phenolic acids, glycosylated flavonoids and their aglycones, triterpenes, and tannins (**Table 1**). The chemical structure of some compounds isolated from *E. uniflora* is shown in **Fig. 1**. Many studies report the analysis of total phenolics found in extracts, as well as the content of flavonoids present in leaves, seeds or fruits of different varieties or stages of maturation of *E. uniflora* (Lazzarotto *et al.*, 2021; Fidelis *et al.*, 2022; Migues *et al.*, 2018). There are compounds which are commonly found in different parts of *E. uniflora*. However, since the amount of these compounds varies depending on the plant (Bezerra *et al.*, 2020; Borsoi *et al.*, 2022), this evidence can direct the scientific interest for a specific part of *E. uniflora*. Qualitative and quantitative profiles of secondary metabolites are influenced by parameters such as storage time, extraction method and solvent. Depending on these external factors and plant physiology, the type and content of metabolites during pitanga's maturation certainly change. It was observed that the anthocyanin content increased, while flavonoid and tannin contents decreased in fruits of red, red-orange, and purple biotypes from *E. uniflora* harvested in Brazil (Chaves *et al.*, 2018). Bellaver *et al.* (2024) evaluated the impact of drying at different temperatures on the retention of phenolic compounds and carotenoids in the pulp of *E. uniflora.* The results indicated the degradation of those compounds, highlighting the importance of optimizing the drying process and maximizing the fruit's nutraceutical value.

Santos *et al.* (2021) reported differences between the content of myricetin, quercetin, and lutein in distinct samples of fruits from the purple variety. This illustrates the variability in the constituents of the fruits belonging to the same plant and highlights the importance of collecting a sample that represents the whole specimen.

Table 1. Chemical composition of *E. uniflora.*

Plant part	Compound class	Compound	Reference
Fruits	Sesquiterpenes	Eugenilones A-N	Chen *et al.*, 2023
Pulp, seeds, and leaves	Phenolic acid	Gallic acid	Borsoi *et al.*, 2022; Tenório *et al.*, 2024
Seeds	Phenolic acids	Protocatechuic acid	Bagatini *et al.*, 2023
		Daucic acid	
		Salicylic acid	
Leaves	Phenolic acids	Quinic acid	Oliveira *et al.*, 2018
		4-hydroxybenzoic acid	
		4-p-coumaroylquinic acid	
		Chlorogenic acid	
Leaves, pulp, and fruits	Phenolic acids	p-Coumaric acid	Borsoi *et al.*, 2022; Rashmi and Neghi, 2022
Leaves and pulp	Phenolic acids	Ellagic acid	Bagatini *et al.*, 2023; Borsoi *et al.*, 2022
		Vanillic acid	Rashmi and Neghi, 2022
		Caffeic acid	
Fruits	Phenolic acids	Syringic acid	
		Homovanillic acid	
		3-Hydroxybenzoic acid	
		Tannic acid	
Pulp		4-hydroxyphenylacetic acid	Chaves *et al.*, 2018
		Gallic acid 3-*O*-[6'-*O*-acetyl-βd-glucoside]	Sobeh *et al.*, 2019
		Gentisic acid 5-*O*-β-D-glucoside	
Leaves, pulp, and seeds	Flavonoids	Myricitrin	Oliveira *et al.*, 2018;
Leaves	Glycosilated flavonoids	Myricetin-3-*O*-(2''-*O*-galloyl)-α-l-rhamnopyranoside	Oliveira *et al.*, 2018
		Myricetin-3-*O*-(2''-*O*-galloyl)-α-L-rhamnopyranoside hydrate	
Pulp	Hydrolysable tannins	Valoneic acid dilactone	Sobeh *et al.*, 2019
		Sanguiin h1	Bagatini *et al.*, 2023
		Tellimagrandin I	
Leaves	Hydrolysable tannins	Tellimagrandin II	
		Tercatain	
		Heterophylliin a	
		Ellagitannin	Oliveira *et al.*, 2018
Fruits	Hydrolysable tannins	Theogallin	Ramalho *et al.*, 2019
		Eugeniflorin D2	Oliveira *et al.*, 2018
Leaves	Dimmeric tannins	Camptothin	Oliveira *et al.*, 2018
		Gemin/dhippomanin A	
		Oenothein B	Oliveira *et al.*, 2018; Ramalho *et al.*, 2019
Leaves	Flavanone	2"-Galloylastragalin	Bagatini *et al.* 2023
		Isosakuranetin	
		Quercetin	
Leaves, seeds	Flavanols	Kaempferol	Borsoi *et al.*, 2022
		Gallocatechin	Bagatini *et al.*, 2023
		Epigallocatechin	
Leaves, seeds	Glycosilated flavone	Luteolin 7-*O*-glucoside	
Fruits[a]	Glycosilated flavonols	Myricetin-galloyl-hexoside	Migues *et al.*, 2018
		Quercetin galloyl hexoside	
		Quercetin-rhamnoside	
		Quercetin-pentoside	
		Quercetin-hexoside	
	Glycosilated Flavones	Myricetin-pentoside	
		Myricetin-rhamnoside	
	Anthocyanins	Delphinidin-3-hexoside	
		Cyanidin-3-hexoside	
Fruits[b]	Anthocyanins	Malvidin-*O*-galactoside	Migues *et al.*, 2018
		Malvidin-*O*-pentoside	
		Malvidin-*O*-acetylhexoside	
		Petunidin-*O*-galactoside	

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Fruits[a,b]	Anthocyanins	Pelargonidin-*O*-rutinoside	Chaves *et al*., 2018
		Delphinidin-*O*-galactoside	
		Pelargonidin-*O*-glucoside	
		Delphinidin-*O*-glucoside	
		Cyanidin-*O*-galactoside	
		Cyanidin-*O*-glucoside	
Pulp	Anthocyanins	Cyanidin 3-glucosyl-rutinoside	Chaves *et al*., 2018
	Glycosylated flavones	Luteolin 7-*O*-glucuronide	
		Kaempferol-3-*O*-glucuronide	
		Kaempferol-3-*O*-sophoroside	
		Isorhamnetin-3-*O*-glucoside	
		Luteolin-6-C-glucoside	
Pulp	Flavones	Isorhamnetin	Chaves *et al*., 2018
		Rhamnetin	
	Flavonol	Catechin	Chaves *et al*., 2018
	Flavanone glycoside	Eriodictyol-7-*O*-glucoside	
Fruits	Stilbene	Pterostilbene	Rashmi *et al*., 2023
	Naphtoquinone	Juglone	
	Lignan	Syringaresinol	
	Isoflavone	Biochanin A	
	Phenylpropanoid	Estragole	
Pulp	Carotenoids	Rubixanthin	Borsoi *et al*., 2022
		Lutein	
		B-carotene	
		Violaxanthin	
		Lycopene	
		Zeaxanthin	

Source: Elaborated by the authors.
Note: a: purple variety; b: red variety.

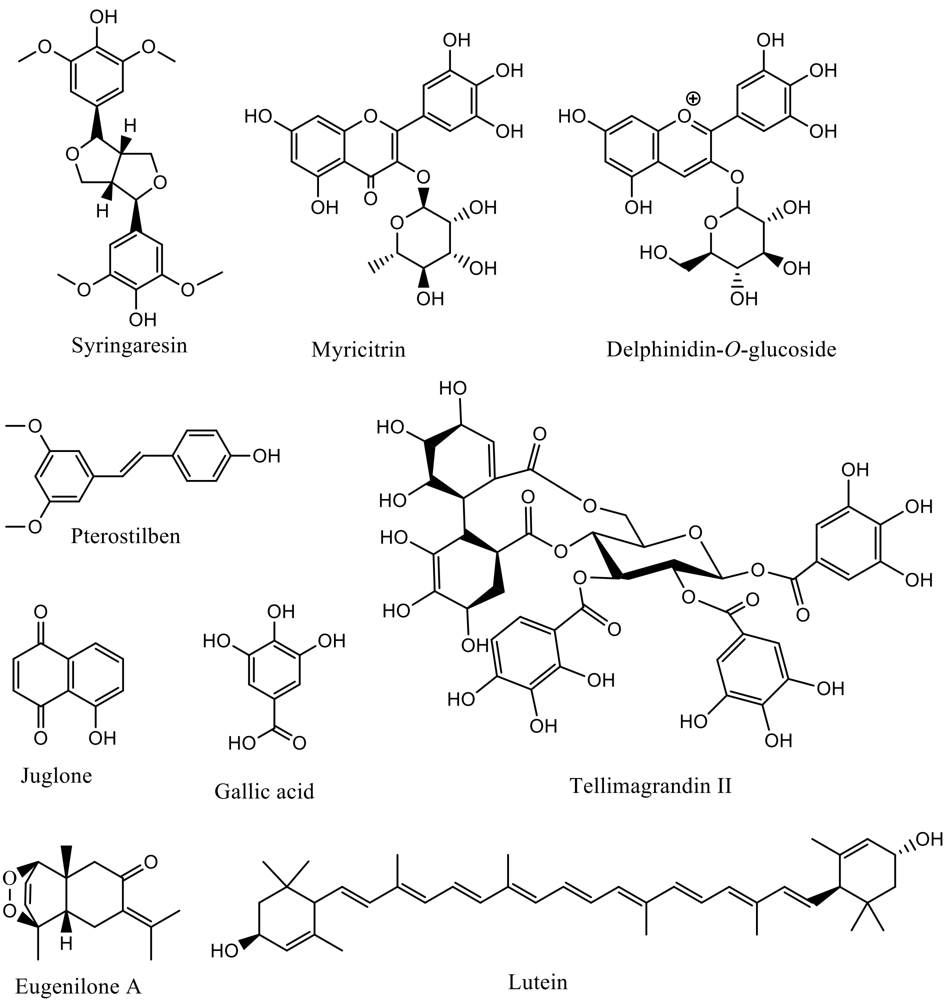

Figure 1. Chemical structures of metabolites isolated from *E. uniflora*.
Source: Elaborated by the authors.

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5. Bioactivity of extracts and isolated substances of *E. uniflora*

The fruits and leaves of *E. uniflora* are used for the treatment of different symptoms and diseases, such as fever, bronchitis, digestive disorders, gout, and hypertension (Chen *et al.*, 2023; Fidelis *et al.*, 2022; Souza *et al.*, 2018). Many published studies support the traditional use of *E. uniflora*, as described in the sequence.

Virulence attributes, such as adhesion and biofilm formation, were tested in cultures of nine *Candida* species in the presence and absence of an *E. uniflora* leaf extract. The results were statistically significant for both *C. albicans* and non-*albicans Candida* isolates (Souza *et al.,* 2018). *Pseudomonas aeruginosa* is an important pathogen for human health, with a great capacity to develop antibiotic resistance. It was chosen to conduct tests with an ethanolic extract of *E. uniflora* leaves. The results obtained describe the interaction of components of the extract with commercial antibiotics. For ciprofloxacin, amikacin and colistin. The presence of the extract does not alter the antibiotic activity. However, with piperacycline or ceftazidime, the extract of *E. uniflora* induced synergistic effects increasing antibiotic activity (Bobadilla *et al.*, 2018).

A crude methanolic extract of *E. uniflora* leaves was evaluated against *Helycobacter pylori* and presented MIC of 128 mg/mL. The composition of the extract was studied by determining total phenolic compounds (19.31%) and total tannins (16.13%) in milligram equivalents of gallic acid per gram of extract, total flavonoids (2.86%) and using FT-ICR-ESI-MS demonstrating the presence of monomeric saccharides, dimers and trimers, ellagic acid, ellagitannin, galloyl-derivatives, and myricetin and as main compounds. (Monteiro *et al.*, 2019).

Serratia liquefaciens is a relevant bacterium because of its ability to form a biofilm that facilitates infection. An extract containing phenolics from *E. uniflora* fruit pulp in sub-inhibitory concentrations for S. liquefaciens significantly reduced biofilm formation by the microorganism (Rodrigues *et al.*, 2020). An ethanolic extract from the pulp of the pitanga was tested against the colorectal bacteria *Streptococcus bovis*, *Enterococcus faecalis*, *E. coli*, and *S. enterica*, demonstrating a significant reduction in the infectious potential of these microorganisms (Indrawati *et al.*, 2019).

Anti-inflammatory and antihyperglycemic activities are linked to traditional uses of the leaves from *E. uniflora* and were evaluated in the crude methanolic extract, isolating and identifying several phenolic compounds. The extract showed strong antioxidant activity in HaCaT cells, reducing ROS and p38 phosphorylation, and increasing GSH levels (Sobeh *et al.*, 2019). The *in vivo* anti-inflammatory activity was evaluated by the considerable reduction in paw edema caused by carrageenan, in addition to the reduction in acid-induced writing and the increase in latency time in the hot plate test, and reduction in rectal temperature in rats after intraperitoneal injection of Brewer's yeast (Sobeh *et al.*, 2019). Antidiabetic activity was demonstrated in rats with streptozotocin-induced diabetes, strongly reducing serum glucose and lipid peroxidation levels and, at the same time, increasing serum insulin concentration (Sobeh *et al.*, 2019).

The fraction obtained with ethyl acetate extract of *E. uniflora* leaves showed a high concentration of phenolic compounds, identifying gallic acid (5.29%), ellagic acid (1.28%) and myricitrin (8.64%) as being the major compounds. This fraction showed anti-inflammatory activity with a significant reduction in paw edema and the number of abdominal contortions induced by acetic acid, and an antinociceptive effect at all doses tested, suggesting the participation of opioid receptors (Candeia *et al.*, 2022).

A recent discovery of novel secondary metabolites in *E. uniflora* occurred in studies of fruits and thus may become important in food production. They are sesquiterpenes with rearranged skeletons called Eugenilones A-H, some of which have moderate anti-inflammatory activity determined in a model using zebrafish (Chen *et al.*, 2022). Between the so-called Eugenilones A-N, two of which (A and E) showed significant anti-inflammatory activity by inhibiting the production of cellular factors such as NO and TFN—alpha (Chen *et al.*, 2023).

Counting on phenolic compounds with strong antioxidant activities, an ethanolic extract of *E. uniflora* and fractions showed promising results in hepatoprotection models (Syama *et al.*, 2020). At doses of up to 2.0 g/kg administered to rats, no toxic effects could be observed. By the other side, the most active fraction of the extract (500 mg/kg) showed antitoxic effects comparable to silymarin (100 mg/kg) in the model of rat intoxication with CCl_4 at the highest dose tested, in the same way that impaired normal bilirubin and alkaline phosphatase levels were restored. The histological study showed the normalization of liver tissues after treatment with the active fraction (Syama *et al.*, 2020).

Cytotoxic activities have been found in different *E. uniflora* extracts from leaves, seeds, fruit pulp, essential oils, and isolated substances. As an example, the cytotoxic potential of an *E. uniflora* leaf extract was studied *in vitro* against dengue virus replication in the Huh7it-1 cell line, showing an IC_{50} of 19.8 μg/mL (Dewi *et al.*, 2019). Furthermore, phenolic compounds from this plant, such as myricetin, cyanidin -3-*O*-glucoside, and galloylastragalin, were evaluated by in silico analysis of toxicity assessment and against the MDM2 and Bcl-xL proteins, which are responsible for promoting cancer cell growth and malignancy. Galloylastragalin showed potent inhibition of those proteins. All the compounds assayed were potentially non-hepatotoxic, non-mutagenic, non-carcinogenic, and non-cytotoxic (Kar *et al.*, 2024), which stimulates further evaluation of the anticancer properties of *E. uniflora*.

Extracts from the seeds and pulp of the *E. uniflora* fruit were tested to determine antitumor activities, cytotoxic potential and inhibitory capacity for α-amylase and α-glucosidase. The extracts were not cytotoxic to peripheral blood mononuclear cells. The seed extract decreased the cell viability of melanoma cells within 24 hours of exposure. At a concentration of 5 μg/mL, the seed extract inhibited α-amylase (7.73%) and α-glucosidase (15.34%) (Borsoi *et al.*, 2022).

A specific extract for phenolic compounds from fresh purple pitanga was obtained by homogenizing the seedless fruits with ethanol in an ULTRA-TURRAX® mixer. The extract was studied using a Parkinson's disease model in which memory impairments are induced by intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in rats. The results of the analyses demonstrated a neuroprotective effect for the fruit phenolic extract, which contains a total phenolic of around 96.5 mg of chlorogenic acid equivalent/mL (Savall *et al.*, 2023).

The aqueous extract and fraction obtained in acetyl acetate of the leaves from *E. uniflora* were evaluated in vitro and in vivo assays for their antiophidic action. Both samples inhibited the enzymatic action of *B. leucurus* and *B. brazili* venoms at low concentrations. In addition, the extract and fraction also demonstrated *in vivo* antiphonic activity by reducing oedema in the first 0.5 h after treatment (Daniele-Silva *et al.*, 2024).

The relevant and diversified biological properties of *E. uniflora* illustrated here stimulate the continuation of research about this species' therapeutic potential, which can lead to the development of new psychotherapies.

6. Food applications

The pitanga tree is well adapted to the Brazilian climate, which allows its cultivation in almost all parts of the country. Due to its ability to thrive in different climatic and soil conditions, the pitanga tree has spread and is currently cultivated in several regions of the world, including South America, Central America, the Caribbean, Florida (where it is the most popular *Eugenia* species), Hawaii, Mexico, China, India, Sri Lanka, Madagascar, South Africa, Israel, and Mediterranean countries (Bezerra *et al.*, 2018; Engela *et al.*, 2021; Griffis *et al.*, 2018). Its high plasticity supports diversified production and stimulates commercial exploitation in different regions of the world.

A part of the human consumption of *E. uniflora in nature, it* was also investigated as a promising ingredient for many food applications. Adding bioactive compounds from plants in films could reduce the need for food preservatives. The application of pitanga leaf extract to cassava starch/chitosan films exhibited antifungal activity against *Aspergillus flavus* and *A. parasiticus* (Chakravartula *et al.*, 2020). The second layer of gelatin-based film application promoted antimicrobial effects against *S. aureus* and *L. monocytogenes*. Furthermore, the addition of phenolic compounds from *E. uniflora* produced an active film with high antioxidant activity. The bilayer technique allowed for the use of lower concentrations of additives without affecting the water vapor permeability characteristics (Luciano *et al.*, 2021a). On the other hand, when the extract was used with a single-layer technique, negative impacts were observed on the physical properties of films derived from cassava starch and chitosan. Gas permeability, including O_2 and CO_2, was elevated compared to the control film (Iaccheri *et al.*, 2023).

The incorporation of a water-in-oil-in-water (W/O/W) emulsion containing hydroalcoholic extract from pitanga leaves into gelatin and/or chitosan films resulted in a film with higher phenolic compounds and antioxidant capacity, able to suppress the growth of *S. aureus*. (Tessaro *et al.*, 2021a; 2021b). The addition of soybean straw crystalline nanocelluloses and the W/O/W emulsion produced a flexible material with high water vapor barriers (Tessaro *et al.*, 2021a). In both uses, a film with excellent UV/Vis light barrier properties was achieved, which could be ideal for packaging lipid-rich foods.

The extract of pitanga leaves was able to prevent lipid oxidation in canola oil (Vargas *et al.*, 2019), fresh pork sausages (Luciano *et al.*, 2021b), pork burgers (Lorenzo *et al.*, 2018; Rocchetti *et al.*, 2020) and lamb burgers (Carvalho *et al.*, 2019). The shelf life of these products was improved due to the inhibition of the oxidation process. In meat products, factors such as greater water retention (Luciano *et al.*, 2021b), control of microbial growth (Lorenzo *et al.*, 2018), pH (Carvalho *et al.*, 2019; Lorenzo *et al.*, 2018; Rocchetti *et al.*, 2020), reduction of protein oxidation, and enhancement of red color (Carvalho *et al.*, 2019; Lorenzo *et al.*, 2018) were perceived with the addition of the leaves extract of *E. uniflora* (Luciano *et al.*, 2021b).

The freeze-dried pulp of orange pitanga could also be added to obtain antioxidant properties against lipid and protein oxidation, but the cooking yield and texture characteristics showed significant changes compared to the standard, as well as the low sensory acceptance concerning color in beef patties (Romero *et al.*, 2021).

Pitanga pulp has the potential to be used as an ingredient by the food industry as an innovative, natural option with a health appeal. The addition of pitanga pulp to diet candies (Vergara *et al.*, 2022) and diet jellies (Tobal and Rodrigues, 2019) showed positive physicochemical characteristics, in addition to the maintenance of phenolic compounds after processing. However, anthocyanins, carotenoids and vitamin C levels decreased significantly during storage. Both the dietary and control formulations, with added sucrose, were well accepted sensorially, suggesting the addition of pulp as an alternative to encourage the consumption of native fruits with added phenolic compounds and replace artificial colors and flavorings (Tobal and Rodrigues, 2019; Vergara *et al.*, 2022).

Because the fruit is physically and chemically delicate, its transport to the final consumer is difficult. Generally, pitanga is consumed only by people with a pitanga tree (pitangueira) nearby. This fact stimulates the realization of research aiming at preserving fruit and, consequently, of the species. The above data showed the versatile application of *E. uniflora* and demonstrated the scientific potential of these species, which can stimulate its production and improve its economic value.

7. Concluding remarks

Due to the metabolites from different classes present in *E. uniflora*, promising pharmacological, nutraceutical, and technological potential are attributed to this species, a part of their application in the food industry. The diversified properties of *E. uniflora* stimulate the research of new applications for this plant, which can improve the economic value of this natural resource and its sustainable cultivation.

Authors' contribution

Conceptualization: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes; **Data curation**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Isabela Maria Macedo Simon Sola; **Formal Analysis**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes; **Funding acquisition**: Not applicable; **Investigation**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes; **Methodology**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes; Isabela Maria Macedo Simon Sola; **Project administration**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes; **Resources**: Not applicable; **Software**: Not applicable; **Supervision**: Cássia Gonçalves Magalhães; Domingos Sávio Nunes; **Validation**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes; **Visualization**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes; Isabela Maria Macedo Simon Sola; **Writing – original draft**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Isabela Maria Macedo Simon Sola; **Writing – review & editing**: Cássia Gonçalves Magalhães; Aline Alberti; Jociani Ascari; Domingos Sávio Nunes.

Data availability statement

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Conflict of interest

The authors declare that there is no conflict of interest.

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The creativity of chemistry education students in the digital age

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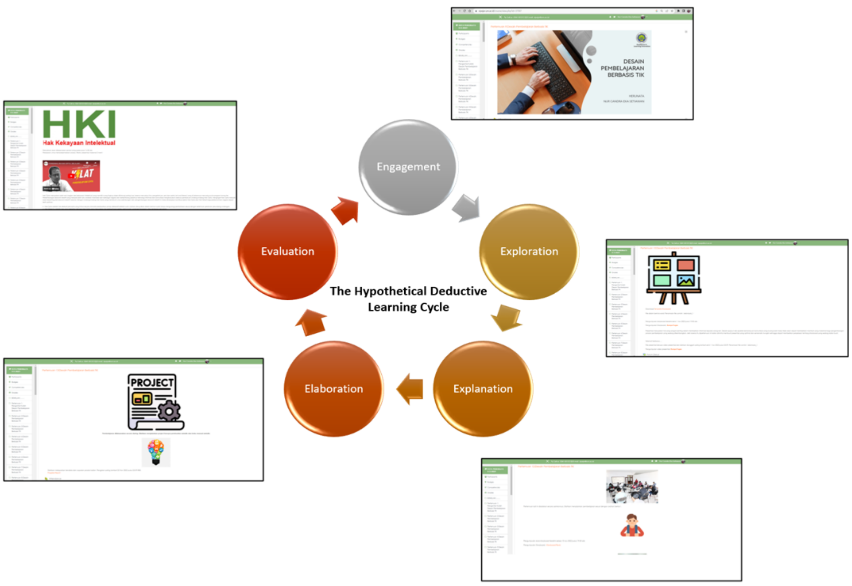
Abstract

The creativity of chemistry education students in the digital era is an exciting topic for discussion, given the critical role of creativity in developing innovations for prospective chemistry teachers. This study explores the creativity of chemistry education students in the digital era. The research method used is descriptive qualitative with data collection techniques through learning with hypothetical deductive learning cycle models, interviews, and observations. The research participants consisted of twenty-nine fifth-semester chemistry education students. The results showed that 70% of chemistry education students were in the creative category, and 30% were in the moderately creative category. This study provides essential information about the creativity of chemistry education students in facing the digital era, as well as challenges and opportunities that must be considered in the development of chemical education innovations in the digital era.



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Highlights

- HDLC model with digital integration enhances chemistry students' creativity.
- 70% of students exhibit high creativity through project-based digital learning.
- Student-created digital products are registered for Intellectual Property Rights.
- AR, virtual labs, and multimedia tools improve understanding of chemical concepts.

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1. Introduction

In an ever-evolving educational landscape, the digital era's emergence has significantly changed how students learn and educators teach (Wilcox *et al.*, 2017). With the rapid integration of technology into the classroom, the dynamics of knowledge acquisition have been reshaped, leading to new challenges and opportunities for creativity across multiple disciplines (Afrianto, 2018). Among these disciplines, Chemistry education is an essential field of study, as it is essential in nurturing the next generation of scientific minds and problem solvers (Pagliaro, 2019). As the world becomes increasingly interconnected and dependent on digital tools, students' demand for creative problem-solving skills is increasing (Astuti *et al.*, 2019; Setiawan *et al.*, 2023).

As we study creatives, we must recognize that creativity is not limited to artistic expression. Creativity refers to generating new ideas, solutions, and perspectives in the educational context, fostering critical thinking and innovation (Oppert *et al.*, 2023). Today, these creative ideas are increasingly intertwined with digital tools and technologies, influencing the way Chemistry is taught and understood (Pont-Niclòs *et al.*, 2023). The tendency of students who studied from home for two years due to the COVID-19 pandemic changed their learning chemistry (Setiawan and Rosli, 2022). Students are used to learning from home with a learning management system provided by education providers (Setiawan and Rosli, 2023). However, the measurement of student creativity is rarely carried out due to the limited instruments and measurement methods used (Haviz *et al.*, 2020).

The study of measuring students' creative abilities in the digital era is essential because digital transformation affects how students create and learn (Oppert *et al.*, 2023). This measure helps understand the role of technology in the creative process, identify students' creative potential, and improve their creative skills. In addition, the results of this measurement help refine the curriculum and encourage educators to create innovative learning environments (Akib and Muhsin, 2019). This is important to prepare students to face future challenges that are increasingly complex in technological developments (Yang *et al.*, 2021). Thus, measuring creativity in the digital era is essential for creating a generation of creative students ready to face changing times (Redhana, 2019).

Relevant learning models can optimize creativity (Weng *et al.*, 2022). One such model is the hypothetical deductive learning cycle (HDLC), which can be adopted with abstract, concrete, and algorithmic chemical concepts (Lawson, 1995). The HDLC learning model is very flexible and can be applied according to technological developments and coupled with existing digital platforms (Setiawan, 2017).

The digital age has ushered in an era of limitless possibilities for teaching and learning. Modern educational institutions integrate multiple digital platforms, software applications, and interactive learning environments to engage students innovatively (Afrianto, 2018; Turiman *et al.*, 2012). Chemistry education has also undergone a paradigm shift, with instructors utilizing digital simulations, augmented reality, online labs, and collaborative platforms to deliver engaging and interactive lessons (Khery *et al.*, 2020; Setiawan *et al.*, 2020). Consequently, students are encouraged to adopt a creative approach to their learning, connecting abstract concepts and real-world applications (López, 2022).

This study aims to determine the digital era's impact on Chemistry education students' creativity. By examining the creative abilities of chemistry education students, we seek to identify ways in which these resources influence students' creative thinking processes, problem-solving abilities, and overall academic performance. In addition, we will explore the challenges and barriers that may arise when integrating technology into the teaching of Chemistry education and potential strategies to overcome these obstacles. The findings of this study have significant implications for educators and policymakers. By understanding how the digital age influences the creativity of Chemistry education students, educators can adapt their teaching methodologies to nurture students' creative potential better. Policymakers can also use this insight to develop practical guidelines for integrating digital technologies into curricula, ensuring that technology is a catalyst for creativity rather than a distraction.

2. Experimental

This research is descriptive and uses qualitative methods. Learning occurs face-to-face and online using a learning management system (LMS). The LMS has arranged learning videos, modules, virtual discussion forums, virtual meeting links, and quizzes. All student activities are fully documented in the LMS. The learning method applied is a hypothetical deductive learning cycle of five phases (Lawson, 1995; Setiawan, 2017).

The research was conducted for five months at the end of 2022 at a public university in Indonesia. Twenty-nine undergraduate chemistry education students who took the school chemistry laboratory management program in their fifth semester participated in this study. The data collected includes questionnaires, interviews, and learning achievement. At the end of the semester, students voluntarily filled out a questionnaire about their opinion of the course, and all students answered the questionnaire. The questionnaire consists of 9 items with a Likert scale of five, from strongly agree to disagree strongly. The question focuses on the participants' views of their interests during lectures.

The interviews were deliberately structured to complement the findings in the questionnaire. Interview participants were selected to represent variations in responses from the questionnaire (Nida *et al.*, 2020). The interview was conducted for 15-20 minutes. It was recorded, transcribed, and qualitatively analyzed (Nida *et al.*, 2021). All interview excerpts are translated from Indonesian. Data is provided by participants voluntarily and handled anonymously. Collecting and handling data complies with legal requirements for empirical research ethics with humans in relevant departments. The dean of the faculty granted permission to use the data.

3. Results and discussion

Several factors should be considered in designing the right teaching approach for Information and Communication Technology (ICT) Based Learning Design courses. One way is to evaluate the appropriate pedagogical model. Among the various learning theories, the HDLC is considered the most appropriate approach to apply in learning in this class. HDLC illustrates that students actively manage the information they acquire and rearrange it to acquire and retain new knowledge. In addition, this approach emphasizes the idea that students can further develop knowledge by building on previous information and experience through a series of different activities and assessments (Barnard *et al.*, 2009; Shetu *et al.*, 2021). In the HDLC learning model, new information is presented in a way that connects and builds on previous concepts. Discussions on technical topics, practical applications, or problems related to ICT-based learning designs are held interactively. Assessments are given to test students' understanding and problem-solving skills. These strategies provide effective learning because this method emphasizes the most relevant and applicable learning concepts in the digital era. Creativity is an essential aspect of the 21st-century learning approach. In this context, Negovan *et al.* (2015) found that students in face-to-face and distance learning highly perceive learning as understanding, which includes increasing memory and knowledge and applying what is already known. The HDLC learning model applied in the ICT-Based Learning Design course supports the development of students' creative abilities because it allows them to connect new information with previous experiences and face challenges in designing innovative learning according to the demands of the times. Thus, the HDLC strategy applied in this lecture aims to maximize students' role in the learning process and equip them with critical, creative abilities in dealing with the complexity of problems in the 21st century.

The ICT-Based Learning Design course was held face-to-face for the first time after the COVID-19 pandemic, which hit the world for about two years. Based on **Fig. 1**, twenty-nine students taking this course are fifth-semester chemistry students, dominated by female students. Lectures are held for 16 weeks, and lessons use the HDLC learning model. In addition to face-to-face lectures, learning also uses a learning management system provided by the university. Utilizing this LMS provides an advantage for educators to monitor the learning process of students in real-time (Setiawan and Rosli, 2023).

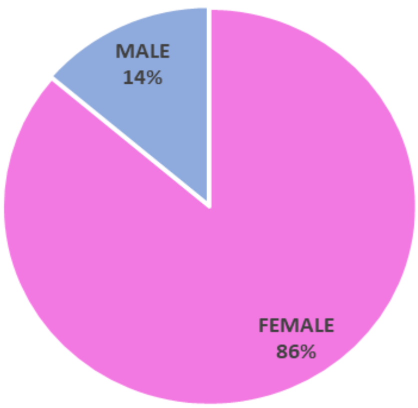

Figure 1. Gender of participants.
Source: Elaborated by the authors.

Based on **Fig. 2**, student learning achievement for one semester following an ICT-Based Learning Design course is good. As many as 14% of students got B+, 41% of students got A-, and 45% of students got A. Learning achievement is obtained from discussion scores, presentation scores, and project exams, with a weighting of 30% for discussion scores, 30% for presentation scores, and 40% for project exams.

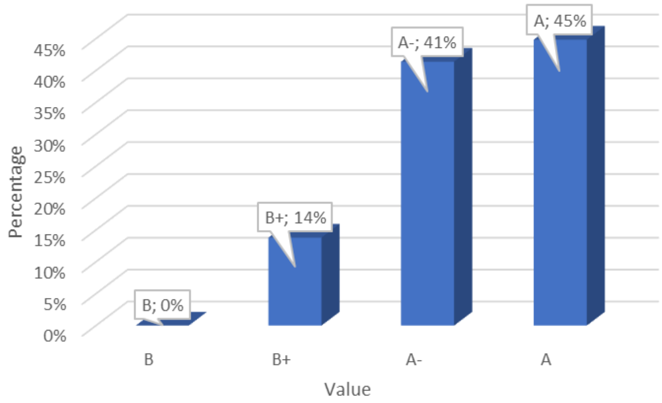

Figure 2. Learning achievement.
Source: Elaborated by the authors.

Most students (72%) agree and strongly agree with groupings in class. Grouping is done randomly, hoping that students can exchange opinions and help each other in this lecture. Only 10% of students disagree with this grouping. The results of the interviews revealed that there needed to be a better match in working together in groups. Typical examples were: "*I have invited group mates to discuss problems in making digital products, but group mates gave a slow response, so I did it myself.*"; "*My group mates are too busy with activities outside the campus, so it is difficult for us to discuss.*"

Based on **Fig. 3**, most students (69%) like learning with the HDLC model, and only 7% of students do not like it. Almost all participants (90%) stated that their creativity had increased in this lecture, and 86% of students stated that they were satisfied with the results of the products that had been made. This is supported by interview data collected at the end of the course. The analysis results show that most students stated that the part they liked the most was the project of making digital media and product presentations. A typical example is: "*I learned a lot about making digital media... In completing the project, I learned how to make moving image animations about abstract chemical concepts and create a website that looks attractive and can be integrated with smartphones. This is a new thing for me, who incidentally is a chemistry education student.*"; "*I like the product presentation session where each group presents their best work and shows the creativity of each group... this gives me ideas to develop the products I have made.*" (Setiawan, 2017) states that HDLC learning allows students to explore the potential within them. The HDLC phase allow teachers to direct students in learning concepts (Lawson, 1995). This positively impacts student learning outcomes in the ICT-Based Learning Design course.

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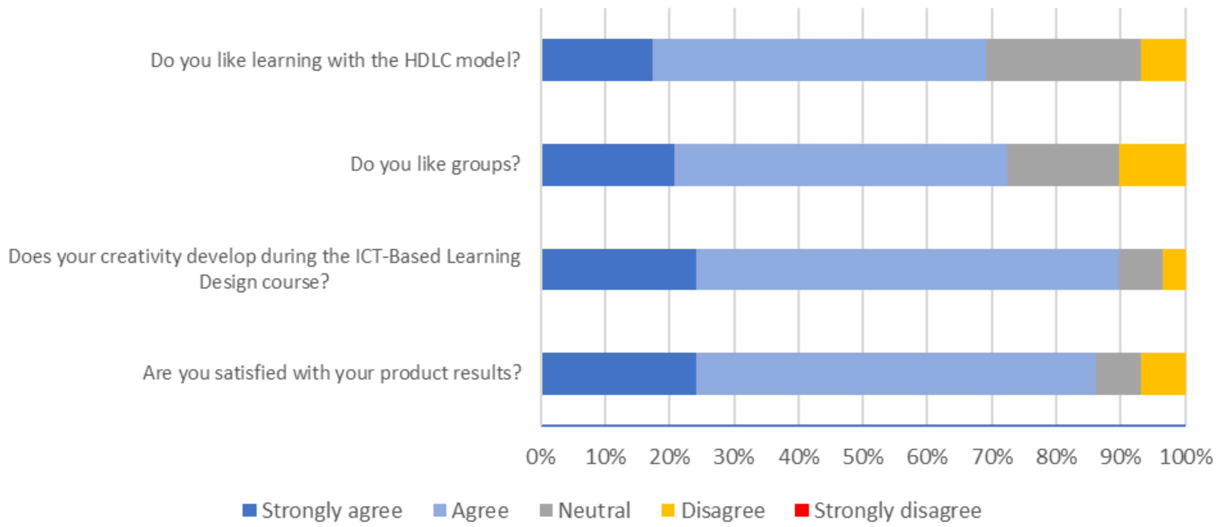

Figure 3. Students' experiences (*N*=29).
Source: Elaborated by the authors.

During the lecture, students explained the learning objectives and class activities using the HDLC learning model. The lesson plan proposed by (Setiawan, 2017) is similar in several ways. The new feature implemented in this unit is an integrated LMS recorded on a university server.

Students carry out learning activities in sixteen meetings, eight of which are asynchronous and eight synchronous, with an asynchronous time of 100 minutes each. The process of these activities can be seen in **Fig. 4**.

Figure 4. Students' experiences.
Source: Elaborated by the authors.

Students are asked to pay attention to the videos prepared in the LMS at the engagement stage. Students are asked to work in groups and discuss videos about problems in chemistry education in Indonesia. From the video, they were asked to identify the advantages and disadvantages of learning chemistry in Indonesia. Groups that have obtained the advantages and disadvantages of learning chemistry are asked to determine the parts that must be by the regulations and solutions that apply to these problems. In the exploration phase, the results of group discussions were responded to by other groups in online discussion forums (chat). Each group will argue with each other about the concepts they understand. The group will test the hypothesis and try to solve the problem by making digital learning media. In this phase, student creativity will be optimized because they must be able to create digital learning media that are in line with current developments. At the explanation stage, each group tested digital learning media on a respondent, so they knew the advantages and disadvantages of digital media products. This data will be used to revise digital media products. At this stage, the lecturer provides reinforcement and synchronization of the concept of chemistry learning media. Each group presents digital learning media products in class in the elaboration phase. This is where creativity between groups will be pitted. The digital media products offered will be discussed in class, and suggestions for further development will be obtained. The last phase is the evaluation phase, where the lecturer will assess the product through presentations and interviews (**Table 1**). The evaluation criteria are product authenticity, accuracy of chemical concepts, suitability of digital media, and fluency and flexibility in discussions.

From the learning results presented in **Fig. 5**, it is known that students' presentation abilities show promising results. The digital media products are of good quality, and all digital media products are registered to obtain Intellectual Property Rights (IPR). **Figure 6** shows that 70% of students have creativity criteria, and only 30% have creative enough criteria. The interviews showed that the students enjoyed the lectures and felt facilitated by them so that their creativity could develop optimally. One example: "*I feel happy with this lecture because I can create a digital application that can be useful for learning. Besides that, this application can be widely marketed and gain profit for the developer. My creativity is maximized in designing the application so that readers do not get bored looking at the application; There are many challenges in developing the digital media that I make, so my creativity is tested to give maximum results*".

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Table 1. HDLC in ICT-based Learning Design courses.

Phase	Description	Activities	Creativity
Engagement	Arouse students' initial understanding and study problems in the world of chemistry education	• The lecturer pointed out the problems faced in education, namely the problem of students' difficulties in learning chemistry. • Students make temporary guesses or hypotheses on existing problems. • Students look for solutions to problems set in their groups.	• Be creative in solving the problems presented in the video
Exploration	Problem-solving exploration	• Students design a problem-solving design according to the problems set by the group. • The study of making digital media suitable for each group. • Make digital media according to the design that has been made.	• Creative in designing problem solving
Explanation	Apply the product that has been made	• Digital media was tested on one respondent, and feedback was received for further improvement and development. • Digital media is improved according to the suggestions of the respondents.	• Creative in digital media application
Elaboration	Got a question? Get connected and participate in classes.	• Presentations made in class. • Discussion of the advantages and disadvantages of digital media. • Making videos for digital media products. • Manufacture of manuals for digital media products.	• Creative in making digital media product videos
Evaluation	Assess your understanding of the problems uploaded through the exam	• The assessment was conducted by interviewing the results of making digital learning media products.	• Creative in making digital media

Source: Elaborated by the authors.

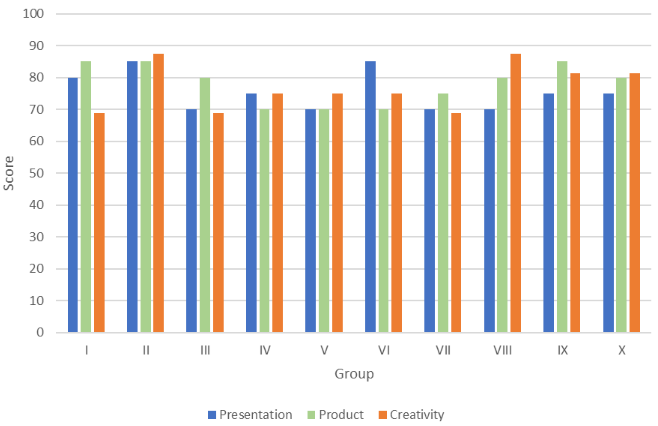

Figure 5. Scores of presentation, product and creativity assessment results.
Source: Elaborated by the authors.

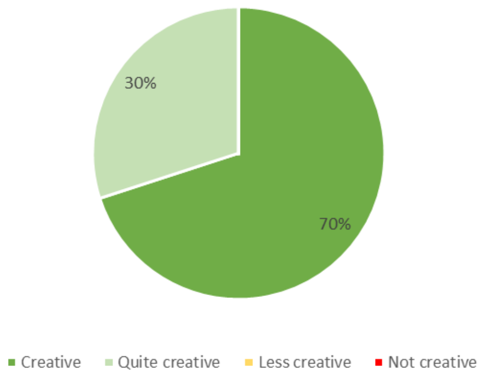

Figure 6. Percentage of creativity.
Source: Elaborated by the authors.

In the explain phase, there are limited trial activities for respondents. This is a new activity for chemistry education students because the product is directly tested on respondents. This experience makes students develop quality products. The results of the interviews show that with this provision in place, they prepare digital media seriously for fear of disappointing the respondents. An example of an interview result: "*I prepared this extra better because I was worried that the respondent would reproach me if the product were bad.*" The elaboration phase requires groups to present digital media products in front of the class. This stage is the stage that students look forward to the most because they can present their product results in front of the class by showing videos of digital media products. Students' creativity in making videos of digital media products is dominant in this phase. The final phase is evaluation; in this stage, students present the final product to the lecturer. The presentation includes product videos, digital media products, manuals, and a statement letter for filing Intellectual Property Rights regarding digital media products.

Research has demonstrated that learning in this manner can be remarkably advantageous for students, as it has been shown to amplify their creativity (Williamson *et al.*, 2015). Students can foster their creative aptitude by furnishing a properly conditioned learning environment (Ojha, 2016; Tubb *et al.*, 2020). Notably, digital learning media products have been found to impact student creativity significantly, as evidenced in **Fig. 7**. Embracing this form of learning can unlock each student's creative potential to its fullest extent (Thompson, 2017).

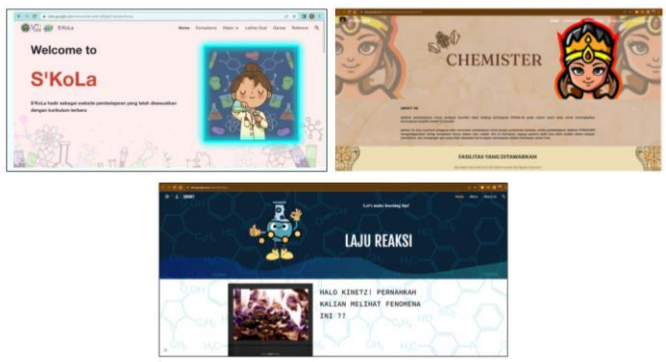

Figure 7. Digital learning media products.
Source: Elaborated by the authors.

4. Conclusions

This research provides insight into the intersection between technology and creativity in the context of chemistry education that is developing in the 21st century. The results showed that 70% of chemistry education students had creative criteria, while 30%

had creative enough criteria. Through the integration of technology and the development of creativity, students are expected to be able to develop competencies that are relevant to the times, support scientific progress, and contribute to solving complex problems faced by global society.

Authors' contribution

Conceptualization: Nur Candra Eka Setiawan; **Data curation**: Nur Candra Eka Setiawan; **Formal Analysis**: Nur Candra Eka Setiawan; **Funding acquisition**: Nur Candra Eka Setiawan; **Investigation**: Nur Candra Eka Setiawan; **Methodology**: Nur Candra Eka Setiawan; **Project administration**: Nur Candra Eka Setiawan; **Resources**: Nur Candra Eka Setiawan; **Software**: Herunata Herunata; **Supervision**: Mohd Shafie Rosli; **Validation**: Herunata Herunata; **Visualization**: Nur Candra Eka Setiawan; **Writing – original draft**: Nur Candra Eka Setiawan; **Writing – review & editing**: Nur Candra Eka Setiawan

Data availability statement

The data will be available upon request.

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Conflict of interest

The authors declare that there is no conflict of interest.

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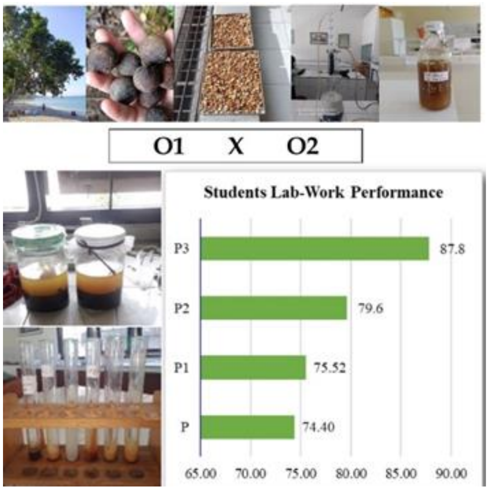
Improving student chemistry laboratory performance through Nyamplung ethnoscience-oriented learning of the *Sasak* tribe

Yusran **Khery**[1,2], Aliefman **Hakim**[1+], Joni **Rokhmat**[1], Aa **Sukarso**[1]

Abstract

Evaluating student performance in open-ended laboratory settings presents challenges compared to the structured format of typical lab exercises, which often resemble recipes. This study aims to enhance student performance by integrating local ethnoscience into chemistry education, using the Nyamplung tree (*Calophyllum inophyllum* L.), part of the Sasak tribe's knowledge, as teaching material. The study employed a pre-experimental pretest-posttest design with 17 chemistry students participating in three lab sessions. All course participants were selected as research subjects using purposive sampling. Performance was assessed through portfolios, and the N-gain method was used to analyze improvement. Results showed consistent performance increases, with scores rising from 75.52 to 87.76 over three sessions and N-gain values indicating positive but low-category improvement (0.015, 0.070, 0.181). These findings suggest that integrating ethnoscience-based materials can improve student performance while offering a culturally relevant learning experience.



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Keywords

1. ethnoscience integration;
2. chemistry education;
3. student performance;
4. Nyamplung tree (*Calophyllum inophyllum L.*);
5. *Sasak* indigenous knowledge.

Section Editors

Habiddin Habiddin
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Highlights

- Innovation skills and chemical concept mastery are linked to student learning.
- Positive correlation between Motivation, teaching strategies, and curriculum design.
- To uncover how innovation/chemistry education intertwines, 31 studies were revised.
- Innovative teaching methods enhance innovation skills and concept mastery.
- Comprehensive insights into the impact of

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1. Introduction

As an archipelagic country rich in multicultural diversity, Indonesia must preserve these values by maintaining the nation's noble heritage. National education aims to develop students' potential by transferring cultural values and excellence, helping them become stewards and innovators of the nation's culture (Ministry of Education and Culture, 2012). Through education, students learn, study, and develop cultural values and practices from the past, adapting them to modern society and the world the student live in (Fasasi, 2017a; Kusniati, 2012; Okechukwu *et al.*, 2014; Rist and Dahdouh-Guebas, 2006). Students can inherit and develop culture effectively if their knowledge, intellectual abilities, attitudes, habits, and social skills provide a strong foundation for their growth as individuals and members of society (Lee *et al.*, 2012; Tauro *et al.*, 2021).

Schools teach various subjects based on the applicable curriculum, including chemistry. Chemistry education helps students understand facts, solve problems, use laboratory skills, and behave scientifically. However, chemistry education often focuses too much on pure science, leaving students unable to see science as part of a broader system that integrates with the environment, technology, and society (Fitria and Widi, 2015). Integrating ethnoscience into chemistry lessons can give students a holistic understanding of how scientific principles connect to their cultural and environmental context (Hastuti *et al.*, 2019; Pieter and Risamasu, 2024). This approach not only enhances students' appreciation of the relevance of science in everyday life but also fosters critical thinking by linking indigenous knowledge to scientific concepts (Yazidi and Rijal, 2024; Zidny and Eilks, 2018).

Culturally oriented learning is crucial, particularly in connecting students to their local environment and heritage. One approach to culturally oriented learning is ethnoscience, which studies knowledge systems derived from cultural practices and natural phenomena. Ethnoscience provides valuable insights into how communities understand the world around them. For example, the Sasak tribe of Lombok has developed unique ethnoscientific knowledge that can be integrated into science education (Dewi *et al.*, 2019). Traditions such as the Sasak house of Sade village, mopping with cow dung, the Bau Nyale festival, "*sesek weaving, gendang belek, poteng reket, and dilah jojor"* offer rich learning opportunities (Hikmawati *et al.*, 2020; Mashami *et al.*, 2023). Among these, one particularly unexplored yet promising ethnoscientific resource for science education is the Sasak community's use of the Nyamplung plant.

Nyamplung in Sasak Society is utilized in three main aspects. First, as raw material for making *dile jojor*. *Dile jojor* Nyamplung is a type of torch whose raw material is made from Nyamplung fruit and is part of the Maleman tradition of the Sasak community (Hayadi, 2021; Khery *et al.*, 2022; Mashami *et al.*, 2023). Traditional bamboo torch lighting equipment generally uses kerosene (fossil fuel) which is not renewable, while Nyamplung oil can be renewed. This shows that Nyamplung seed oil holds potential as a renewable fuel. Many studies have studied the potential of Nyamplung seed oil into biodiesel and biofuel (Ansori *et al.*, 2019; Chasani *et al.*, 2015; Dewajani *et al.*, 2016; Fadhlullah *et al.*, 2015; Handayani *et al.*, 2017; Kurniati *et al.*, 2018). Making biodiesel from Nyamplung oil generally applies a transesterification reaction (Musta *et al.*, 2017; Muhammad *et al.*, 2017; Sudrajat *et al.*, 2007). The possibility of chemical and natural catalyst modifications is also studied to optimize the transesterification reaction of Nyamplung oil (Dewajani *et al.*, 2016; Enggarwati and Ediati, 2013; Juwono *et al.*, 2013; Muhammad *et al.*, 2017; Qadariyah *et al.*, 2017).

Second, *Sasak* people use Nyamplung seed oil to heal and remove scars (Khery *et al.*, 2022). The oil in its seeds can treat skin pain and grow hair and other diseases. In Central Kalimantan, People are accustomed to using the yellow sap of anchor dragon rods (*C. inophyllum*) when they are attacked by scabs or itch. Meanwhile, the Dayak Suru community uses it as a dye (Emilda, 2019). In Chinese society, this plant is commonly used to treat eye pain, rheumatism, inflammation, and wounds (Kainuma *et al.*, 2016; Safrina and Murtini, 2021). Third, Nyamplung is planted or allowed to grow by Sasak communities on the banks of rivers and beaches. This is because they realize that with the Nyamplung plant, riverside erosion and coastal aberration can be prevented (Khery *et al.*, 2022). Nyamplung can be a soil-fattening agent to make the soil around it grow more fertile (Khamidah and Darmawan, 2018). Nyamplung plants can live in areas with high salinity and become a composition of mangrove ecosystem plants (Danu *et al.*, 2011).

The Nyamplung plant (*Calohyllum inophyllum* L.) includes members of the family Clusiaceae. The family consists of 20 genders with 1200 species. Calohyllum comes from the Greek words 'kalos' –beautiful and 'phullon'– leaf. It means beautiful leaves (Hegde and Warrier, 2015). In Indonesia *Calohyllum inophyllum* L is known as Camplung, Nyamplung, Bintanguru, Benaga, Bintangur Laut, Menaga, Naga, and Penaga Anchorkar. Nyamplung is a medium to large tree species. This plant has a wide distribution, ranging from Africa, India, Southeast Asia, Northern Australia, and others. It is found in almost all regions in Indonesia, especially coastal areas. Its habitat is 0 to 200 meters above sea level, with rainfall between 1,000-3,000 mm/year. Nyamplung grows in non-swampy habitats and sandy beaches. The tree is dark, leafy, between 10-30 m high. It generally grows rather bent or even parallel to the ground. Has a white or yellow sticky sap (Danu *et al.*, 2011; Emilda, 2019). Nyamplung plant characteristics are shown in **Fig. 1**.

The ethnoscience of Nyamplung has the potential to be developed into a science learning topic to improve student performance. Performance in science learning is the skill in observing, recording data and information, understanding the instructions, taking measurements, implementing procedures and using equipment, making predictions and inferences, selecting procedures, designing investigations, conducting investigations, and reporting on the results of investigations (Asy *et al.*, 2017; Khery *et al.*, 2013; Nufida *et al.*, 2020). Science learning includes learning science competencies, namely identifying scientific issues, explaining scientific phenomena, and using scientific evidence; learning science knowledge competencies, namely learning using content knowledge, using compiling knowledge, and using epistemological knowledge; learning the context of science, namely the application of science in everyday life; and learning the attitude of science, which is learning to show an attitude towards science which plays an important role in decision making (Rubini *et al.*, 2016). Topics exploring natural materials are perfect for project learning (Hakim and Jufri, 2018). Through investigating and exploring natural materials, project learning can improve process skills (Wildan *et al.*, 2019) and critical thinking (Hakim *et al.*, 2016).

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Figure 1. Characteristics of Nyamplung **(a)** Trees, **(b)** Flowers, **(c)** Leaves, Fruits, **(d)** Seed Flesh and **(e)** Nyamplung Seed Oil (*Calophyllum inophyllum L.*).
Source: Elaborated by the authors.

Integrating science with local knowledge is crucial for preparing prospective science teachers, as it allows them to engage in authentic and open investigations of local knowledge. Hsu *et al.* (2009) suggest that when implemented authentically, scientific inquiry involves students in ways that closely mirror the practices of professional scientists. Bruck *et al.* (2008) support the practical application of open inquiry at both school and university levels. In open inquiry, learning occurs through investigation, with the instructor posing a question or problem and providing relevant background information. This approach is particularly suited for preparing future science teachers, emphasizing research skills essential for developing teaching materials and strategies. Employing open inquiry can enhance prospective science teachers' abilities to conduct independent investigations and scientifically validate local knowledge (Parmin *et al.*, 2022, 2016). However, many aspects of local knowledge in Indonesian society remain scientifically unverified, posing challenges to their integration into science education.

Engaging in open investigations to explore local knowledge can enhance creative thinking skills among prospective science teachers, enabling them to effectively design and implement such methods in their teaching activities (Hikmawati *et al.*, 2020; Sudarmin *et al.*, 2019). Chinn and Malhotra (2002) emphasize that open investigations are crucial for fostering the creative thinking employed by scientists. Lee *et al.* (2012) further support this, noting that student involvement and motivation in science learning are significantly improved through the application of open inquiry. Zion (2008) asserts that university-level learning activities should equip students with skills in sourcing scientific information, developing experimental techniques, and conducting independent scientific investigations. Instructors should guide students in formulating preliminary conclusions and structuring learning activities to closely resemble expert research practices. Sadeh and Zion (2012) identify the investigative component of open inquiry as the most complex, as it requires students to design their investigative processes.

However, development research that utilizes and explores the ethnoscience of Nyamplung plants in learning to improve students' scientific performance still needs to be carried out. Validation and design of teaching materials, as well as evaluation of the effectiveness and reliability of the application of learning to the performance of science, need to be evaluated. This article will describe aspects of science learning that can be obtained from Nyamplung ethnoscience. This article can be a recommendation for preparing teaching materials or worksheets for students towards Nyamplung ethnoscience-oriented learning, which aims to improve students' performance.

The lack of student engagement and declining performance in chemistry laboratories is a growing concern, particularly when traditional learning methods are used. Many students struggle to see the relevance of scientific concepts to their everyday lives, leading to disengagement and poor performance. Furthermore, conventional approaches often fail to incorporate cultural contexts that could enhance students' connection to the material. This study addresses these issues by integrating culturally relevant materials into chemistry education. Specifically, it explores the use of the Nyamplung plant, an element of the Sasak tribe's indigenous knowledge, in laboratory sessions. Ethnoscience offers an authentic way to engage students by linking scientific inquiry with local cultural practices, fostering deeper learning and motivation. This research aims to determine whether incorporating Nyamplung ethnoscience into laboratory activities can improve student engagement and performance. This research suggests that incorporating Nyamplung ethnoscience may enhance student performance and provide a meaningful connection between scientific theory and the cultural environment. This study contributes 1to science education and preserving indigenous knowledge, offering a practical model for improving student outcomes in culturally diverse contexts.

2. Methodology

This research employed a pre-experimental design with a pretest-posttest non-control group, as shown in **Fig. 2** (Cohen *et al.*, 2007; Sugiyono, 2010). This design was chosen because this study is a limited trial involving a small but sufficient subject to answer the research problem.

O1	X	O2

Figure 2. Scheme of pretest-posttest non-control group design.
Note: O1: Students initial laboratory performance; **X:** Nyamplung ethnoscience-oriented learning; **O2:** Students' laboratory performance after treatment.
Source: Adapted from Sugiyono (2010).

The study involved 17 students participating in the chemistry of natural product laboratory session, the study program of chemistry education, Mandalika University of Education, Mataram, Lombok Island, Indonesia. The research sample was taken in a saturated manner in purposive sampling by involving all

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the students participating in the natural products chemistry course as the subject of the study. The subject is divided into six groups of two or three students to facilitate observation during learning activities. The learning takes place from April to May 2023. The activities carried out consist of three themes as presented in **Table 1**.

Table 1. Laboratory activity's theme.

Laboratory session	Code	Theme
Initial session	P	Student performance in previous laboratory activities
Laboratory session 1	P1	Nyamplung seed extraction
Laboratory session 2	P2	Secondary metabolite screening of Nyamplung seed extract
Laboratory session 3	P3	Sunscreen activities evaluation of Nyamplung seed extract

Source: Elaborated by the authors.

Participants in an open laboratory inquiry environment are afforded considerable autonomy. Students can select and experiment with various extraction methods, phytochemical screening techniques, secondary metabolite analyses, and methods for evaluating sunlight-blocking activities.

Student laboratory performance during P1, P2, and P3 was assessed through performance observation and portfolio evaluations. Observation methods and portfolio assessments evaluate student performance. The things assessed are presented in **Table 2**. Performance assessment items are observed according to the science process skills Subali (2009) suggested. Each item was scored as 1 (achieved) or 0 (not achieved).

Learning activities during P1, P2, and P3 process consist of three major activities **Attempt direction (A)**, students exploring information (A1), defining problems (A2), and constructing hypothesis (A3); **Designing and executing projects (B)**, students create an observation plan (B1), collect observational data (B2), and drawing up conclusions (B3); **Discussion (C)**, students presenting products (C1); product excellence argumentation (C2); argue the environmental and social impact of products (C3). Initial laboratory performance (P) obtained from the assessment of previous laboratory session activities. The student used similar standards of reciprocal assessment to those of researchers on P1, P2, and P3. The data was collected by employing six observers who monitored each group of students during the laboratory sessions. Student performance was analyzed using percentage (**Eq. 1**), average (**Eq. 2**), and N-gain (**Eq. 3**) formulas as presented below (Cohen *et al.*, 2007).

$$P\% = \frac{score}{score\ max} \quad (1)$$

$$Average = \frac{Sum\ of\ all\ observations}{Total\ number\ of\ observation} \quad (2)$$

$$N - gain = \frac{postest\ score - pretest\ score}{maximum\ score - pretest\ score} \quad (3)$$

The score and N-gain are interpreted consecutively by category as presented in **Tables 3** and **4**.

Table 2. Students' performance assessment items.

Item observed	Activities assessed	
	Observation	Portfolio
Students gather information from a variety of sources		A1
Students make a summary		A1
Students set investigative / research variables		B1
Students raise the background of the importance of conducting investigation/research		A2
Students formulate investigation/research objectives		A2
Students make hypotheses		A3
Students select appropriate variables, collect relevant data, and select a form of presentation of results appropriate for a chosen investigative procedure		A3, B2
Students document pictures of observation objects		B2
Students present observations in a chart, graph, or histogram	C1	B2
Students compile and complete an investigative procedure		B1, B2
Students prepare units/measuring devices to take measurements	B2	
Students take measurements according to the measurement scale	B2	B2
Students use the appropriate measuring instruments correctly	B2	
Students make observations and collect data with measuring instruments	B2	
Students choose laboratory equipment that is following the task at hand	B2	
Students adopt laboratory procedures by minimizing risk		B1
Students move materials/materials/equipment using the right way/container	B2	
Students separate substances based on their form	B2	
Students do sample preparation	B1	
Students make/mix materials according to certain standards/concentrations	B2	
Students maintain work safety using glassware and hazardous chemicals	B2	
Students make observations and collect data using the five senses	B2	B2
Students convert units from a legible measure into another quantity		B2
Students recognize objects based on their characteristics	B2, B3	B2, B3
Students identify objects to match specific references/reading sources	B2, B3	B2, B3
Students identify similarities/differences between objects	B2, B3	B2, B3
Students match an object with a variety of visible characteristics	B2, B3	B2, B3
Students make reasonable generalizations/conclusions based on observations		B3
Students use observations to confirm or prove errors/refute existing hypotheses		B3
Students distinguish between observations and references/literature sources		B3, C1
Students generating ideas and conduct investigations related to everyday life		C3
Students formulate the benefits of investigation for the environment and society and promote innovation		A2, C2, C3
Students present observations in group discussions	C1, C2	C1, C2
Students demonstrate the excellence of their product/investigation results and pursue ideas to conduct new product	C1, C2, C3	C1, C2, C3

Source: Elaborated by the authors.

Table 3. Students' performance category.

Score	Category
80—100	Excellence
60—79.99	Good
40—59.99	Poor
20—39.99	Fail

Source: Adapted from Arikunto (2016).

Table 4. N-gain category.

N-gain	Category
g>0.7	High
0.3≤g≤0.7	Medium
G<0.3	Low

Source: Adapted from Selis *et al.* (2023).

3. Results and discussion

3.1. Student performance in ethnoscience-oriented laboratory session learning Nyamplung

In the first session of learning implementation, students are invited to make direct observations about local knowledge in the village of Gunungsari, West Lombok district, West Nusa Tenggara province, Indonesia. The students observed and met directly with the local community to learn more about using Nyamplung. The student learned that the local community used the Nyamplung plant as a barrier to the erosion of rice paddy land around the river. In contrast, the flesh of the Nyamplung seeds was used to make dilah jojor (a kind of fire lamp) and used the seed oil as a disguise for scars on the skin. The student also got information about the local community who roasted the meat of the Nyamplung seeds and then pounded and squeezed mechanically to get the oil. This information is consistent with what has been presented by (Khery *et al.*, 2022). The results of the photos captured by students while exploring the activities of the Nyamplung cultivation community in Gunungsari village are presented in **Fig. 3**.

Figure 3. (a) Nyamplung cultivation community; **(b)** collect old Nyamplung seeds in Gunungsari.
Source: Elaborated by the authors.

Laboratory activities carried out by all class participants departed from the fact that Nyamplung oil was used as a scar treatment. Many research results in the field of chemistry support this idea. Nyamplung (*Calophyllum inophyllum* L.) contains a lot of secondary metabolite compounds, mainly the group of Santon, coumarin, triterpenoid and flavonoid compounds. Based on several studies, the bioactivity exhibited by Nyamplung is quite diverse. The chemical compounds present in Nyamplung are varied, resulting in a wide range of bioactivities. Studies exploring this bioactivity have generally been conducted in vitro (Emilda, 2019). These include antioxidant, anticancer (Raju and Victoria, 2015), antiviral, anti-HIV, anti-inflammatory, antibacterial, antidiuretic, antidiabetic activities, and more (Artanti *et al.*, 2020; Hasibuan *et al.*, 2013; Kainuma *et al.*, 2016; Oo, 2021; Ragasa *et al.*, 2015). The results of the image capture by students related to the process they carried out in their laboratory activities are presented in **Fig. 4**.

Figure 4. Students use the extraction method. **(a)** sample preparation **(b)** alcohol maceration; **(c)** soxhletization of n-hexane; **(d)** mechanical press.
Source: Elaborated by the authors.

In implementing the first laboratory session, the extraction of Nyamplung oil, six groups of students were divided into 3 groups based on the methods used to adjust the state of laboratory facilities. The extraction methods are maceration techniques using alcohol, Soxhlet extraction using n-hexane solvents, and mechanical press methods. Samples of the flesh of the Nyamplung seeds are prepared by drying to reduce moisture content and ground to improve the contact surface. The image students captured during the Nyamplung extract phytochemical test is presented in **Fig. 5**.

Figure 5. Phytochemical test observation of Nyamplung oil sample **(a)** alcohol maceration extract; **(b)** soxhletization of n-hexane extract; **(c)** mechanical press extract.
Source: Elaborated by the authors.

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In the next laboratory session, students conducted a semi-qualitative phytochemical analysis to detect the presence of secondary metabolites in the alkaloids, flavonoids, terpenoids and steroids, tannins, and saponin tests. The student's methods are presented in Khery *et al.* (2023). The tests were carried out uniformly due to the limitations of laboratory facilities. Nevertheless, the students get different observation experiences. In the last laboratory session, students conducted SPF, erythema, and pigmentation tests in the sun protection activity test. The students were divided into three groups to test their respective samples. The first and second groups use Sayre *et al.* (1979; 2013). The first group executed the method by varying the type of solvent used when testing the sample. The second group executed the method by varying the sample concentration when testing it in an alcohol-chloroform solvent (1:1). The third group used the recommended method by Rejeki and Wahyuningsih (2015).

Figure 6 shows student performance in attempting direction activities, designing and executing projects, and discussing learning during the first, second, and third laboratory sessions.

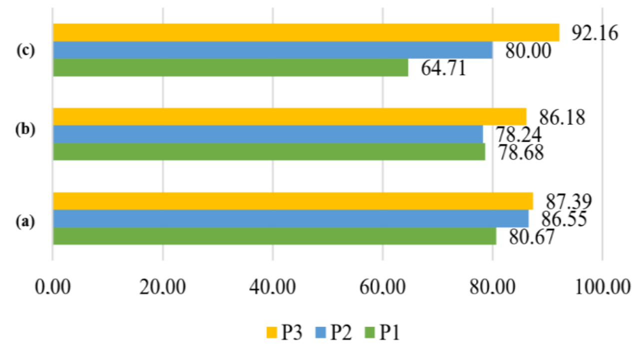

Figure 6. Student laboratory performance during **(a)** attempt direction, **(b)** designing and executing projects, and **(c)** discussion.
Source: Elaborated by the authors.

The results showed that, in general, students experienced improved performance in both attempt direction, designing and executing projects, and discussion activities. This can be caused by students getting used to the learning methods applied. The student also showed better attitudes during laboratory sessions. Ethnoscience-oriented learning does allow for increasing student interest, motivation, and learning outcomes in science learning (Fasasi, 2017b; Muliadi *et al.*, 2022; Munandar *et al.*, 2022). Increased interest and motivation will likely cause this increased performance (Lizzio *et al.*, 2002; Shakir, 2014).

A very sharp increase occurred in discussion activities. There are 64.71, 80.00, and 92.18 respectively. It enhances from good into the excellence category of performance. In this section, students present products, discuss product excellence, and argue about the environmental and social impact of products. Student performance has improved sharply, indicating that from the first laboratory session to the last, Students are increasingly confident in presenting the results of their experiments. Their orientation towards the resulting innovative product is getting better. An overview of product development ideas and how students affect the economic and social environment for the better can be presented in a better way. Discussion activities can positively influence students' motivation and critical thinking skills (Cholisoh *et al.*, 2015). Discussion activities motivated by investment and inquiry experiences result in better student motivation and perception of science learning (Gouvea *et al.*, 2022; Rahayu *et al.*, 2020).

The next interesting result of this study is the occurrence of a very small decrease in designing and executing projects during the first and second laboratory sessions. The average score in the good category is 78.68 and 78.24, respectively. This is due to the student's lack of ability to develop an investigation plan that allows for maximizing laboratory equipment or measuring instruments. The second laboratory session job is easier than the first. Previous research has shown that the more students engage in practical activities in the laboratory, the higher their motivation and learning performance in science learning (Ateş and Eryilmaz, 2011; Corter *et al.*, 2011; Sesen and Tarhan, 2013; Shana and Abulibdeh, 2020).

3.2. Improving student performance in ethnoscience-oriented laboratory session learning Nyamplung

Student performance in Nyamplung ethnoscience-oriented laboratory session learning is shown in **Fig. 7**.

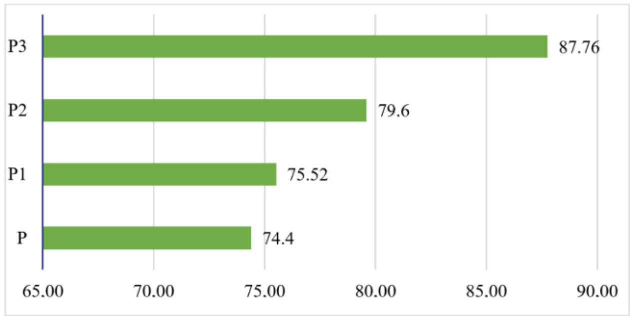

Figure 7. Student laboratory performance on the chemistry of natural product laboratory session with Nyamplung ethnoscience-oriented learning in P1, P2, and P3.
Source: Elaborated by the authors.

The results of this study show a gradual improvement in student performance throughout the Nyamplung ethnoscience-oriented laboratory sessions, with average scores increasing from 75.52 to 87.76. This demonstrates that implementing Nyamplung ethnoscience-oriented laboratory methods positively impacts student performance. The ethnoscience approach has enhanced students' attitudes toward science learning (Fitria and Widi, 2015; Muliadi *et al.*, 2022; Munandar *et al.*, 2022). These findings align with research by Zidny *et al.* (2020), which demonstrated that integrating indigenous knowledge into science education can increase student engagement and understanding of scientific processes. Pieter and Risamasu (2024) also reported that ethnoscientific learning materials enhance students' science process skills.

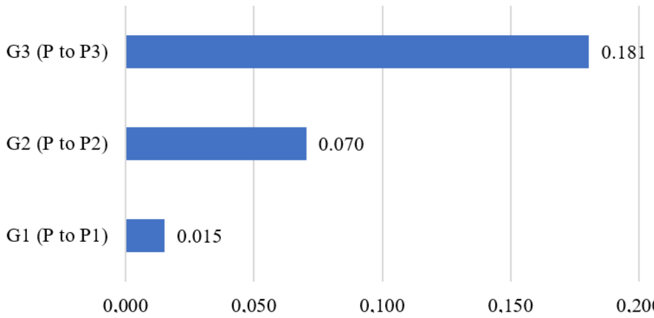

Figure 8. Student laboratory performance on the chemistry of natural product laboratory session with Nyamplung ethnoscience-oriented learning in P1, P2, P3.
Source: Elaborated by the authors.

Despite the overall improvement, the N-gain values remained in the low category, as shown in **Fig. 8** (0.015; 0.070; 0.181), indicating a moderate level of progress compared to studies like Hastuti *et al.* (2019), where medium N-gain scores were achieved. This suggests that while ethnoscience-based learning has positive effects, certain contextual or instructional factors may have limited the extent of improvement. These differences indicate that the ethnoscience approach offers clear benefits. Still, adjustments, such as extending the learning period or providing additional support, might be needed to enhance students' science process skills fully.

Previous research conducted by Ibe and Nwosu (2017) and Mudana (2023) emphasized the importance of active student involvement in ethnoscientific learning environments, which leads to significant gains in reasoning and inquiry skills. The findings of this study, particularly the improvement in discussion activities, support these conclusions, as students demonstrated a greater ability to articulate the scientific and societal relevance of their work. However, the lower gains in investigative skills, such as designing and executing projects, suggest challenges that may not have been as evident in previous research. These challenges could be due to the relatively short duration of the laboratory sessions or the novelty of the Nyamplung ethnoscience approach for students.

The application of Nyamplung ethnoscience-oriented learning improved student performance and supported the preservation of local knowledge and sustainable practices. Integrating indigenous knowledge into the curriculum helps preserve cultural heritage while promoting sustainability. For instance, students explored the potential of Nyamplung seed oil, traditionally used in the Sasak community, as a renewable energy source during the laboratory sessions. This connection between local knowledge and modern scientific inquiry enriches the curriculum and prepares students to address real-world problems. Zidny *et al.* (2020) argue that science education should aim to offer a balanced perspective by introducing students to diverse ways of understanding science. Incorporating indigenous knowledge broadens students' perspectives, enabling them to understand scientific concepts more holistically and recognize the influence of social and cultural contexts in shaping scientific knowledge (Zidny and Eilks, 2018; Zidny *et al.*, 2021).

This study provides insights into the potential for broader applications in science education. While it focused on the Nyamplung plant, the ethnoscience-based learning model can be adapted to other culturally significant plants or natural resources from various regions. Emphasizing the integration of local knowledge into science education allows this approach to be applied in different educational contexts, fostering a deeper understanding of science concerning students' cultural and environmental backgrounds. Elias *et al.* (2009) and Yazidi and Rijal (2024) advocate for educational reforms that integrate indigenous knowledge as a key element in science education, emphasizing its role in achieving sustainability goals by equipping students to tackle complex environmental and societal challenges.

The proposed teaching practice, which integrates Nyamplung ethnoscience into chemistry education, is believed to offer several benefits for student learning and the broader educational context. Integrating ethnoscience into learning can enhance student engagement and motivation. Using culturally relevant materials, such as the Nyamplung plant, allows students to connect their learning to their local environment and traditions. This relevance increases student interest and motivation, as demonstrated by the significant improvement in discussion activities, where students confidently presented their work and connected their findings to environmental and social impacts. Ethnoscience-oriented learning has been shown to improve motivation (Hariyono *et al.*, 2023; Munandar *et al.*, 2023), cognitive achievement (Fasasi, 2017a), and attitude toward science (Fasasi, 2017b). According to research by Hastuti *et al.* (2019) and Saija and Tahya (2023), ethnoscience-oriented learning effectively improves students' science process skills. Pieter and Risamasu (2024) also found that this approach enhances students' scientific skill development. Additionally, Ibe and Nwosu (2017) and Mudana (2023) recommend that education stakeholders, particularly teachers, actively engage students in the learning process by incorporating ethnoscientific strategies, as these methods have proven more effective for developing science process skills.

4. Conclusions

The main findings demonstrate a consistent improvement in student performance across three laboratory sessions, with average scores increasing from 75.52 in the first laboratory session to 87.76 in the third. Although the N-gain values indicated a low level of improvement (0.015, 0.070, and 0.181), the upward trend suggests that ethnoscience-based learning positively impacts student engagement, motivation, and skill development in the laboratory environment. The most significant gains were observed in discussion activities, where students exhibited increased confidence and ability to articulate their experiments' environmental and social relevance. This study contributes to the growing body of literature on ethnoscience and its application in science education, particularly in the context of chemistry learning. By incorporating local knowledge, such as using Nyamplung seed oil, the study demonstrates how culturally relevant materials can enhance the learning experience and improve student outcomes in scientific disciplines. The study emphasizes the potential of Indigenous science to enrich science education and recommends further research in broader educational settings.

Recommendations: Teachers should consider integrating ethnoscience-based teaching materials into their curricula to foster deeper student engagement and enhance practical laboratory skills. Specifically, utilizing local knowledge not only makes science more relevant and accessible to students but also promotes the preservation of cultural heritage. To maximize the effectiveness of this approach, teachers should provide opportunities for open inquiry, allowing students to explore and apply local knowledge in scientific contexts. Future studies should consider implementing this model in larger student populations and across various cultural settings to further validate its effectiveness and broaden its application.

Limitations: Several limitations of this study should be acknowledged. **Small sample size:** The study involved only 17 students, which limits the generalizability of the results to a broader student population. While the findings are promising, a larger sample would provide more robust evidence of the effectiveness of ethnoscience-oriented learning. **Lack of control group:** As mentioned, the study did not include a control group that followed a traditional laboratory approach without ethnoscience content. This limits the ability to determine whether the observed improvements in performance were directly due to the ethnoscience-based methods or other factors, such as increased familiarity with laboratory work overtime. **Short duration:** The study was conducted over a relatively short period, with three consecutive laboratory sessions. While the results show improvement, a longer-term study would provide more insight into the sustainability of these gains and whether the student persists beyond the initial learning phase. **Cultural specificity:** The ethnoscience approach is closely tied to the cultural knowledge of the Sasak tribe. While this is a strength in promoting local knowledge, it also limits the transferability of the approach to other regions with different ethnoscience traditions. A more diverse exploration of ethnoscience from multiple cultures could provide a broader application of this teaching method.

Authors' contribution

Conceptualization: Aliefman Hakim; Yusran Khery; **Data curation**: Yusran Khery; **Formal Analysis**: Yusran Khery; Aliefman Hakim; Aa Sukarso; **Funding acquisition**: Alieman Hakim; **Investigation**: Yusran Khery; **Methodology**: Yusran Khery; Joni Rokhmat; **Project administration**: Yusran Khery; **Resources**: Not applicable; **Software**: Not Applicable; **Supervision**: Aliefman Hakim; **Validation**: Joni Rokhmat; Aa Sukarso; **Visualization**: Yusran Khery; **Writing – original draft**: Yusran Khery; **Writing – review & editing**: Yusran Khery; Aliefman Hakim.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Enhancing student motivation in reaction rate topics through the integration of Instagram-based learning media and the Student Teams-Achievement Divisions cooperative model

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Abstract

Instagram-based learning media can be applied in chemistry learning because it can be accessed at any time, it is not limited by distance or time if you have an internet network and adequate hardware and can increase student learning motivation. This study aimed to ascertain whether applying the Instagram-integrated Student Team-Achievement Divisions learning model and multi-representation strategies enhances student motivation on reaction rate topics. The Nonequivalent Control Group Design with the Quasi-Experimental technique is the research design. Using a random sampling technique, students from Malang Senior High School's class XI-Science were selected as the research sample. Student learning motivation data was descriptively and quantitatively examined, and statistical tests, such as prerequisite analysis tests and hypothesis testing, were used to analyze data on learning outcomes. The results of this experimental research found that in the extremely high category, the percentage of students who were motivated to learn rose from 56 to 85%. These findings show the experimental class's improvement in learning motivation outperformed that of the control class.

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1. Instagram;
2. learning motivation;
3. reaction rate;
4. Student Teams-Achievement Divisions;
5. cooperative learning.

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Highlights

- Instagram-based learning can be effectively applied to chemistry education.
- STAD model integrated with Instagram enhances collaborative student learning.
- Multi-representation strategies boost motivation in learning reaction rates.
- Student motivation increased significantly, from 56% to 85% after the treatment.

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1. Introduction

Education stands as a cornerstone in the development of individuals, crucial for fostering intelligence, honing skills, nurturing character, fortifying personality, and fostering a sense of communal unity, thereby contributing to the nation's advancement (Saptono, 2017). This field gets more attention because the country's quality of education is used as a benchmark for the country's progress. In the era of digitalization, it brings many positive impacts on people in various sectors of life, especially in education. According to Fadlillah (2016), the field of education has experienced significant developments in its direction, which is now not only limited to "education" but has led to "edutainment". These two approaches essentially do not change the nature of the teaching and learning process in the classroom, but there are differences in planning, strategies, techniques, and teaching methods (Afif, 2019). Furthermore, the contemporary landscape offers an array of diverse learning outlets, with the advent of numerous mass media platforms, notably the Internet and electronic media, emerging as vital reservoirs of knowledge and educational hubs. This will impact on teachers who are no longer the only primary source of knowledge in teaching and learning activities. This change will also create a new paradigm for educators regarding the use of time that must be as efficient as possible to facilitate students' maximum learning outcomes. This can be achieved if educators can make a teaching innovation, i.e., balanced with the increasing quality of teachers (Harsanto, 2014).

Apart from the educators, this all-digital era will also impact students who are born as "digital native generations", a term for the generation who was born, grew up, grew up and interacted with various kinds of digital media. This condition may impact the psychological aspect, which affects the cognitive map. According to Widianingsih (2019), students' needs, changes and habits tend to follow what they see through the media they see and use the most. The students also have broad opportunities to operate in the digital world anywhere and anytime if there is an internet network and adequate hardware. With this opportunity, from now until the future, education can be implemented without being limited by space and time. This transformation will change the strategy in teaching from conventional media to digital-based so that educators will find it easier to manage classes in learning. Thus, student learning activities in the digital era will find a structured and sustainable pattern with a predetermined curriculum flow.

The "digital native" generation has a great opportunity to utilize the digital world in education. With the digital world that can be accessed anytime and anywhere and the trend of internet and mobile phone developments, this generation has great potential to learn to explore their potential independently anywhere and anytime. Although students learn independently, educators remain responsible for guiding and facilitating students. Learning that is carried out independently needs to be directed to stimulate a sense of responsibility and creativity and build logical and critical thinking skills. According to Wibowo (2018), independent learning autonomy is rooted in regulated learning, which emphasizes the individual's capacity to autonomously regulate their learning journey through various modalities, to achieve peak academic achievement. In addition, educators need to develop independent learning by choosing the right and creative learning model that emphasizes the activities of students as actors in learning tasks. This creative learning activity is expected to foster new creativity in students' thinking, feelings, and attitudes. In the digital era, choosing a diverse and creative learning model is about acquiring skills and fostering social cohesion and national unity with a spirit of inclusivity (Anwar, 2004). These models serve as a foundation for educational practice, derived from theories in educational psychology and learning, which are carefully crafted to guide curriculum implementation and its practical implications in the classroom environment. Thus, to encourage a spectrum of learning experiences, creativity in designing various learning models becomes a must (Warsono, 2013). This is supported by the current state of the education world, which is changing the traditional paradigm to include classroom-based learning, especially the lecture method. As a result, there is a need for alternative methodologies, among which the cooperative learning model stands out as a promising path.

Cooperative learning emerges as a pedagogical approach wherein students collaborate towards shared objectives. This structured method aims to enhance student engagement, foster leadership qualities, encourage decision-making within groups, and promote cross-cultural interaction and learning (Afandi *et al.*, 2013). Extensive research underscores the manifold benefits of cooperative learning, transcending mere academic achievement. It cultivates self-assurance, nurtures interpersonal skills, and fosters mutual trust among peers, both individually and collectively. Moreover, it nurtures a culture of assistance and collaboration among classmates, eschewing individual competition and fostering a collaborative ethos within the classroom. There are several techniques in the cooperative learning method, including jigsaw, Student Teams-Achievement Divisions (STAD), NHT (Numbered Heads Together), and others. This cooperative learning is suitable for use in all subjects. One of the subjects taught in this era was chemistry. Chemistry is one of the science subjects that is usually taught in high school.

Chemistry is a science discipline that studies matter's structure, properties, and transformation (Artini and Wijaya, 2020). Chemistry subjects in high school provide a comprehensive understanding of substances, covering aspects such as composition, structure, properties, changes, dynamics, and energetics, all explored at the molecular level (Astuti, 2020). Teaching chemistry lessons in schools can improve thinking skills and stimulate the creative thinking patterns of students (Rachman and Ahsannunisa, 2017). The large scope of material studied in chemistry makes it a complex and not simple subject, making students' views of it negative. Many students often complain and consider this subject difficult to learn. Students' difficulty in understanding chemistry learning is because it is abstract and complex concepts that require a deep understanding to study it (Sariati *et al.*, 2020). In addition, chemistry is also often considered a difficult and boring subject by most students (Muderawan *et al.*, 2019). Some of the difficulties experienced by students in studying chemistry tend to be caused by students not knowing how to learn, having difficulty connecting between concepts, and requiring the ability to utilize logic, mathematics, and language skills (Zakiyah *et al.*, 2017). In learning chemistry, students may have difficulty learning complex chemistry materials and use a lot of mathematical calculations to solve problems. One of the chemistry materials often considered difficult to understand is the reaction rate material. Based on research conducted by Marthafera *et al.* (2018), it is stated that the low understanding of students' concepts on the reaction rate material is caused by several factors, one of which is the media and teaching materials that are not by the conditions of students in the classroom so that it affects students' conceptual understanding. In learning chemistry, students have difficulty learning complex chemistry materials and use a lot of mathematical calculations to solve problems. The concept of reaction rates poses a significant challenge for students studying chemistry. Research conducted by Marthafera *et al.* (2018)

highlights that students' difficulties in grasping the intricacies of reaction rate material stem from various factors, including inadequacies in instructional media and teaching materials that fail to align with classroom dynamics, thus hindering conceptual understanding. Furthermore, students encounter hurdles when tackling complex chemistry topics, particularly those involving extensive mathematical calculations. This underscores the need for tailored instructional strategies and supportive learning environments to enhance students' comprehension and mastery of chemistry concepts, particularly those related to reaction rates.

In tandem with technological advancements, the landscape of learning media is undergoing exponential growth and diversification. A notable innovation in the digital era is the emergence of internet-based learning platforms. This surge is propelled by the prevalence of internet usage among teenagers, as evidenced by research conducted jointly by the National High School of Passwords (STSN) Indonesia and Yahoo, revealing that adolescents aged 15-19 constitute a significant portion, accounting for 64% of internet users in Indonesia. Consequently, there has been a notable shift towards using social media platforms as engaging online learning tools, particularly accentuated during the pandemic due to heightened social media usage among students (Annur, 2021). Besides, video and image-based learning innovations are interactive and interesting learning media solutions that ask students to learn during the COVID-19 pandemic (Adawiyah *et al.*, 2021). The existence of these innovations can encourage educators and students who become teachers and content creators to learn chemistry that creates interesting and easy-to-understand content for students (Maeskina and Hidayat, 2022). Media creations can be in the form of images, videos, or writing that will become complete content and be distributed through platforms, one of them being social media (Sundawa and Trigartanti, 2018).

Instagram emerges as a promising platform for educational advancement, offering a range of features conducive to learning. This application was first launched in October 2010 and experienced significant user growth after it was officially launched. As of April 2015, the number of users has reached 300 million active users (Costill, 2014). The popularity of Instagram has reached Indonesia, it was recorded that in 2019, Indonesia had the most active Instagram users (Riyanto, 2019). With a total number of active users of as many as 56 million or 20.97% of the total population of the entire Indonesian community. Instagram facilitates various features that can be developed for students to learn critically and reflect on the meaningful learning process (Chun et al., 2016). These include live streaming and facilitating direct teacher-student interaction to comprehensively monitor learning needs. Post feeds provide a convenient avenue for students to gather and submit assignments and work, fostering collaboration and engagement within the learning community. Moreover, the platform's video reels offer a creative outlet for presenting educational content in engaging video formats, enhancing comprehension and retention.

Additionally, Instagram TV is a valuable resource for students to access longer-form educational content. It enables them to explore assignments and works in video or animation formats, thus enriching the learning experience with diverse multimedia resources. This application is suitable for keeping teachers or educators connected so they can always relate to students.

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Instagram's diverse features and its great potential for integration into learning follow the opinion of Mandja (2016), who discovered a statistically significant difference between classrooms that used traditional learning methods and classes that used Instagram media. Instagram media could enhance learning outcomes suggesting motivation. The study by Widarti (2024) echoed similar outcomes, revealing a substantial improvement with a standard gain of 0.82 in the higher performance bracket. It showed a notable surge in students' learning motivation after incorporating Instagram as an integrated learning tool for independent study. These findings underscore the imperative for further investigation to elucidate the potential efficacy of implementing the Instagram-integrated STAD learning model alongside a multi-representation approach. This forthcoming research explores whether such pedagogical strategies will bolster student motivation and augment learning outcomes, particularly in chemistry, focusing on reaction rate material.

2. Experimental

2.1. Research design

The study conducted falls under the category of Quasi-Experimental research. It was carried out during the odd semester of the 2022/2023 academic year at Senior High School (SMA/Sekolah Menengah Atas) Negeri 8 Malang. Employing a random sampling technique, subjects were selected from all six XI (second year of a three-year study period) mayor Mathematics and Natural Sciences (MIPA/Matematika dan Ilmu Pengetahuan Alam) classes at SMA Negeri 8 Malang, with the control group comprised of XI MIPA 1 (consisting of 13 male and 20 female students) and the experimental group comprised of XI MIPA 2 (comprising 13 male and 18 female students). The research design adopted is a control group design, specifically a Non-equivalent Control Group Design, allowing for the inference of causal relationships. The experimental class implemented the STAD learning model supplemented with Instagram media. The same learning model is used in the control class without additional treatment, utilizing PowerPoint as the learning medium.

2.2. Research instruments and procedures

The research comprised three sessions, each lasting 2 JP or 2 x 45 minutes for both classes. Various instruments were utilized, including a questionnaire to assess learning motivation before and after the study. Additionally, learning materials such as lesson plans and student worksheets (LKPD/Lembar Kerja Peserta Didik) were employed, having undergone validation by chemistry experts from SMA Negeri 8 Malang and chemistry lecturers. Data collection encompassed both test and non-test instruments. The test instrument comprised *pre-* and *post-test questions* featuring 10 multiple-choice items with 5 response options, whereas the non-test instrument consisted of a student learning motivation questionnaire utilizing a 4-point Likert scale. The scale utilized in the learning motivation questionnaire presented statements followed by four response choices. The research measurement scale was adapted from Riduan's framework. The values given are one to four for responses that are very less agreeable, less, good, and very good, which describes a very negative position to a very positive position. The level of scale measurement in this study uses intervals.

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At the outset of the learning sessions, a non-test instrument in the form of a learning motivation questionnaire was administered to both classes utilizing Instagram and PowerPoint media. This initial step aimed to gauge the level of student motivation towards learning reaction rate material at the onset of the session. Subsequently, a pre-test was administered before the commencement of the learning process to assess students' baseline understanding of the reaction rate material. While the experimental class received Instagram media as a form of treatment, the control class utilized PowerPoint media without any additional treatment. Following the learning sessions, a post-test was administered, and a learning motivation questionnaire was distributed to both the experimental and control classes. This post-test and questionnaire aimed to evaluate the increase in motivation and learning outcomes in the experimental class, which received treatment through Instagram social media, compared to the control class. The data processing methodology for the student learning motivation questionnaire involved descriptive quantitative analysis.

3. Results and discussion

3.1. Learning implementation data

Information regarding the execution of the learning process was gathered through observations conducted throughout the instructional sessions. Two observers, observer I and observer II, were tasked with this observation process, and the resulting data is presented in Fig. 1 (results in percentage).

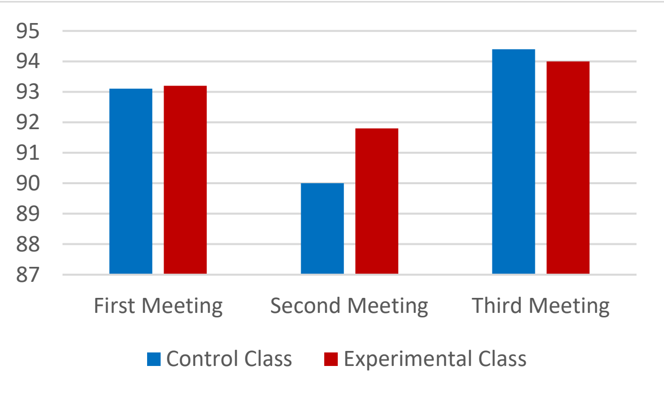

Figure 1. Histogram of Learning Implementation.
Source: Elaborated by the authors.

The assessment of learning implementation indicated that the control and experimental classes exhibited implementation rates of 92.5 and 93%, respectively. These findings suggest a minimal disparity in the execution of learning activities between the two classes. The learning implementation deficit occurred in the control class due to students' tardiness in participating in learning both during learning and discipline which was slightly less than in the experimental class, so it affected the enthusiasm for learning in the control class in contrast to the experimental class.

3.2. Implementing an Instagram-integrated STAD learning model

The integration of Instagram social media into the STAD learning model was implemented in class XI MIPA 2 at SMA Negeri 8 Malang, comprising 31 students of both genders. STAD is a cooperative learning model emphasizing student interaction to help and motivate each other. Instructions on reaction rate material occurred during three sessions per meeting, each lasting 45 minutes, throughout September 2022. The experimental and control classes utilized the STAD learning model; however, the experimental class employed Instagram media, while the control class used PowerPoint. The Instagram learning media, integrated into the @chemsquad.kimia account, offered accessibility across various devices such as laptops, smartphones, and tablets. Content included infographics and videos covering reaction rate material, with each sub-material featuring real-life examples, explanations, sample problems, and discussions. The appearance of the @chemsquad.kimia Instagram account utilized in the instructional process is depicted in Fig. 2.

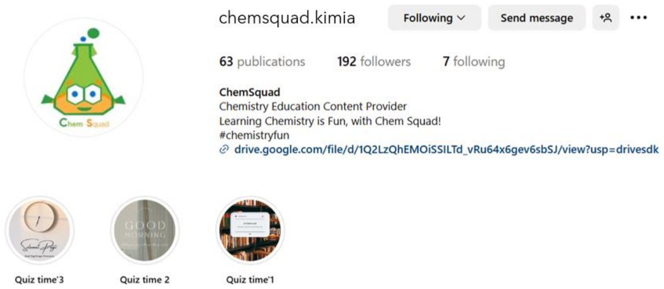

Figure 2. Depicts the front view of @chemsquad.kimia *Instagram* account.
Source: Elaborated by the authors.

The utilization of Instagram-integrated learning media in education involves several features. One such feature is the presentation of teaching media for reaction rate material in the feeds feature, illustrated in Fig. 3.

Figure 3. The presentation of material on *Instagram Feeds* Feature.
Source: Elaborated by the authors.

Users can upload infographics as images within the Instagram Feeds feature, with each post accommodating up to 10 images. Additionally, the content or material presented can include supplementary information in the form of a concise narrative, provided through a feature known as caption. Furthermore, each post includes a comment feature, enabling students to contribute comments about the material presented or pose questions to the teacher regarding any unclear concepts. This

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allows for collaborative interactions among students, fostering courage in expressing opinions in public forums and promoting independent learning during and outside formal educational settings. **Figure 4** illustrates these aspects.

Figure 4. Depicts of comment columns across various Posts.
Source: Elaborated by the authors.

The material was provided through the Instagram Reels feature, as illustrated in **Fig. 5**. This feature presents short videos with a maximum duration of 2 minutes each. The video uploading system in the reels feature differs from the feeds, allowing for a maximum of one video per upload. Despite this distinction, student interaction with teachers remains feasible, as students can comment or pose questions through the comment column.

Figure 5. Depicts the presentation of materials on the Instagram reels feature.
Source: Elaborated by the authors.

Two primary considerations in implementing learning through social media on Instagram are the potential improvements in student learning motivation, particularly regarding reaction rate material. Learning motivation is crucial in achieving student learning objectives, which originate from within each student. The level of learning motivation varies among students, influenced by numerous internal and external factors, driving students to learn for diverse reasons. Students with heightened learning motivation are more inclined to exert greater effort towards achieving their learning objectives. Conversely, the effort required to attain these goals decreases when learning motivation is low.

3.3. The impact of Instagram media on enhancing learning motivation in the experimental group

The study was centered on leveraging Instagram media to bolster learning motivation. A motivation questionnaire was administered to 31 students in the experimental class, who were exposed to Instagram as a learning medium. The questionnaire aimed to assess learning motivation before and after utilizing Instagram-based media. The effectiveness of Instagram media was evaluated based on the increased motivation levels pre- and post-exposure to the media in learning reaction rates. The learning motivation data, which comprises quantitative information, was subjected to descriptive analysis to elucidate its implications.

3.3.1. The average analysis of the student learning motivation questionnaire

Figure 6 graphically depicts the findings, showcasing the average student learning motivation results (results in percentage).

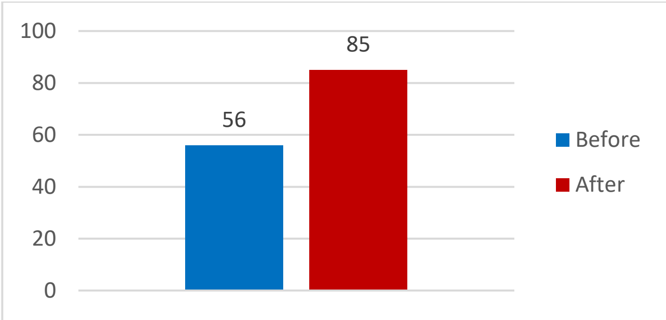

Figure 6. Illustrates the average outcomes of student learning motivation.
Source: Elaborated by the authors.

Before utilizing media, the average learning motivation stood at 56%, surging by 29% to 85% post-implementation. These results suggest a significant improvement in student learning motivation, attributed to the integration of Instagram media. This observation aligns with prior research conducted by Widyastuti and Puspitasari (2020), which reported a similar increase in student learning motivation upon utilizing Instagram as an independent learning resource. The study yielded a Z value of -3.300 with an asymp.sig (2-tailed) value of 0.001, further corroborating the efficacy of Instagram media in fostering enhanced learning motivation. Other social media, such as TikTok, which has been studied by Caballero (2024), got positive results. However, the research conducted is still limited to microlearning and small samples. In addition, there are more posts on Instagram than on TikTok, according to Dubin's (2024) research. With this comparison, Instagram is superior in social media development, and the number of posts and views is expected to increase interaction between users more effectively. With its diverse features, Instagram emerges as a promising alternative learning medium, offering meaningful engagement opportunities for students in chemistry education.

3.3.2. Analysis of factors affecting the improvement of student learning motivation

Students' motivation to learn can come from internal and external factors. This study used three indicators of learning motivation to show the increase in students' learning motivation.

3.3.2.1. Indicator of encouragement of student morale in learning

In this indicator, the results of the learning motivation questionnaire on four supporting aspects consisting of aspects of student interest, activeness, and curiosity about the reaction rate material are presented in **Table 1**.

Based on **Table 1**, in the aspect of interest in the reaction rate material, there was a significant increase in students who felt very interested in the reaction rate material after being given Instagram media, where initially only 8.57% of students then increased to 50.72%. The learning motivation of students who use Instagram media as learning media is greater in terms of student interest in learning and the reaction rate. This was also shown during teaching and learning activities, where the experimental class actively discussed the reaction rate material. Using Instagram as a learning media increases students' enthusiasm, activeness and motivation (Fidian, 2017). In the aspect of student activeness during the learning process, there is an increase in students who have very high activeness when learning the reaction rate, with an initial percentage of 27.59% rising to 48.28%. Based on this description, the learning motivation of students who use Instagram media as learning media has increased in student activeness in the learning process. This was also shown during teaching and learning activities, where the experimental class showed increased activeness by asking more questions when they did not understand and asking many questions during discussions to make learning more meaningful. Regarding student curiosity in learning, before being given Instagram-integrated learning media, students who were very curious had a percentage of 5.71%. Giving Instagram afterwards can significantly increase the percentage to 28.57%. Using Instagram media to display images and videos on the reel and feed features can increase student curiosity about the exposure of reaction rate material. In addition, the comment column can provide a wide platform for students who want to ask questions about material they do not understand. Furthermore, other students who understand better can answer these questions, thus creating an open discussion space between one student and another.

3.3.2.2. Indicator of desire to succeed in learning

In this indicator, the results of the learning motivation questionnaire are obtained in two supporting aspects, namely, there are aspects of student confidence in mastering the material and student confidence in completing the exam presented in **Table 2**.

3.3.2.3. Indicators of student interest in using learning media

In addition to internal factors, external factors can increase learning motivation, in this case, the use of learning media. Students' interest in the learning media is presented in **Table 3**.

Based on **Table 3**, students feel very interested in the learning media provided in the reaction rate material, which has a percentage of 57.23%. Providing learning media significantly increases students who feel interested in Instagram media up to 47.33%. Instagram-integrated learning media can attract attention and help students understand reaction rate material. There is a caption feature that contains a written explanation of the material presented in addition to the presentation of material in the form of images and videos contained in the *feeds* and *reels* feature. The combination of explanation in the form of writing and interpretation in the form of images makes the presentation of material more varied and attracts students' attention. The fact that they do not use paper is unrelated to the possibility of using other media. This distinguishes Instagram learning media from traditional learning media such as lectures that use notebooks continuously. Instagram learning media can be an alternative to creative and environmentally friendly learning innovation because it minimizes paper use.

The data analysis of the questionnaire results of student learning motivation above, which increasingly shows changes in the good direction, can be interpreted as increasing student motivation in the learning process. The fulfilment of each aspect of the learning motivation indicator is directly proportional to the appearance of several student attitudes shown during learning such as the emergence of a sense of comfort, helping each other in understanding lessons, not feeling alone so that self-confidence grows, growing attitudes willing to work together, responsibility and a healthy competitive spirit. Students motivated during learning will feel encouraged to actively participate in every learning process, such as asking questions, discussing, and expressing opinions during the question-and-answer process at the presentation stage. Students' focus on following the teacher's instructions during learning will also be maintained by showing it at the stage of presenting material through Instagram learning media, where students listen carefully to the presentation of the material in it, and students can make good use of the provided media.

According to Andrew *et al.* (2005), two factors influence motivation: attitudes and needs. If students have a positive attitude towards learning, learning motivation will increase, and vice versa. Likewise, with students' needs, if lessons are considered meaningful in students' lives, then students' learning motivation will increase, and vice versa. Based on the observation results in the initial condition before implementing the STAD-type cooperative learning model, students' learning motivation was still lacking, as indicated by students' activities during learning tended to be low where most students were still passive, did not dare to ask questions, were not confident in answering questions asked by the teacher, and still lacked cooperation in completing tasks. Changes from these conditions began to appear after implementing the STAD learning model integrated with Instagram learning media, where student motivation to learn began to appear from the initial stage of presenting the material to the final stage of learning by giving awards and quizzes.

At the material presentation stage, student activity began to appear as shown by students who actively asked questions about the reaction rate material that was not understood. This attitude appeared in both classes, but the aspect of curiosity about the reaction rate material was more prominent in the experimental class, which was shown by most of the group representatives asking questions, compared to the control class, which needed to be given an additional question stimulus from the teacher first before they arose curiosity. The attitude of daring to ask questions was also shown in the experimental class, which was very prominent during the discussion when answering the exercise questions in the LKPD. Students in this class were not afraid to raise their hands to ask questions or ask for directions in working on problems. This is slightly different from the control class where the class tends to be passive in asking questions, so the teacher must check each group to ensure students understanding and the course of discussion in working on exercise questions. Regarding cooperation between group members, the experimental class was more organized and organized regarding the division of tasks for each group member. A slight difference was shown in the control class, where several group members did not work on the division of tasks and were only charged to several members. Based on this, the control class is still dominated by several passive students and does not show any motivation to learn during the learning process.

At the publication stage, one group representative presented their group discussion results, followed by a question-and-answer process. At this stage, student activeness in the experimental class was seen with all representatives of groups that did not present asking questions to the presenter group. When clarifying the answers, students did not hesitate to enthusiastically express their opinions. Student activeness at this stage in the control class was also visible. Still, those who dared to ask questions and express opinions were only dominated by a few students, and it seemed that other students began to be distracted by other things, so they did not focus on paying attention to the question-and-answer process during the presentation. However, those who dared to ask questions and express opinions were only dominated by a few students, and it seemed that other students began to be distracted by other things, so they did not focus on paying attention to the question-and-answer process during the presentation. Followed by the awarding stage, where the accumulation of award stickers obtained from the *Reward Chart* of the two classes was different, where in the experimental class the most active group was successfully fulfilled by the award sticker in the table given, while in the control class, the most active group was not fully fulfilled by the sticker in it. From these results, students' motivation during the discussion session to ask and answer questions in the experimental class increased in terms of learning activities.

The increase in student motivation to learn is due to the application of STAD learning. This follows the results of research conducted by Yudiasa et al. (2016) which states that using the STAD model can increase student learning motivation. Increased motivation occurs during learning following the stages of the STAD learning process. Students focus when the teacher explains the material, cooperate in doing group assignments, actively ask questions during group discussions and presentations, and utilize learning resources during the learning process. The following research by Supriyo (2008) shows that the STAD cooperative learning model can increase students' motivation. Previous research conducted by Widarti (2022) showed that the increase in student learning motivation after using the *Chem Squad Instagram* account learning media as an independent learning resource for chemistry learning on Acid and Base material showed an increase in the standard gain value of 0.40 in moderate criteria.

Banerjee *et al.* (2014) argue that when students learn with a self-learning approach, it will create an environment where they can learn. A supportive learning atmosphere, as well as innovations in learning that involve the active role of students, will further increase student learning motivation. Learning requires creative strategies when using technology, especially for novice students. The most important feature that is directly influenced by engagement is self-concept. Instagram social media encourages creative learning. The use of Instagram social media in education as an innovative, interactive and creative learning media shows that this application can increase students' learning motivation which is also very important in achieving maximum learning outcomes.

Table 1. Indicators of encouraging student enthusiasm in learning.

Aspects	Average percentage of each score (%)							
	Before				After			
	1	2	3	4	1	2	3	4
Interest in the Material Rate Reaction	2.86	8.57	68.57	8.57	0	3.57	45.71	50.72
Student Activity in Learning Reaction Rate	6.90	20.69	31.03	27.59	6.90	13.79	27.59	48.28
Students' Curiosity in Learning Reaction Rate	2.86	28.57	51.43	5.71	0	17.14	42.86	28.57

Source: Elaborated by the authors.

Table 2. Indicator of desire to succeed in learning an average percentage of each score.

Aspects	Average percentage of each score (%)							
	Before				After			
	1	2	3	4	1	2	3	4
Student Self-Confidence in Mastering the Material of the Rate Reaction	17.24	37.93	27.59	6.90	2.86	11.43	20	54.23
Student Self-Confidence in Completing an Exam	5.71	22.86	48.57	14.29	0	22.86	51.43	17.14

Source: Elaborated by the authors.

Table 3. Indicators of student interest in using learning media average percentage of each score (%).

Aspects	Percentage of each score (%)							
	Before				After			
	1	2	3	4	1	2	3	4
Student interest in using learning media	17.24	37.93	27.59	6.90%	2.86	11.43	20	54.23

Source: Elaborated by the authors.

4. Conclusions

Considering the findings and subsequent discussion, it is evident that integrating Instagram applications positively affects student motivation and learning outcomes. The research outcomes revealed a notable increase in learning motivation, with students utilizing the Instagram-integrated STAD learning model registering a remarkable 85% motivation level in the very high category. These findings collectively affirm the efficacy of the Instagram-integrated STAD learning model in enhancing chemistry learning, particularly regarding reaction rate material, and in augmenting student motivation and learning outcomes. Future research efforts are encouraged to explore the application of Instagram application in chemistry education across various materials and to investigate further to identify which Instagram features are most conducive to the chemistry learning process or other social media that may be able to develop better than Instagram.

Authors' contribution

Conceptualization: Hayuni Retno Widarti; **Data curation**: Elvira Risva Firda Amalia; **Formal Analysis**: Elvira Risva Firda Amalia; **Funding acquisition**: Hayuni Retno Widarti; **Investigation**: Elvira Risva Firda Amalia; Hayuni Retno Widarti; **Methodology**: Elvira Risva Firda Amalia; **Project administration**: Hayuni Retno Widarti; Elvira Risva Firda Amalia; Deni Ainur Rokhim; **Resources**: Elvira Risva Firda Amalia; **Software**: Deni Ainur Rokhim; **Supervision**: Hayuni Retno Widarti; **Validation**: Hayuni Retno Widarti; **Visualization**: Elvira Risva Firda Amalia; **Writing – original draft**: Elvira Risva Firda Amalia; **Writing – review & editing**: Deni Ainur Rokhim

Data availability statement

All data were acquired and analyzed in the present investigation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Systematic literature review on the application of Problem-Based Learning model in chemistry education

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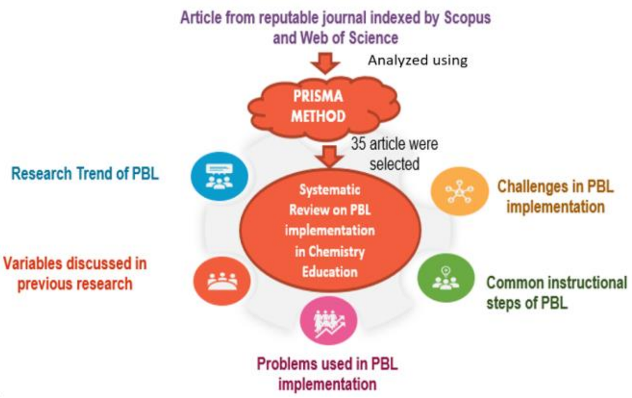
Abstract

Problem-based learning (PBL) has seen significant growth in recent years, but its effective implementation remains a challenge. Therefore, this study undertakes a systematic literature review to synthesise the research patterns, challenges, and approaches employed in chemistry education. PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) methodology was employed in analysing publications within journals indexed in Scopus and Web of Science. A total of 523 papers have been found using some keywords. Following a thorough assessment, 35 publications were selected for in-depth analysis. The study found that PBL implementation varies depending on four primary variables: thinking skills, problem-solving abilities, conceptual comprehension, and argumentation skills. PBL concerns include issues like malnutrition, obesity, waste management, climate change, pollution, and green chemistry. Common instructional steps of PBL include problem orientation, student organisation, independent study, group investigation, reporting, and evaluation. Several challenges were found in applying PBL, including time limitation, instructor proficiency, student characteristics, and technical implementation of PBL. These findings are expected to complement the references for future research on PBL.



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Highlights

- Describe the trends of PBL research in the period 2013-2023.
- Analysing the common steps of PBL used by researchers in the period 2013-2023.
- Exposing the problems that were addressed by previous researchers applying PBL.
- Explain the challenges in using PBL in learning chemistry.

Contents

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1. Introduction

Chemistry is one of the branches of natural science that studies the structure, composition, properties, and changes of matter and the reactions accompanying the changes (Chang, 2010; Jespersen *et al.*, 2012). Everyday items such as foods, medicines, and cosmetics are practical manifestations of the applications derived from chemistry principles (Rusmansyah *et al.*, 2019). It is believed that through the study of chemistry, people can be trained to solve complex real-life problems. While learning chemistry poses challenges for students (Yerimadesi *et al.*, 2023), teaching chemistry at the school level fosters meaningful learning experiences that empower students to apply their knowledge in addressing everyday problems. The learning approach to fostering such meaningful learning is Student-Centred Learning (SCL).

SCL has garnered significant attention from educators in Indonesia and globally (Kaput, 2018). Recognising its pivotal role, educators across diverse nations acknowledge the significance of SCL in actively engaging students throughout the learning process (Estes, 2004). This approach not only fosters problem-solving, collaboration, and the development of critical thinking skills (Brush, 2000) but also brings enjoyment and sparks interest among students in their studies (Chairam *et al.*, 2015; Murni *et al.*, 2022). The approach is also considered effective in enhancing students' learning outcomes.

Problem-based learning (PBL) is one of the learning models encompassed within the framework of student-centred learning (SCL) (Ediansyah *et al.*, 2019; Ernawati *et al.*, 2022; Naji *et al.*, 2020). PBL uses complex real-world problems to encourage students to identify essential ideas in planning the problem solution (Flynn and Biggs, 2012), to train students in solving the problems given by the teacher and to find the optimal solution (Silver-Hmelo, 2004; Simamora *et al.*, 2017). Rooted in the constructivist learning theory, PBL facilitates the development of students' thinking skills through analysing real-life problems during the learning process (Abdullah and Panai, 2022). The students are provided with an ill-structured problem that they must solve, which becomes the starting point for them to obtain and integrate new knowledge.

Previous studies have revealed the positive impact of PBL on various educational facets, including enhanced learning outcomes, improved thinking ability, elevated problem-solving skills, strengthened argumentation skills and increased conceptual understanding. However, some studies on PBL reported that the learning model still cannot be applied optimally in the classroom, especially in learning chemistry. Teachers faced challenges in implementing PBL, with key problems revolving around constraints such as limited time for PBL implementation (Ayyildiz and Tarhan, 2018; Ernawati, 2021; Ernawati *et al.*, 2022; Hugerat *et al.*, 2021; Kartamiharja *et al.*, 2020; Seçgin and Sungur, 2021). Additionally, other challenges were identified, including teachers lacking proficiency in using PBL, student characteristics affected the learning process (Ayyildiz and Tarhan, 2018; Seçgin and Sungur, 2021; Tosun and Taskesenligil, 2013), and teachers challenges in supporting PBL implementation (Batdi, 2014). Consequently, these obstacles hinder the attainment of learning objectives. Therefore, a Systematic Literature Review (SLR) on PBL is necessary to provide an overview of how the previous researchers have implemented PBL and the obstacles encountered in the process.

Previous researchers have conducted SLR on PBL within various fields. Noteworthy examples of these SLRs include the study of the effectiveness of PBL in developing critical thinking skills of nursing students (Kong and Qin, 2014), PBL's role in mathematics learning (Merritt *et al.*, 2017), trends in PBL models within university science courses (Azhar *et al.*, 2023), PBL assessment in medical schools (Nendaz and Tekian, 1999), the effect of PBL during medical school on physician competence (Koh *et al.*, 2008), and bibliometric analyses on the implementation of PBL models in science education (Nurhayati *et al.*, 2023).

However, our search did not find any SLRs specifically addressing the implementation of PBL in chemistry education. Consequently, a systematic, step-by-step review is needed to explore the trend and applications of PBL in chemistry education over the past decade. This review aims to provide readers and prospective researchers with insights into PBL research trends in chemistry education, highlighting variables that have received much attention, issues raised by PBL, common PBL procedures employed by previous researchers, and challenges encountered during PBL implementation. This SLR intends to provide necessary information for future researchers interested in implementing PBL in science or chemistry classrooms, enabling them to consider the weaknesses and obstacles identified by prior researchers in their future endeavours.

1.1. Research aim

The primary objective of this SLR is to review, examine, and draw comparisons among prior studies that have used PBL models in science education, with particular emphasis on chemistry education. This review encompasses studies published in reputable journals indexed by Scopus and Web of Science during the period from 2013 to 2023. Serving as an initial point of reference, this SLR is intended to be a valuable resource for future researchers keen on exploring and applying PBL to their work. The result of our study aims to provide insights into the historical implementation of PBL and the challenges encountered. By doing so, we hope to assist future researchers in anticipating these challenges, thereby optimising the outcomes of their research endeavours.

1.2. Research question

This review is oriented towards addressing the following research questions:

RQ 1. What characterizes the research trends of PBL application in chemistry education from 2013–2023?

RQ 2. Which variables have been studied by researchers in the exploration of PBL within the past studies?

RQ 3. How is the context of the problem discussed in PBL learning described in previous studies?

RQ 4. What are the common steps of PBL implemented in chemistry learning within the past studies?

RQ 5. What challenges have previous researchers encountered when applying PBL in chemistry education?

2. Experimental

This research is characterized as a Systematic Literature Review (SLR), which is an investigative approach that systematically, clearly, and comprehensively synthesizes prior research findings, allowing for replicability (Snyder, 2019; Xiao and Watson, 2019). Employing a method that is both systematic and transparent in addressing research questions, SLR can provide a brief overview of the scientific topics under consideration (Kurniati *et al.*, 2022; van Dinter *et al.*, 2021). This study aims to identify and analyse various articles on PBL engaging in an in-depth examination of 35 articles that align with predefined criteria.

The review and analysis of articles in this study used the PRISMA model (Preferred Reporting Items for Systematic Review and Meta-Analysis). PRISMA is a tool and guide for evaluating systematic reviews and meta-analyses (Zarate *et al.*, 2022).

In this study, the authors chose to use the PRISMA model because PRISMA has a clear and structured framework at each research stage. Adopting the PRISMA model is advantageous as it facilitates the effective synthesis of articles meeting the criteria while mitigating potential biases that may arise during the study (Moher *et al.*, 2009; 2010). In addition, PRISMA also encourages authors to report every step of their research in detail. This allows other researchers to replicate our research and verify the results obtained, thus increasing the credibility and quality of the research results. The PRISMA model consists of several processes, including identification, screening, eligibility, and quality assessment of articles.

2.1. Identification

Identification is finding, considering, and diversifying appropriate keywords for article searches. This keyword assignment was done before the literature search so that the resulting articles would be more accurate and aligned with the desired topic. The phrases "problem-based learning, science learning, PBL effectiveness, and PBL implementation" were used as keywords to search articles in the Scopus, ERIC, and Publish or Perish 8 databases to obtain reputable articles indexed by Scopus and Web of Science. The identification of keywords for this research is described in **Table 1**.

Table 1. Keywords search of articles in the database.

Database	Keyword
Scopus by ERIC	"Problem-based learning*" OR "PBL*" AND "Science Learning" AND "PBL effectiveness*" OR "Implementation of PBL" AND "PBL in chemistry"
Scopus by Publish or Perish 8	TITLE-ABS-KEY (("problem-based learning*" OR "PBL*") AND PUBYEAR > 2013 AND PUBYEAR < 2023 AND LIMIT-TO (SUBJAREA, "SOCI") AND LIMIT-TO (DOCTYPE, "ar") AND (LIMIT-TO (EXACTKEYWORD, "Chemistry learning") OR LIMIT-TO (EXACTKEYWORD, "Implementation of PBL") OR LIMIT-TO (EXACTKEYWORD, "PBL Effectiveness*") OR LIMIT-TO (EXACTKEYWORD, "Science Learning")) AND LIMIT-TO (LANGUAGE, "English")
Web of Science	TS = (("Problem based learning*" OR "PBL*") AND "Science Learning" AND ("PBL effectiveness*" OR "Implementation of PBL" OR "PBL in chemistry"))

Source: Elaborated by the authors.
Notes: **AND / OR** are Boolean operators used to combine keywords logically; ***represents a wildcard**, including different word endings (e.g., "learning*" covers "learning", "learned", "learners" etc.); **TITLE-ABS-KEY** searches within the **title**, **abstract**, and **keywords** fields in Scopus; The **Scopus (via PoP 8)** search uses advanced filters: publication year, subject area, document type, language, and specific keywords.

The search terms outlined in **Table 1** resulted in the identification of 523 articles. Among these, 369 articles were in the ERIC database, 130 in the Scopus database using Publish or Perish 8, and 24 in Web of Science. The search parameters were limited to articles published within the last ten years. The literature search in this study was conducted in March 2023, meaning that the articles included in the criteria of this study were Scopus and Web of Science-indexed articles published from January 2013 to March 2023. Scopus and Web of Science were chosen as the primary databases for determining the inclusion criteria in this study due to their several advantages. Firstly, Scopus and Web of Science offer search results that are more stable, functional, and comprehensive compared to others (Gusenbauer and Haddaway, 2021). In addition, the articles indexed by Scopus and Web of Science demonstrate higher quality due to their emphasis on quality control and a systematic indexing system (Martín-Martín *et al.*, 2018). The better the quality of a paper, the more it will be cited by other researchers (Nugroho, 2022). Therefore, papers published in Scopus-indexed journals and Web of Science are more likely to be cited (Schafmeister, 2021), thereby enhancing the author's prestige.

2.2. Screening

The process of determining the inclusion and exclusion criteria of the articles studied to form a systematic literature review is carried out in this screening stage (Mohamed Shaffril *et al.*, 2020). Before the screening, the researchers excluded five duplicate articles from the initial 523 articles gathered in the previous stage. The screening process was applied to the remaining 518 articles obtained during the identification stage, employing various inclusion criteria. The first criterion was the year of publication, with inclusion limited to articles published in the last ten years, from 2013 to 2023. The study will likely include the most recent articles within this period to describe the trend of PBL research in science education, especially chemistry.

The second criterion considered when selecting articles is publication type. In this study, only articles from reputable journals were considered. Such articles are deemed of higher quality, having undergone a rigorous review process before publication. Furthermore, the study exclusively includes research articles based on empirical data. This criterion was pivotal in selecting articles, with proceedings, book chapters, and review articles excluded from the collected data as they did not meet these specified criteria.

The next criterion considered in the data selection is the language employed in the article. The analysis was restricted to articles written in English. This measure is implemented to mitigate potential errors in transcription and comprehension of the article's content, ensuring clarity in delivering reviews associated with the studied articles. The final criterion is focused on thematic relevance, specifically examining articles related to PBL in science or chemistry education, implementation of PBL, effectiveness of PBL, and description of PBL steps taken. For a more detailed overview, the inclusion criteria for the articles selected are outlined in **Table 2**.

Following the screening process according to the inclusion criteria outlined in **Table 2**, 419 articles were deemed ineligible and excluded. Subsequently, the remaining 99 articles underwent the eligibility assessment for inclusion in this study.

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Table 2. Inclusion criteria of the articles.

Category	Inclusion Criteria
Publication year	Januari 2013-March 2023
Publication type	Journal articles only
Language	English
Type of Finding	Research based on empirical data
Focus of finding	Data related to PBL in science or chemistry education, Implementation of PBL, effectiveness of PBL, instructional steps of PBL in previous research

Source: Elaborated by the authors.

2.3. Eligibility

The eligibility stage aims to identify articles that align closely with the study objectives, ensuring that the selected articles make substantive contributions to the study. The second stage of the screening (the eligibility stage) is carried out by analysing the abstract, title, methodology, research outcomes, and discussion of the remaining 99 articles. An evaluation was then conducted to determine whether the articles were consistent with the subject matter of the ongoing study. During this process, 64 articles were excluded for various reasons, including the research type being qualitative or classified as research & development (R&D). This is because both types of research (qualitative and R&D) are often conducted on a small scale and in a particular context. They also provide in-depth qualitative data about a process. In contrast, SLR requires research with larger and diverse samples with quantitative data that can be generalised from multiple studies to infer broadly applicable trends or patterns.

This study exclusively analysed articles falling within experimental, mixed-methods, and action research categories. The primary aim is to identify how PBL is implemented in chemistry education based on previous studies and to investigate challenges encountered during the implementation of PBL. Articles that are not based on empirical data were excluded from consideration. Articles discussing PBL in medical, social, language, and art fields were excluded from the study. The review focused solely on articles addressing PBL in science and chemistry, given that science falls within the same scientific cluster as chemistry, the main subject of this study. Consequently, the instructional steps related to PBL identified in these articles can be inferred and applied to the context of chemistry education. Furthermore, articles lacking discussion on implementing PBL in their research were excluded, as they were considered to require more detailed information. Following the eligibility process, 35 articles remained for further analysis in the subsequent quality assessment stage. The systematic literature review process is illustrated in **Fig. 1**.

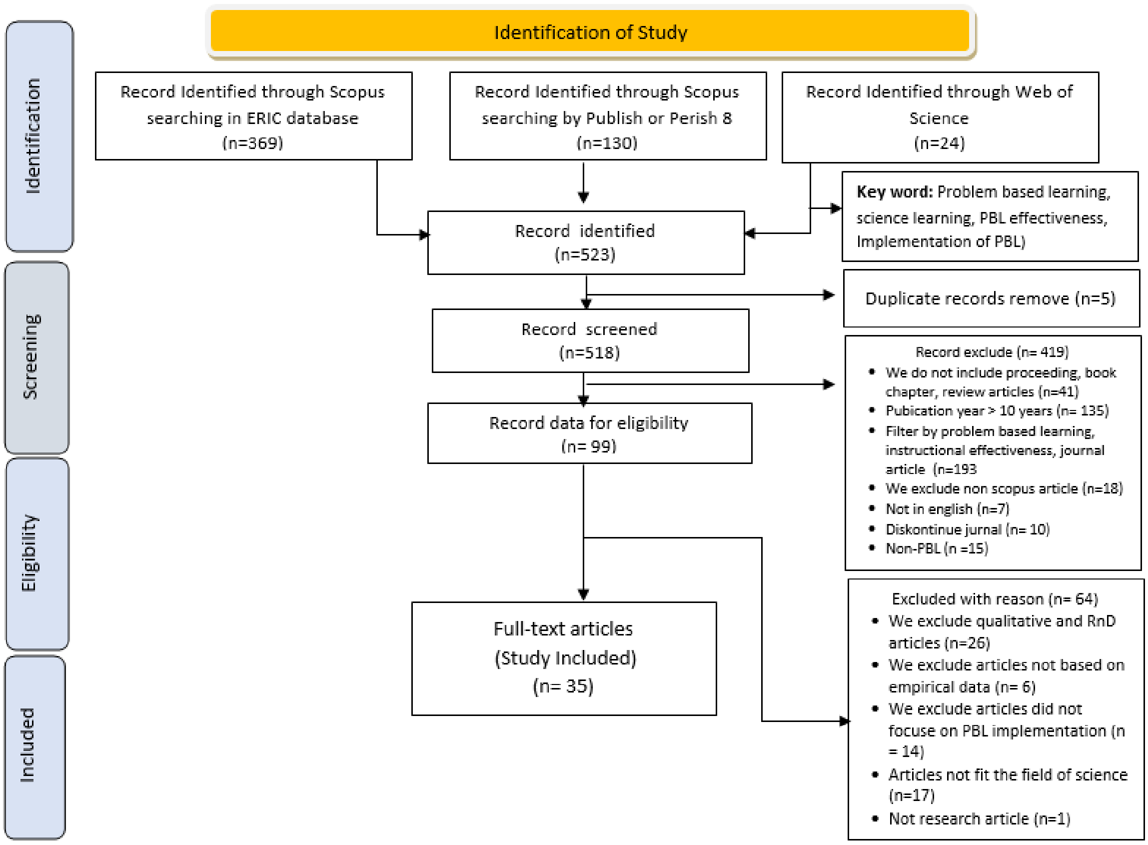

Figure 1. Systematic literature review flowchart using the PRISMA model.
Source: Elaborated by the authors.

2.4. Quality assessment

To ensure the quality of the articles selected for study and to mitigate potential biases in the research outcomes, the remaining 35 articles were evaluated by two raters who were PhD students in chemistry education. In this context, the two raters were tasked with categorising the articles based on the methodology into three quality tiers: high, medium, and low (Petticrew, 2008). The parameters used in this assessment included research design, research sample, research instruments, research procedures and data analysis. Only articles categorized as high and medium quality were considered for inclusion in the research.

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Furthermore, consensus between the two raters was crucial, demanding an agreement that the selected articles must possess at least medium quality. In the event of discordance between the raters, any differences were resolved through in-depth discussion before deciding to include or exclude the article from the study.

The evaluations conducted by the two raters were subsequently subjected to analysis using SPSS 27 to compute the kappa agreement value (k). Based on the SPSS 27 calculation, as illustrated in **Fig. 2**, the resulting kappa value is 0.723. This value signifies that the strength of agreement between raters in this study falls within the "good" category. Through this assessment process, 18 articles were rated as high quality, while 17 articles were rated as medium quality by both raters. Thus, all the remaining articles were deemed eligible for further analysis in this study.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.723	.110	4.511	<.001
N of Valid Cases		35			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Figure 2. Kappa agreement value between two raters using SPSS 27.
Source: Elaborated by the authors.

2.5. Data extraction and analysis

Articles that successfully passed the quality evaluation stage were then further analysed to identify patterns that address the predetermined research question. This data analysis process involved carefully examining the 35 articles selected as research objects, wherein data were extracted and organized into a table to facilitate the following analysis process. This SLR focuses on identifying research trends related to PBL, reviewing prior research findings on PBL in science/chemistry education to obtain common PBL steps, analysing the contextual aspects of PBL problems employed, and analysing the challenges encountered by previous researchers during the PBL implementation. To address these objectives, the data extraction process focused on the three main components of each article: the abstract, results, and discussion.

Several data analysis tools were utilized by the authors, including Mendeley Reference Manager, VOS Viewer, and MS Excel. The complete article that became the object of research was downloaded and uploaded to Mendeley. Subsequently, Mendeley files are exported in ".ris" format, so they can be exported to the VOS Viewer application to illustrate PBL research trends in terms of publication year data, authors, keywords, and variables used in previous studies. Simultaneously, MS Excel was utilized to present the data through graphs and tables, enhancing comprehension and interpretation.

2.6. Information on Artificial Intelligence (AI) Tools

This article was written using artificial intelligence tools like Quilbot for paraphrasing, Grammarly for improving sentence structure, and Turnitin for detecting plagiarism.

3. Results and discussion

3.1. Research trend of PBL application in chemistry education

Concerning the categorization of journals, the 35 articles under examination in this study exhibit a distribution across various tiers: 11 articles published in Scopus Q1 journals, 12 articles published in Scopus Q2 journals, five articles published in Scopus Q3 journals, two articles published in Scopus Q4, and the remaining five articles published in a journal indexed by Web of Science (WoS). This distribution implies that most of the studies reported in these journals are considered reputable. Furthermore, the articles were analyzed and classified based on the following categories to distinguish the research trend related to the implementation of PBL in chemistry education.

3.1.1. Distribution year

Figure 3 illustrates the annual publication trends of articles on Problem-Based Learning (PBL) in chemistry education from 2013 to 2023.

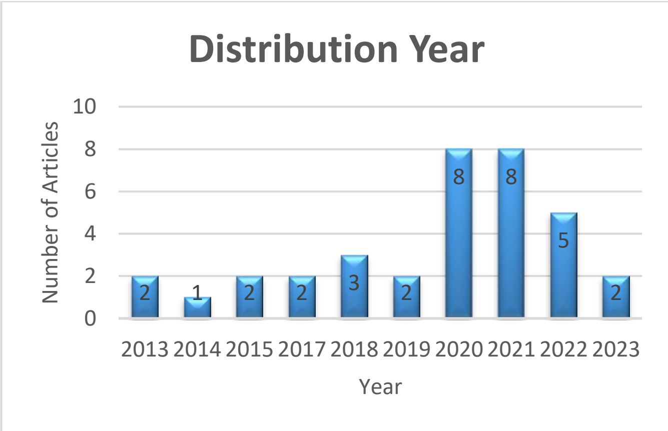

Figure 3. Research distribution by publication year.
Source: Elaborated by the authors.

Based on **Fig. 3**, the number of publications on implementing Problem-Based Learning in chemistry education has fluctuated over the past decade. This aligns with findings from earlier studies that indicate an increasing trend in the number of PBL research articles in chemistry education between 2013 to 2018 (Nurhayati *et al.*, 2023). The average number of articles addressing the theme of PBL in science learning published per year was 2 from 2013 to 2018.

The results of our study reveal that the peak of PBL publications occurred in 2020 and 2021, with both years showcasing the publication of 8 PBL articles in the field of education, featured in reputable journals. In 2022 and 2023, there was a decrease in the number of publications with PBL topics published in similar journals, although additional data for 2023 may be imminent. It is noteworthy that, at the time of data collection in March 2023, there remains a nine-month window until the end of 2023, allowing for the potential emergence of more publications on the topic of PBL implementation in science and chemistry education within other reputable journals.

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Moreover, several studies underline the potential for future research on PBL, highlighting its relevance and capacity for promoting 21st-century skills (Azhar *et al.*, 2023; Rahayu, 2017). These skills are fundamental in preparing students to face the challenges of the ever-changing modern world (Ayyildiz and Tarhan, 2018; Roza *et al.*, 2023; Sudarmin *et al.*, 2019). The ongoing global changes across various domains aim to enhance the quality of life in modern societies. Consequently, there is a growing focus among educators, educational institutions, and stakeholders on incorporating efforts to infuse 21st-century skills into the learning process. Universities recognize the importance of producing graduates who are academically intelligent and ready to navigate real-world challenges. By providing inclusive education centred on 21st-century skills, students stand a greater chance of success in the future and can contribute to the diversity and complexity of the global society (Zubaidah, 2020).

3.1.2. Geographical distribution

Concerning the geographical distribution of research on PBL in chemistry education, investigations have taken place in various countries worldwide, including Indonesia, Turkey, the USA, Australia, Spain, the Philippines, India, and South Korea, as illustrated in **Fig. 4**. Notably, Indonesia and Turkey are prominent contributors to PBL research, constituting 37.14 and 31.4% of the 35 articles examined in this study. Additionally, 14.29% of the research was conducted in the USA, while the remaining 17.17% was distributed across India, the Philippines, South Korea, Israel, and Spain. This distribution differs from the findings of Samosir *et al.* (2023), who focused on PBL research at the junior high school level and identified the United States as the leading country in applying PBL in that educational context.

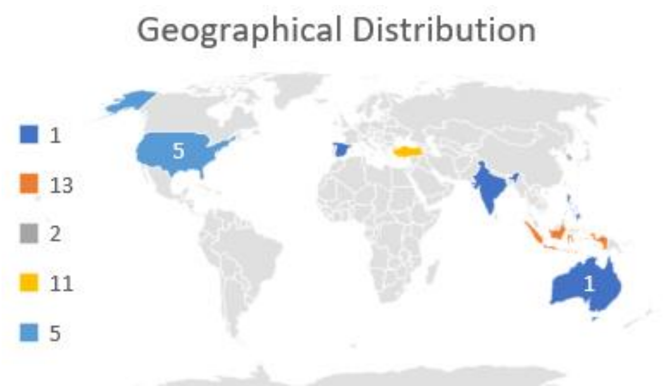

Figure 4. Distribution of studies across countries.
Source: Elaborated by the authors.

Further analysis reveals the distribution of PBL research implementation in terms of continents, as depicted in **Fig. 5**. Within this categorization, the Asian continent takes the lead, contributing more than half (65.71%) of the articles reviewed in this study. Europe follows with the second-highest representation at 17.14%, followed by the Americas, accounting for 14.29% of the total articles examined. Notably, there is an absence of PBL articles or research in science and chemistry education on the African continent and oceanic countries, marking an interesting aspect of this data.

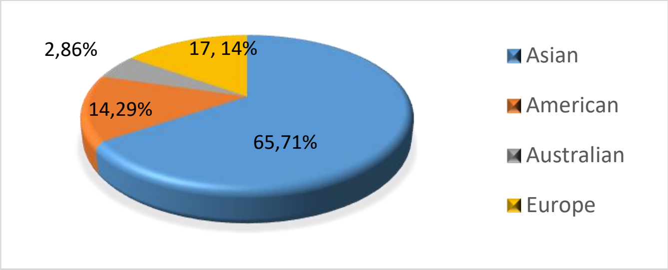

Figure 5. Distribution of research across the continent.
Source: Elaborated by the authors.

3.1.3. Distribution by educational level

Figure 6 illustrates that most of the research on implementing PBL in science and chemistry education was conducted at the university and senior high school levels. Based on the analysis of the article, most of the studies, encompassing 16 articles (45.71%) out of 35 papers, were conducted at the university level. Meanwhile, PBL research at the senior high school level ranked second with 37.14%. The PBL model suits senior high school and college students (Yin Peen and Yusof Arshad, 2014). Notably, PBL implementation requires students to be independent in solving problems through individual and group learning.

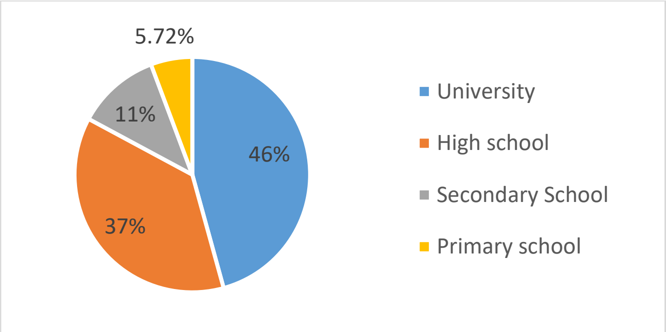

Figure 6. Distribution of research across educational levels.
Source: Elaborated by the authors.

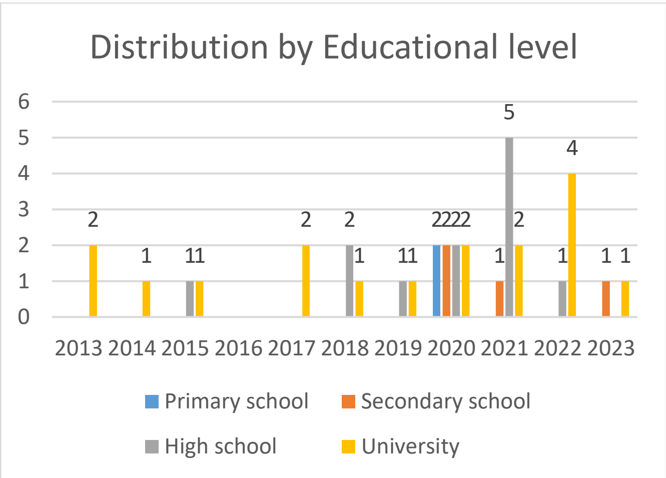

Figure 7. Distribution of research by educational level annually.
Source: Elaborated by the authors.

On the other hand, PBL at the junior high school level was discussed in only four articles (11.43%). An intriguing finding from this review is that researchers have explored the application of PBL learning to elementary school students. Even though elementary school students may not possess complete independence in learning, requiring guidance from the teacher as a facilitator, there are limited studies on PBL at the primary school level (Wandira *et al.*, 2023). Therefore, implementing PBL models in primary schools may present an opportunity for future PBL research.

The distribution of PBL research based on the educational level of the participants per year is depicted in **Fig. 7**. The figure illustrates that, over the years, PBL research on senior high school and university students has been dominant. This result is consistent with the study by Azhar *et al.* (2023), which concluded that PBL application in higher education is more prevalent and successful in enhancing students' 21st-century skills. In concurrence, Roza *et al.* (2023) revealed that the PBL approach was effectively implemented in teaching biology, chemistry, and other science subjects at the college level.

The peak of PBL research occurred in 2020 and 2021 when researchers were able to publish eight PBL-related articles per year. However, there was a decline in 2023 as our data collection only extended until March 2023.

3.1.4. Research methods used in previous PBL implementation

Patterns in the research methodologies on PBL application in science, especially chemistry education, as published in reputable international journals, are outlined in **Table 3**.

Table 3. Distribution of research-by-research methods.

Research Methods	n	P (%)	References		
Quantitative	20	57.14	Akhdinirwanto *et al.* (2020) Ayyildiz and Tarhan (2018) Batlolona and Souisa (2020) Cahyono *et al.* (2021) Ernawati (2021) Günter *et al.* (2017) Hugerat *et al.* (2021)	Jumadi *et al.* (2021) Kuvac and Koc (2019) Marthaliakirana *et al.* (2022) Palupi *et al.* (2020) Rehmat and Hartley (2020) Seçgin and Sungur (2021) Simanjuntak *et al.* (2021)	Tosun and Senocak (2013) Tosun and Taskesenligil (2013) Ulger (2018) Valdez and Bungihan (2019) Yoon *et al.* (2014)
Qualitative	2	5.72	Kim *et al.* (2019)	Varadarajan and Ladage (2022)	
Mix-methods	10	28.57	Aslan and Duruhan (2021) Baran and Sozbilir (2018)	Costa *et al.* (2023) Kartamiharja *et al.* (2020)	Kim *et al.* (2022) Liu *et al.* (2018) Ramlo *et al.* (2021)
Action research	3	8.57	Laksmi *et.al.* (2021)	Overton and Randles (2015)	Pritasari *et al.* (2015)

Source: Elaborated by the authors.

Table 3 shows that the quantitative method is the most used research methodology across the 35 articles under study. Quantitative research methods constitute 57.14% of the total object study. Almost 90% of the total quantitative research identified was experimental research. This indicates that over half of the investigations in the examined papers used experimental methods for data collection. This outcome is consistent with the findings of Azhar *et al.* (2023), who asserted that the experimental method is the most popular in the PBL application at the university level. Experimental research holds broader usage than other methodologies in educational and learning research. Typically, experimental research aims to assess the impact of implementing the PBL model in science education on variables related to 21st-century skills, including critical thinking (Cahyono *et al.*, 2021; Marthaliakirana *et al.*, 2022), creative thinking (Nuswowati *et al.*, 2017; Simanjuntak *et al.*, 2021; Yoon *et al.*, 2014), problem-solving (Tosun and Taskesenligil, 2013), scientific argumentation (Akhdinirwanto *et al.*, 2020; Cahyono *et al.*, 2021), metacognitive awareness (Tosun and Senocak, 2013) and others.

Meanwhile, mixed methods constitute the second-highest percentage at 28.57%. This approach combines qualitative and quantitative methodologies within a study (Creswell and Clark, 2018), enabling the collection and analysis of both data types to provide an understanding of a phenomenon (Halcomb and Hickman, 2015). Researchers employing mixed-method studies are not restricted to a singular research approach or data type; rather, they must integrate quantitative and qualitative methods to explore diverse aspects comprehensively. The research examined the impact of PBL supported by Augmented Reality (AR) on improving reflective thinking for problem-solving skills (Arici and Yilmaz, 2023), the influence of the PBL model with scaffolding on students' creative thinking skills and their response to scaffolding in PBL (Ernawati, 2021), and the effectiveness of PBL in chemistry education concerning tofu wastewater treatment (Kartamiharja *et al.*, 2020). In 2021, a study was conducted to investigate the influence of problem contexts on students' creative thinking skills (Ernawati, 2021), while another study investigated the PBL influence on context on student achievement, knowledge retention, motivation (Hugerat *et al.*, 2021), and attitudes towards chemistry, as reported by Baran and Sozbilir (2018).

Moreover, Hugerat *et al.* (2021) explored the impact of PBL combined with Jigsaw Discussion (PBL-JD) on student motivation in learning science. Ramlo *et al.* (2021) examined students' perspectives on PBL-based chemistry laboratories in science courses. Liu *et al.* (2018) investigated the effects of PBL learning environments enriched with multimedia on students' science knowledge by administering pretests and post-tests before and after learning. The outcomes of the mixed-method research in this review suggest a favourable inclination towards utilizing PBL for chemistry education to enhance learning achievement and life skills among students, following the requirements of modern education.

Qualitative research methods were the least popular in this review, each accounting for 5.72% of the total. The lack of qualitative studies is attributed to the eligibility stage, where qualitative methods were considered ineligible for the study's objectives. The focus was on assessing PBL implementation, the process of learning chemistry with PBL, and its impact on the observed variables. Thus, most of the PBL qualitative studies in the sample were excluded based on the criteria set for this study.

3.2. Variables studied by PBL researchers

The identification of frequently occurring keywords in the articles examined in this study was conducted utilising the Mendeley Reference Manager and VOS Viewer tools. Article data that has been downloaded is stored in Mendeley and then exported in RIS* format. Afterwards, the file is imported into the VOS viewer, and keyword analysis is carried out. VOS viewers display for the analysis type "co-occurrence--> keywords" analysis type (**Fig. 8**) reveal four primary keywords that previous researchers commonly used in articles addressing "the implementation of PBL in learning science". The four themes are indicated by large circles in the VOS Viewer visualization. The identified keywords are problem-based learning, problem-solving skills, argumentation skills, and cooperative learning. When the keyword problem-based learning is designated as the main keyword, the VOS viewer visualisation transforms, as shown in **Fig. 9**. The keyword problem-based learning frequently appears and demonstrates a connection to other keywords such as motivation, creative thinking, argumentation, and problem-solving skills.

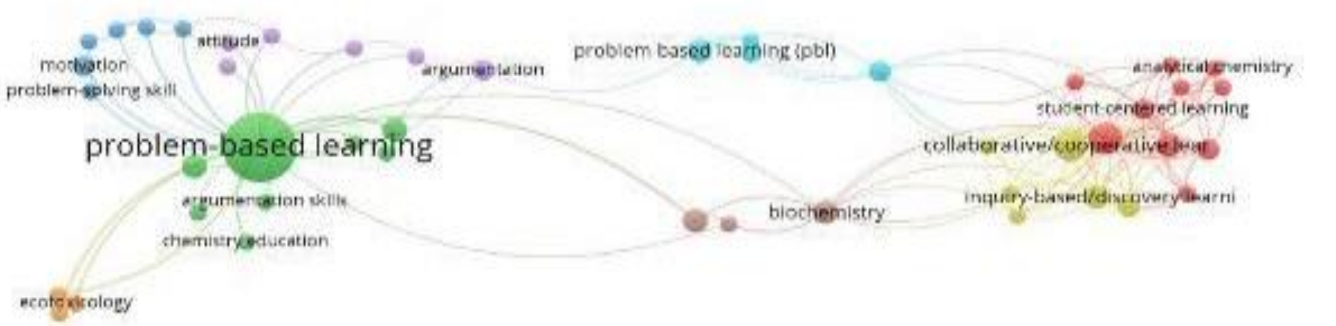

Figure 8. VOS Viewer presentation for the type of analysis "Co-occurrence--> keywords".
Source: Elaborated by the authors.

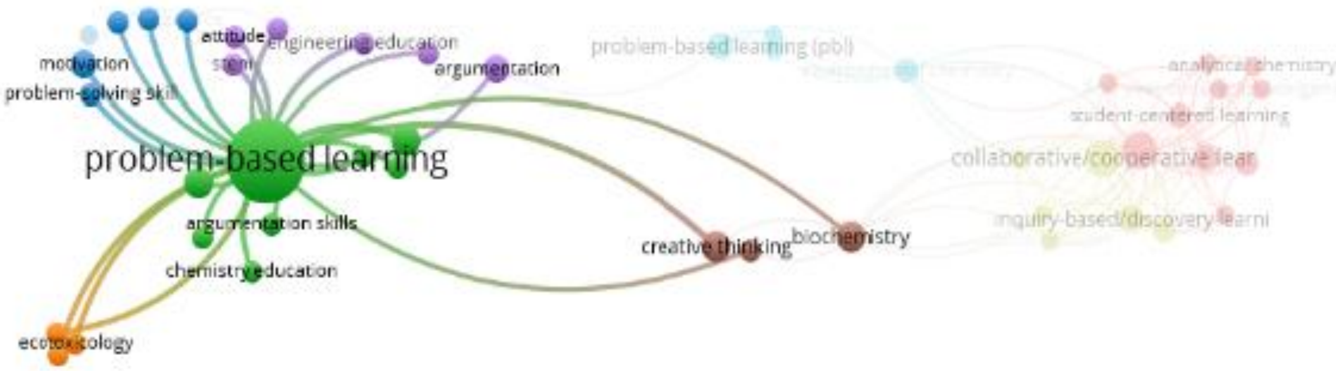

Figure 9. VOS Viewer presentation for the type of analysis "co-occurrence-->keywords: problem-based learning".
Source: Elaborated by the authors.

Apart from utilising the VOS Viewer tool, we also conducted an in-depth analysis of the articles by inputting data from the articles into analysis tables tailored to the study's requirements. The outcomes of the analysis of variables commonly employed by previous researchers are shown in **Table 4**. Thinking ability emerges as a frequently used variable, with a prevalence of 20.48%. Subsequently, problem-solving skills (9.64%), argumentation skills (8.43%), conceptual understanding (8.43%), and various other variables are also frequently employed by researchers in PBL studies.

Table 4. Distribution of variables used in PBL.

Variable	Frequency	%
Thinking skills	17	20.48
Problem-solving skill	8	9.64
Argumentation skill	7	8.43
Conceptual understanding	7	8.43
Learning achievement	6	7.23
Scaffolding	6	7.23
Motivation	6	7.23
Scientific behaviours	6	7.23
Metacognitive awareness	4	4.83
Students' knowledge	3	3.61
Self-regulated learning	3	3.61
Academic background	3	3.61
Decision making	3	3.61
Age, gender	2	2.41
Scientific process skill	1	1.21
Information literacy	1	1.21

Source: Elaborated by the authors.

3.3. Problems in PBL discussed by previous researchers

The problems used in PBL learning are usually related to students' daily lives. The article analysis results showed that not all articles studied explained the kinds of problems raised by educators when implementing problem-based learning (PBL) models in science learning, especially chemistry. **Table 5** presents some issues that can be recorded from analysing the articles on the research object.

According to Table 5, PBL has been applied in science education in various fields, including general science, biology, physics, chemistry, and its subfields, such as organic chemistry, analytical chemistry, environmental chemistry, and biochemistry. PBL learning is mainly concerned with problems students commonly face daily. Incorporating authentic, real-world problems into chemistry learning is crucial in fostering students' skill development and affective growth (Rahayu, 2019). Based on the reviews, examples of problem scenarios employed for PBL-based chemistry instruction are health issues like malnutrition and obesity (Marthaliakirana *et al.*, 2022); waste management (Yoon *et al.*, 2014), (Varadarajan and Ladage, 2022); environmental pollution (air, water, dan soil) (Kartamiharja *et al.*, 2020; Kuvac and Koc, 2019); climate change (Pritasari *et al.*, 2015); and Green chemistry and sustainability (Günter *et al.*, 2017). This finding aligns with the study (Qamariyah *et al.*, 2021), which states that chemistry is closely related to social problems such as global warming, pollution, nuclear power plants, additives in food and health problems. Therefore, for students to understand chemistry well, teachers must be able to design contextual and actual problems for students and connect them with the chemistry material being studied so that students are actively involved in each PBL step implemented.

Table 5. The problems context employed by previous researcher in PBL.

Level academic	Subject	Problems context
Elementary School	Science	Respiratory system and its disturbances (Palupi *et al.*, 2020) Natural disaster, Living things and their habitats (Rehmat and Hartley, 2020)
Junior High school	Science	Temperature and heat (Akhdinirwanto *et al.*, 2020) Force and Energy (Aslan and Duruhan, 2021)
Senior High School	Chemistry	Atomic structure, chemical bonding, Carbonic compound, organic compound, mol concept (Valdez and Bungihan, 2019) Enthalpy change in the system (Ayyildiz and Tarhan, 2018)
	Science	Environmental pollution, the environmental change/climate and waste recycling (Pritasari *et al.*, 2015)
	Biology	Environment (Laksmi *et al.*, 2021) Blood circulation system (Hugerat *et al.*, 2021)
University	Biochemistry	Amino Acid and protein (Ernawati, 2021; Ernawati *et al.*, 2022; Hugerat *et al.*, 2021) Mendel's law and genetics (Seçgin and Sungur, 2021)
	Environmental chemistry	Waste treatment (Varadarajan and Ladage, 2022) Tofu liquid waste treatment, river water pollution due to settlement waste (Kartamiharja *et al.*, 2020) Environmental problem, green chemistry (Nuswowati *et al.*, 2017) 'Ecosystems and Biodiversity'; Environment and Water'; 'Environment and Air'; 'Environment and Soil' and 'Environment and Energy' (Kuvac and Koc, 2019)
	Fundamental chemistry	Thermodynamic, high quality water resources, Analysis of heavy metal content in water and soil samples; Identification of organic compounds in petroleum samples; Analysis of nutrient content in sports food; Case studies of carbon monoxide gas poisoning (Baran and Sozbilir, 2018) Solution and its physical properties (Tosun and Senocak, 2013; Tosun and Taskesenligil, 2013) Air quality, Air pollution (Kim *et al.*, 2022)
	Analytical Chemistry lab	Green chemistry and sustainability (Günter *et al.*, 2017) Treatment of wastewater contaminated with acids and bases, Identification of metal and non-metal Ions (Yoon *et al.*, 2014)
	Physics	Temperature and heat (Batlolona and Souisa, 2020)
	Biology	Healthy diet, the benefits of food for health, cases of obesity, and malnutrition (Marthaliakirana *et al.*, 2022)
	Organic Chemistry	NMR spectroscopy data analysis, programming, and processing (Costa *et al.*, 2023)

Source: Elaborated by the authors.

3.4. Common instructional steps employed in applying PBL in chemistry learning

The article review was conducted to identify the steps of PBL implementation in chemistry learning in previous studies. Based on the results of this identification, we found several PBL steps carried out by past researchers, as presented in Appendix 1. Based on the PBL Steps that previous researchers have done, we found some common steps that must be present in the PBL model presented in Table 6. However, from the results of the analysis, it turns out that 28.57% of the articles that are the object of study do not explain explicitly or implicitly the PBL learning steps applied in their studies.

Based on these steps, the author concluded that the common steps of PBL performed by previous researchers consisted of six steps: problem orientation, student organizing, independent study, group investigation and group discussion, reporting and presenting the results, and evaluation and reflection. In PBL, students are involved in the problem-solving process by conducting investigations through the stages of the scientific method (Asmi *et al.*, 2019). Applying the PBL model, lecturers are no longer the only source of information; they also help students direct problems to learning by providing learning materials and resources (Munzil and Mentari, 2021). So, the stages of PBL provide opportunities for students to question existing phenomena and actively build an understanding of the concepts being studied.

Table 6. Common step PBL conducted by previous researchers.

No	Common Step PBL	Activity
1	Problem orientation	a. Presenting ill ill-structured problem b. Generating ideas for questions c. Develop a problem scenario. d. Collect information needed for problem-solving. e. Consider potential solutions to solve the problem.
2	Students organizing	a. Forming heterogeneous small groups b. Determine the roles of group members.
3	Independent study	a. Out-of-class learning b. Assessing information from various learning sources, ex, mass media, social media, internet, articles, books, etc.
4	Group investigation and group discussion	a. Sharing information with group members b. Critically evaluate the resources and knowledge they have collected. c. Investigate to solve the problem. d. Data analysis
5	Reporting and presenting the results.	a. Making a group report b. Presenting the results of the discussion in front of the class c. Class discussion
6	Evaluation and reflection	a. Improve the group report according to teacher and audience suggestions b. Strengthening the concept

Source: Elaborated by the authors.

3.5. Challenges in using PBL in learning science/chemistry

Through analysing 35 articles that became the focus of our study, we found several challenges in implementing the PBL in the classroom. These challenges are related to time, teacher ability, student characteristics and technical implementation of PBL. Some of the obstacles include: First, the implementation of PBL in chemistry learning requires much time for lecturers or educators because they have to make careful preparations such as preparing issues/problem contexts related to the material being studied, preparing scaffolding to direct students before working and discussing in groups, and briefing students about the implementation of PBL learning that will be carried out (Ayyildiz and Tarhan, 2018; Ernawati, 2021; Ernawati *et al.*, 2022; Hugerat *et al.*, 2021; Kartamiharja *et al.*, 2020; Seçgin and Sungur, 2021). Time constraints like this can be reduced using media in PBL (Pertiwi *et al.*, 2021).

The second challenge that occurred during the implementation of PBL in previous studies was the class interruption caused by students who were still unfamiliar with the PBL model, so what the teacher and instructor planned did not run correctly (Ayyildiz and Tarhan, 2018; Seçgin and Sungur, 2021; Tosun and Taskesenligil, 2013). Students also do not understand their role and the role of the lecturer in PBL (Ayyildiz and Tarhan, 2018). Therefore, teachers should socialize the PBL model and explain students' tasks and roles in PBL before implementing this model. The third challenge is the limited skills of teachers in implementing PBL in learning, so the expected improvement in students' skills is not very significant (Ayyildiz and Tarhan, 2018; Kim *et al.*, 2019; Marthaliakirana *et al.*, 2022).

The unequal distribution of tasks among group members is the fourth challenge in implementing PBL. This causes one group member to dominate the activity while the other members become only followers, while the assessment given is the same for both passive and active group members (Baran and Sozbilir, 2018). Fifth, the emergence of alternative conceptions is caused by students' failure to connect or integrate their prior knowledge with the new concepts they are learning (Ayyildiz and Tarhan, 2018). Therefore, teachers must create situations that can activate their students' prior knowledge to change their mindset from remembering information to a meaningful learning process (Gazali and Yusmaita, 2018).

Students lacking scientific argumentation skills during the PBL process is a noteworthy challenge (Batdi, 2014). This arises because students are not explicitly guided in formulating accurate scientific arguments; instead, students are encouraged to solve problems without clear direction toward the criteria for developing proficient scientific argumentation skills (Akhdinirwanto *et al.*, 2020; Jumadi *et al.*, 2021). Another challenge is related to the existence of varying levels of problem understanding among students, accompanied by negative reflection on aspects such as problem formulation, data collection, group work, and all the possibilities that may arise throughout the learning process when implementing the PBL model (Palupi *et al.*, 2020).

4. Conclusions

This SLR examines prior research concerning the implementation of PBL models in science or chemistry education. The study focuses on 35 reputable articles, indexed by Scopus and Web of Science, and published between 2013–2023. The analysis reveals the following findings: **a)** The research trend in PBL implementation for science learning, particularly in chemistry, exhibits variations across different years. Predominantly, PBL research is conducted at the high school and university levels, with only a limited amount of research being done on PBL implementation at the elementary school level. The results of the study indicate that the majority of PBL research is concentrated in Asia (67.71%) and Europe (17.14%); **b)** the four dominant variables that emerged from previous research, namely thinking skills (20.48%), problem-solving skills (9.64%), conceptual understanding (8.43%), and argumentation skills (8.43%); **c)** Issues raised in PBL predominantly revolve around everyday problems faced by students, encompassing themes such as malnutrition and obesity, waste management, climate change, water, air, and soil pollution, green chemistry, and sustainability; **d)** common steps identified in PBL application by previous researchers encompass 6 stages: problem orientation, student organisation, independent study, group investigation and discussion, reporting and presenting the results, and evaluation and reflection; **e)** Several challenges encountered by previous researchers in the implementation of PBL in chemistry education. These challenges are related to time constraints, teacher proficiency, student characteristics, and the technical implementation of PBL.

This SLR contributes to providing insights into the application of the PBL model in chemistry education and clarifying the challenges encountered by researchers in earlier studies. In addition, we have identified common steps crucial to PBL through the investigation of the study's objects. These findings serve as valuable references for future researchers aiming to conduct studies on PBL in chemistry education and devise new strategies to address the shortcomings identified in prior studies.

Authors' contribution

Conceptualization: Fauzana Gazali; Sri Rahayu; **Data curation**: Munzil; Fauzana Gazali; **Formal Analysis**: Fauzana Gazali; **Funding acquisition**: Not Applicable; **Investigation**: Not Applicable; **Methodology**: Surdjani Wonorahardjo; **Project administration**: Fauzana Gazali; **Resources**: Munzil; **Software**: Fauzana Gazali; **Supervision**: Sri Rahayu; **Validation**: Sri Rahayu; Surdjani Wonorahardjo; **Visualization**: Fauzana Gazali; **Writing – original draft**: Fauzana Gazali; **Writing – review & editing**: Muhammad Dimar Alam; Sri Rahayu.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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APPENDIX 1

Table A1. Step of Problem-based learning in previous research.

Step of Problem-based learning	Article Reviewed	Common Steps PBL
1. Identifying problem (problem orientation) 2. Analyzing and formulating problems (problem orientation) 3. Designing problem solution (problem orientation) 4. Implementing solution 5. Presenting (reporting), Evaluating, and reflecting (evaluating)	Kartamiharja *et al.* (2020) Laksmi *et al.* (2021) Pritasari *et al.* (2015)	1. Problem orientation c. Presenting ill structure problem d. Generating ideas for questions e. Develop a problem scenario f. Collect information needed for problem-solving. g. Consider potential solutions to solve the problem 2. Students organizing c. Forming heterogeneous small groups d. Determine the roles of group members 3. Independent study c. Out-of- class learning d. Assessing information from various learning source ex: mass media, social media, internet, articles, book, etc. 4. Group investigation and group discussion e. Sharing information with group member f. Critically evaluate the resources and knowledge they have collected g. Investigate to solve the problem h. Data analyzing 5. Reporting and presenting the results 6. Evaluation and reflection
1. Problem orientation (problem orientation) 2. Developing a problem scenario (problem orientation) 3. Out-of-class learning (independent study) 4. Expert group formation (students organizing) 5. Compile a problem-solving report and present it (reporting and presenting the result)	Tosun and Senocak (2013) Tosun and Taskesenligil (2013)	
1. group formation and problem presentation (problem orientation) 2. brainstorming (clarify the nature of the problem and identify their learning needs) 3. students delegate roles within the groups and share existing knowledge (students organizing) 4. independent study (independent study) 5. Students gather in groups to share and critically evaluate the resources and knowledge they have gathered (group investigation and group discussion) 6. Students work to solve a problem (group investigation and group discussion)	Overton and Randles (2015) Costa *et al.* (2023)	
1. Problem identification (problem orientation) 2. Students grouping (students organizing) 3. Developing the hypothesis 4. Finding the information (problem orientation) 5. Data analysis 6. Evaluate the solution (evaluation) 7. Presenting the results (reporting)	Günter *et al.* (2017) Batlolona and Souisa (2020)	
1. Orientation (problem orientation) 2. Discuss problem in the worksheet (group discussion) 3. help students by asking probing questions and fostering collaboration 4. design a study plan and use online and library resources outside of class (independent study) 5. Sharing their knowledge in group (group investigation and group discussion) 6. Lab work and subject presentations (reporting and presenting the results) 7. To introduce the next subject, the tutor offers the pupils another worksheet, briefly explains it, and presents the issue	Ayyildiz and Tarhan (2018)	
1. Minilecture 2. group formation (students organizing) 3. introduction of problem scenarios related to the topic studied 8. Group discussion (group investigation and group discussion) 4. Presenting group discussion results to another group (reporting and presenting the results) 5. Assessment according to the rubric (evaluation)	Valdez and Bungihan (2019) Baran and Sozbilir (2018)	
1.problem identification and motivation (problem orientation) 2.organization and investigation (students organizing) 3.argumentation building 4.argumentation session 5. Evaluation-reflection (evaluation)	Akhdinirwanto *et al.* (2020)	

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Step of Problem-based learning	Article Reviewed	Common Steps PBL
1. Problem orientation (problem orientation) 2. Group investigation (group investigation and group discussion) 3. Develop and present results (reporting and presenting the results) 4. Analyzing and reviewing each step that has been done in overcoming the problem (evaluation)	Cahyono *et al.* (2021)	
1. Illstructure problem identification (problem orientation) 2. Design a problem solution 3. Group structured inquiry (group investigation and group discussion) 4. Develop and present artifacts and exhibit (reporting and presenting the results) 5. Evaluation and reflection (evaluation)	Marthaliakirana *et al.* (2022)	
1. The teacher designs a problem scenario about amino acid and present it to the students in the form of case study (problem orientation) 2. Students were required to analyze the problem, identify the central issues, and propose solutions in groups (problem orientation) 3. Teachers guided, advised, and supported students to build core concepts and creative thinking skills 4. Students were encouraged to ask questions, clarify, and work together to solve the problem (group investigation and group discussion) 5. Teachers assess students' creative thinking using pre- and post-tests, interviews, and observations (evaluation)	Ernawati (2021) Ernawati *et al.* (2022)	
1. Understanding the problem 2. Explore the problem 3. Generate possible solution 4. Determine the best fit solution 5. Solve the problem by performing experiment 6. Evaluation	Yoon *et al.* (2014)	
1. Directing students to authentic problems 2. Facilitate student learning by forming collaborative groups that share ideas 3. Individual and group investigation 4. Presenting the results 5. evaluate the results of the problem-solving process	Simanjuntak *et al.* (2021)	
1. problem presentation (problem orientation) 2. problem identification (problem orientation) 3. self-investigation (independent study) 4. Data organization 5. Sharing finding	Rehmat and Hartley (2020)	
1. Defining Problem (problem orientation) 2. Determining information for addressing the problems (problem orientation) 3. Finding, evaluating, and utilizing information as evidence for their solutions 4. Generating an argument in support of the solution	Kim *et al.* (2019; 2022)	

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Original Article

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Immunoinformatics designing of peptide-based vaccine for malaria infection

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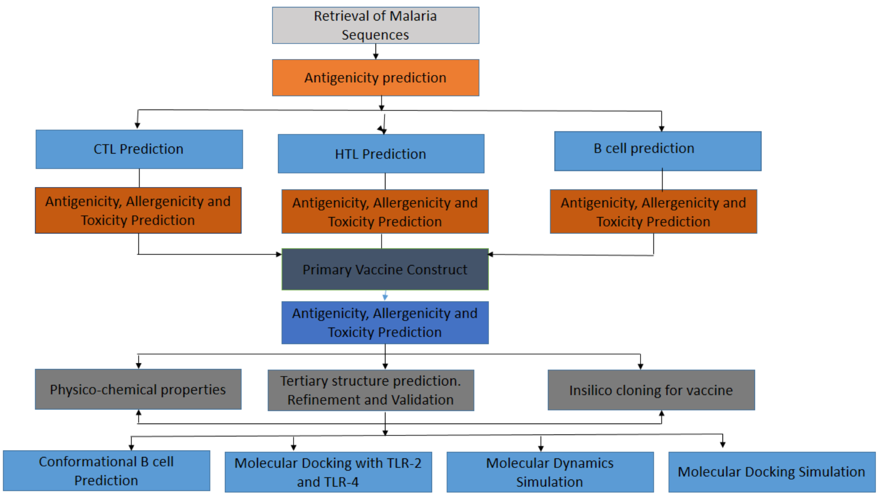
Abstract

Malaria, a life-threatening disease prevalent in tropical regions, primarily affects infants, children under five, pregnant women, travelers, and individuals with HIV/AIDS. This study utilized an immunoinformatics approach to design a peptide-based malaria vaccine targeting antigenic proteins, including Apical Membrane Antigen 1, Knob-Associated Histidine-Rich Protein, Merozoite Surface Protein 1, and Sporozoite Surface Protein 2. Antigenic protein sequences were screened for antigenicity, allergenicity, toxicity, and immune responses involving CTLs, B-cells, and HTLs. Selected epitopes were linked with appropriate linkers and an adjuvant to enhance immunogenicity, forming a vaccine construct. The construction, comprising 1473 amino acids, exhibited a molecular weight of 15.21 kDa, a theoretical pI of 8.94, an aliphatic index of 60.01, and an instability index of 31.66, indicating stability. It was hydrophilic (GRAVY: –0.385) with favorable half-lives in mammalian, yeast, and *E. coli* systems. Docking studies showed strong binding affinity to human TLR2 and TLR4. In silico cloning indicated a CAI value of 0.92 and a GC content of 59.31%. Further studies are needed to validate its efficacy and safety.



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Highlights

- Malaria is caused by Plasmodium parasites, transmitted by Anopheles mosquitoes.
- It is lethal and affects vulnerable populations like children and pregnant women.
- Current vaccines, like RTS, S, offer limited protection.
- New vaccine approaches focus on multi-epitope designs to enhance immune responses.
- Vaccines incorporate CTL, HTL, B-cell epitopes, enhancing protection against malaria.

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1. Introduction

Malaria is regarded as one of the most lethal and incapacitating infectious diseases, marked by intermittent bouts of high fever. Its cerebral form is particularly concerning, as it can lead to severe neurological complications such as brain damage and coma (Pandey *et al.*, 2017). The global incidence of malaria cases has been alarmingly high, reaching 247 million in 2021, 245 million in 2020, and 232 million in 2019 (WHO, 2022). Specific populations are particularly vulnerable to malaria infection, including children under the age of 5, HIV-positive individuals, pregnant women (Schumacher *et al.*, 2012), and travelers (Chaves *et al.*, 2017). The protozoan parasite Plasmodium is the causative agent of malaria, transmitted to humans through the bite of previously infected female Anopheles mosquitoes (Biamonte *et al.*, 2013). Among humans, malaria infection is caused by several species of the Plasmodium genus, including *P. falciparum*, *P. ovale, P. malariae,* and *P. vivax* (Amir *et al.*, 2022). A newly identified species, *P. knowlesi*, has been recognized in Southeast Asian countries, particularly Malaysia, as the sixth species causing malaria infection (Singh *et al.*, 2004). *P. vivax* is commonly associated with the sub-Saharan region of Africa and contributes significantly to morbidity, while *P. falciparum*, the most severe type of parasite, is prevalent in Africa and is responsible for most malaria-related deaths (Amir *et al.*, 2022). The malaria parasite's life cycle consists of four phases: the liver stage, blood stage, human transmission stage, and mosquito stage. Each stage must be considered for effective therapy to achieve complete eradication of the disease (Crompton *et al.*, 2010).

After being released from the mosquito bite, sporozoites migrate into the bloodstream from the bite site and invade hepatocytes in the liver (Lindner *et al.*, 2012). In the liver stage, the parasites undergo proliferation for approximately seven days (a week) before releasing exo-erythrocytic merozoites into the bloodstream to initiate the blood stage of infection (Sinnis *et al.*, 2008). Recurrent cycles of replication during the blood stage of infection lead to an exponential increase in the number of malaria parasites and the manifestation of all clinical symptoms associated with malaria (Kristian *et al.*, 2016). Before the initiation of the symptomatic stage of the disease, the parasite can be eliminated by targeting the asymptomatic sporozoite and liver stage parasites when parasite populations are low (Kristian *et al.,* 2016). Additionally, peptides or antibodies that block the AMA1-RON2 connection reduced the *Plasmodium merozoites'* capacity to colonize the host cells (Srinivasan *et al.*, 2011). The protein known as AHRP, or knob-associated histidine-rich protein (KAHRP) is usually produced when *Plasmodium falciparum* initiates infection in the erythrocytes (Maier *et al.,* 2009). A glycosylphosphatidylinositol-anchored protein known as merozoite surface protein 1 (MSP1), which constitutes the larger part of the merozoite surface protein, has been identified by researchers as a potential vaccination candidate (Holder *et al.,* 2009). This 190–200 kDa protein is attached to the surface of the merozoite by a glycosylphosphatidylinositol at its C-terminus (Holder *et al.*, 2009). Although it is yet unknown how the MSP1 merozoite surface complex affects erythrocyte invasion, recombinant fragments and variants of MSP1 produced from parasites have been associated with the binding of erythrocyte receptors (Kadekoppala *et al.*, 2008).

A key challenge in developing an effective malaria vaccine is the parasite's multistage life cycle complexity. This complexity indeed imposed a significant obstacle to vaccine development, as targeting each life cycle stage requires a deep understanding of the parasite's biology and host immune responses at various stages (Amlabu *et al.*, 2018). The initial malaria vaccine, RTS, S (also known as Mosquirix), which is based on recombinant proteins, has demonstrated only modest effectiveness in protecting young children against the disease. (Amir *et al.*, 2022). Despite considerable endeavors, this vaccination approach has drawbacks, as it only averts 39% of malaria infections and 30% of severe malaria cases (Amir *et al.*, 2022). After several months, its effectiveness diminishes, requiring four doses for optimal protection (Laurens *et al.*, 2020).

This research recommends creating a peptide-based vaccine containing immune-stimulating epitopes capable of eliciting both humoral and cell-mediated immune responses to prevent malaria infection.

2. Experimental

2.1. Systematic workflow

This study adhered to a systematic workflow, as depicted in **Fig. 1**. The diagram outlines each step involved in formulating this multi-epitope vaccine.

2.2. Protein sequence retrieval

The protein sequences of Plasmodium falciparum were retrieved from the National Center for Biotechnology Information (NCBI) (Oladipo *et al.*, 2024a) and the Universal Protein Resource (UNIPROT) Server. Apical membrane antigen 1(A0A0X8II02), Apical membrane antigen 1(A0A193PBV5), Knob associated histidine-rich protein (A0A0L7KKR3), Knob associated histidine-rich protein (W7FDY3), Merozoite surface protein 1(Q25971), Sporozoite surface protein 2(A0A0L0CV98), Sporozoite surface protein 2(A0A0L7KJ49).

2.3. Prediction of protein sequence antigenicity

The antigenicity of the *Plasmodium falciparum* protein was predicted using ANTIGENpro (Magnan *et al.*, 2010) and VaxiJen (Pandey *et al.*, 2016). The threshold of ≥0.8 (ANTIGENpro) (Magnan *et al.,* 2010), and ≥ 0.5 VaxiJen (Doytchinova *et al.,* 2007) were considered for the selection of the protein sequence.

2.4. Cytotoxic T lymphocytes (CTL) epitopes prediction

NetCTL 1.2 tool was utilized in the prediction of CTL epitopes of the proteins (Oladipo *et al.*, 2020). The tool was set at a threshold value of 0.75, while the weights on C-terminal cleavage and TAP transport efficiency were set at 0.15 and 0.05, respectively (Zhao *et al.,* 2017).

2.5. Helper T-Cell (HTL) epitope prediction

The HTL Epitopes of the protein sequence were predicted using the Immune Epitope Database (IEDB) (Oladipo *et al.*, 2022). Three mouse alleles, which are H2-IAb, H2-IEd and H2-IAd, were selected for the Major Histocompatibility Class II (MHC II). MHC-II affinity and percentile rank of ≤ 0.2 were used as criteria for selecting the HTL epitopes (Zhang *et al.,* 2008). The best six epitopes were chosen for each allele.

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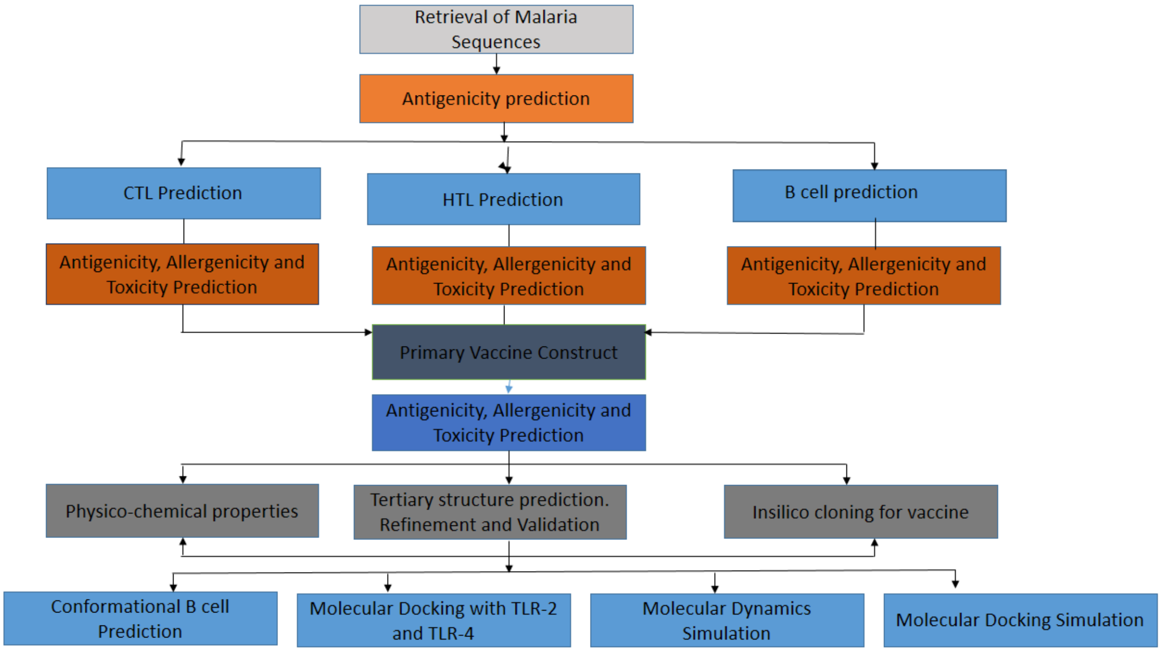

Figure 1. Study workflow for malaria vaccine construct design.
Source: Elaborated by the authors.

2.6. B-Cell epitopes prediction

B-cells are an important component of the immune system for long-term protection against pathogens and antigens (Oladipo *et al.*, 2022). The linear B-cell Epitopes of *Plasmodium falciparum* protein were predicted using ABCPred server (Saha and Raghava, 2006). Eight epitopes with a score > 0.9 were selected and subjected to further analysis.

2.7. Construction of multi-epitope vaccine sequence

A multi-epitope vaccine was developed by combining cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL), and B-cell epitopes, joined with an adjuvant using appropriate linkers. The adjuvant APPHALS was incorporated to enhance the vaccine's immunogenicity. AAY linkers were utilized to connect the CTL epitopes, whereas GPGPG linkers were employed to connect the HTL and B-cell epitopes (Kalita *et al.*, 2019).

2.8. Antigenicity, allergenicity and toxicity prediction of the vaccine construct

The antigenicity, allergenicity, and toxicity of the vaccine construct were assessed using the VaxiJen (Doytchinova *et al.*, 2007), Allertop (Dimitrov *et al.*, 2014), and Toxinpred2 (Sharma *et al.*, 2022) servers, respectively. Antigenicity testing confirmed the vaccine's ability to stimulate antibody production. Allergenicity assessment was conducted to verify the absence of allergic reactions triggered by the vaccine. Toxicity testing was performed to ensure the vaccine's safety by confirming the absence of adverse effects on humans.

2.9. Physiochemical properties and domain identification

The physicochemical properties of the vaccine construct, including molecular weight, theoretical protrusion index (PI), hydropathicity (GRAVY), aliphatic index, instability index, extinction coefficients, atomic composition, charged residues, and in vitro and in vivo half-life, were analyzed using protparam (Garg *et al.*, 2016).

2.10. Prediction of secondary structure

The self-optimized prediction method (SOPMA) was utilized to predict the secondary structure of the vaccine construct. SOPMA analyzes the amino acid composition and predicts the relationships within the construct's secondary structure (Lee *et al.*, 2013).

2.11. Prediction of tertiary structure of the vaccine

The tertiary structure of the multi-epitope vaccine construct was predicted using I-TASSER (Zhou *et al.*, 2022). The I-TASSER tertiary structure prediction server is designed to generate protein tertiary structures and employs a quantitative scoring system to produce models. Additionally, the server predicts estimated TM-score, confidence score, standard deviation, and root mean square deviation (RMSD) values for the generated models.

2.12. Prediction of 3D configuration and discontinuous B-cell epitopes

The tertiary composition of the vaccine construct was utilized to predict the 3D conformational structure of B-cell epitopes using Ellipro. Subsequently, Ellipro was employed to determine the conformational 3D structure of the predicted linear B-cell epitopes. The Ellipro results include the number of residues in each epitope, with higher residue numbers indicating greater solvent availability. The Jmol viewer was utilized for visualizing the predicted antibody epitopes (Ponomarenko *et al.*, 2008).

2.13. Refinement of the tertiary structure

To enhance the quality of the local structure in the multi-epitope vaccine construct, the Galaxy Refine web tool, based on the CASP 10 version, was employed (Heo *et al.,* 2013). Galaxy Refine is a validated algorithm known for refining protein structures and improving the quality of local structural elements (Oladipo *et al*., 2023a).

2.14. Validation of tertiary structure

The ProSA-web server was utilized to validate both the projected and refined 3D configuration of proteins, an essential step in sequence modeling. Additionally, a Ramachandran plot was obtained by submitting the PDB file of the vaccine structure to the PROCHECK server. This process serves to validate the vaccine and authenticate its potential functionality (Laskowski *et al*., 2006).

2.15. Molecular docking of the vaccine with toll-like receptors (TLRs)

Stimulating an immune response is the primary objective in vaccine design. Therefore, it is crucial to evaluate the interactions between an antigen and Toll-like receptors (TLRs). The HDock server was employed to predict the binding sites between TLR-2 (PDB ID: 3a7c) and TLR-4 (PDB ID: 4g8a) in their most stable complex forms. Docking analysis facilitated the examination of binding affinity between the complexes, specifically the vaccine and TLRs (Yan *et al*., 2020).

2.16. Molecular dynamics simulation of the receptor-ligand complex

The iMOD server was utilized to conduct dynamics simulations, which focused on studying the physical basis, structure, and function of biological molecules to determine the stability of the complex. Results obtained from this approach, such as deformability, eigenvalues, and covariance, provide insights into the stability of the complex (López-Blanco *et al*., 2014).

2.17. The *in silico* cloning and optimization of the vaccine protein

The vaccine construct underwent codon optimization using the JCAT Java tool. This tool translates protein sequences into the expression system of another biological host to adapt the codon usage for the new host. JCAT provides the GC content and codon adaptation index (CAI) values of the adapted codons. Additionally, the tool back-translates protein sequences into DNA sequences, which are then used for silicon cloning. In this study, JCAT was employed to adjust the final vaccine sequences to fit the *E. coli* K12 strain expression system. The construct of the final vaccine was input into JCAT for adaptation processing (Grote *et al*., 2005). The DNA sequences obtained from back-translation were cloned into the *E. coli* K12 pET-28a (+) vector expression system at specific restriction enzyme sites, with the assistance of Snap Gene software (Li *et al*., 2016).

3. Result and discussion

3.1. Antigenicity prediction of *Plasmodium falciparum* proteins

The NCBI and Uniprot servers were used to obtain the *Plasmodium falciparum* protein sequence for this work. A critical component of vaccinology was achieved when the chosen sequences underwent antigenic screening and were proven to be both antigenic. The antigenicity of the *Plasmodium falciparum* proteins retrieved was predicted, and the sequences passed the AntigenPRO server and VaxiJen server at a threshold of 0.5 and 0.8, respectively (Table 1). This shows that the sequence can elicit antibodies (Oladipo *et al.,* 2022). The sequences that passed were then subjected to further analysis.

Table 1. Selected proteins of *Plasodium falciparum* and their accession number.

S/N	PROTEIN	ACCESSION NO	VAXIJEN	ANTIGENIC PRO
1	Apical membrane antigen 1	A0A0X8II02	0.6195	0.940153
	Apical membrane antigen 1	A0A193PBV5	0.5807	0.936320
2	Knob associated histidine-rich protein	A0A0L7KKR3	0.7862	0.940365
	Knob associated histidine-rich protein	W7FDY3	0.7880	0.922167
3	Merozoite surface protein 1	Q25971	0.5735	0.810633
4	Sporozoite surface protein 2	A0A0L0CV98	0.6279	0.902585
	Sporozoite surface protein 2	A0A0L7KJ49	0.6223	0.905200

Source: Elaborated by the authors.

3.2. Prediction of novel cytotoxic T lymphocytes (CTL), helper T lymphocytes (HTL) and B-cells epitopes

Different servers were employed to project CTL, HTL, and B-cell epitopes using the selected Plasmodium falciparum protein sequences that successfully passed antigenicity screening. CTL epitopes were identified based on their high scores, which fell within the threshold of 0.75 (Table 2a). Helper T lymphocytes (HTL) epitopes were also predicted, and those with low percentile ranks were chosen for further analysis (Table 2b). B-Cell epitopes falling within the threshold of 0.90 were selected (Table 2c) and incorporated into the vaccine construction alongside the HTL and CTL epitopes. The prediction of CTL, HTL, and B-Cell epitopes was conducted because a multi-epitope vaccine necessitates the inclusion of CTL, HTL, and B-Cell epitopes (Chauhan *et al.*, 2019). This is significant because T-cells recognize surface antigens presented by MHC molecules. MHC class II molecules present surface antigens to T-helper cells, while B-cell epitopes aid in eliciting antibody and memory cell responses (Oladipo *et al.,* 2022). The results obtained from these analyses were employed in building the vaccine candidate using appropriate linkers.

Table 2a. Selected epitopes of CTL.

Protein	CTL epitopes	Score	CTL epitopes	Score	CTL epitopes	Score
Apical membrane antigen 1	TLDQMRHFY	32.272	AQENNGPRY	0.9554	LLSAFEFTY	17.715
	ATILMVYLY	22.792	QYEQHLTDY	0.8786	DAEVAGTQY	14.733
	VLATILMVY	15.535	MVSNSTCRF	0.8368	AKDKSFQNY	11.252
	DISFQNYTY	15.338	VKEEYKDEY	0.8326	TLNGMRDFY	10.820
	AKDISFQNY	11.520	NTETHKCEI	0.7820	MLDPEASFW	0.8858
	ISDDKDSLK	11.379	IIENSNTTF	0.7664	GQNYWEHPY	0.7521
	SASDQPKQY	10.603	YMGNPWTEY	17.963		
Knob associated with histidine-rich protein	LDEYQNQLY	11.135	YAFSEECPY	0.8247	YVPPHGAGY	10.598
Merozoite surface protein 1	HLEAKVLNY	28.435	YLKPLAGVY	12.527	QLENNVMTF	0.8661
	NLEKKKLSY	19.055	LLILMLILY	10.159	KRDKFLSSY	0.8435
	GIADLSTDY	15.242	QTEDNYASL	0.8765	ESIQTEDNY	0.7781
Sporozoite surface protein 2	LLACAGLAY	24.876	LNENAIHLY	14.074	LTDGIPDSI	0.9307

Table 2b. Selected epitopes of HTL.

Protein	Alleles	HTL epitope	Score	Alleles	HTL epitope	Score
Apical membrane antigen 1	H2-IAb	KDGGFAFPPTKPLMS	0.26	H2-IAd	IIIASSAAVAVLATI	0.73
	H2-IAb	DGGFAFPPTKPLMSP	0.37	H2-IAb	DLKDGGFAFPPTKPL	0.84
	H2-IAb	GGFAFPPTKPLMSPM	0.39	H2-IAd	DNMKIIIASSAAVAV	0.85
	H2-IAb	SMIKSAFLPTGAFKA	0.39	H2-IAb	GFAFPPTKPLMSPMT	0.87
	H2-IAb	MIKSAFLPTGAFKAD	0.40	H2-IAd	IIASSAAVAVLATIL	0.90
	H2-IAd	MKIIIASSAAVAVLA	0.46	H2-IAd	PTYDNMKIIIASSAA	0.91
	H2-IAb	IKSAFLPTGAFKADR	0.48	H2-IAd	YDNMKIIIASSAAVA	0.93
	H2-IAd	KIIIASSAAVAVLAT	0.58	H2-IAd	KPTYDKMKIIIASSA	0.37
	H2-IAb	LKDGGFAFPPTKPLM	0.58	H2-IAb	KSAFLPTGAFKADRY	0.60
	H2-IAb	KSAFLPTGAFKADRY	0.60			
Knob associated histidine-rich protein	H2-IEd	ENGPNIFALRKRFPL	0.65	H2-IEd	QENGPNIFALRKRFP	1.75
	H2-IEd	GPNIFALRKRFPLGM	0.79	H2-IAb	GSTTGATTGANAVQS	1.65
	H2-IEd	PNIFALRKRFPLGMN	0.84	H2-IAb	STTGATTGANAVQSK	1.70
	H2-IEd	SFKNKNTLRRKKAFP	1.65	H2-IAb	AGSTTGATTGANAVQ	1.95
	H2-IEd	FKNKNTLRRKKAFPV	1.75	H2-IAb	TTGATTGANAVQSKD	1.95
Merozoite surface protein 1	H2-IAb	KNKNYTGNSPSVNNT	1.00	H2-IAb	KNYTGNSPSVNNTDV	1.51
	H2-IAb	IKNKNYTGNSPSVNN	1.10	H2-IEd	KDPYKFLNKEKRDKF	1.90
	H2-IAb	VIKNKNYTGNSPSVN	1.10	H2-IEd	DPYKFLNKEKRDKFL	2.00
	H2-IAb	NKNYTGNSPSVNNTD	1.26			
Sporozoite surface protein 2	H2-IAb	LAYKFVVPGAATPYA	0.10	H2-IAb	DRYIPYSPLPPKVLD	0.23
	H2-IAb	AGLAYKFVVPGAATP	0.12	H2-IAb	RYIPYSPLPPKVLDN	0.30
	H2-IAd	KNKEKALIIIKSLLS	1.90	H2-IAb	YKFVVPGAATPFAGE	0.46
	H2-IAd	NKEKALIIIKSLLST	2.00	H2-IAb	KFVVPGAATPFAGEP	1.95
	H2-IAb	LAYKFVVPGAATPFA	0.10			

Table 2c. Selected epitopes of B-cell.

Protein	B-cell epitope	Position	Score
Apical membrane antigen 1	RFFVCKCVERRAEVTS	355	0.92
	MKIIIASSAAVAVLAT	397	0.90
	AFKADRYKSHGKGYNW	236	0.90
	MDEPQHYGKSNSRNDE	579	0.93
	ADIPEHKPTYDKMKII	533	0.91
Knob-associated histidine-rich protein	DNKGSEGYGYEAPYNP	290	0.90
Merozoite surface protein 1	SESGSDTLEQSQPKKP	100	0.90
Sporozoite surface protein 2	DRYIPYSPLPPKVLDN	410	0.94
	ERKQSDPQSQDNNGNR	426	0.92
	PEDSEKEVPSDVPKNP	365	0.91
	HGRNNENRSYNRKYND	454	0.90
	AGLAYKFVVPGAATPF	511	0.90

Source: Elaborated by the authors.

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3.3. Construction of novel multiple epitope subunit vaccine

A multi-epitope vaccine was assembled using the predicted CTL, HTL, and B-cell binding epitopes. Linkers were utilized to connect these epitopes (Oladipo *et al.*, 2022), and an adjuvant was linked to the construction to enhance its potency and elicit a robust immune response (Oladipo *et al.*, 2022). Specifically, AAY linkers were employed to connect CTL epitopes, GPGPG linkers were used to link HTL and B-cell epitopes, and EAAAK linkers were utilized to attach the adjuvant to the vaccine construct (Ito *et al.*, 2017) as shown in **Fig. 2**.

Figure 2. The schematic representation of the malaria vaccine construct.
Source: Elaborated by the authors.

3.4. Physicochemical properties of the vaccine construct

The physicochemical properties of the vaccine construct predicted by Protparam indicate that the vaccine has a molecular weight of 15.21 kDa, falling within the range for an accepted vaccine candidate (Garg *et al.*, 2016). Our findings suggest that the vaccine construct is antigenic, non-toxic, and non-allergenic, indicating its safety. The theoretical pI value of 8.94 suggests a structurally favorable vaccine. The aliphatic index score of 60.01 indicates the presence of aliphatic side chains in the vaccine. In contrast, the instability index score of 31.66 predicts the vaccine to be stable (Oladipo *et al.*, 2023b). The GRAVY result of -0.385 indicates the hydrophobicity of immunization (Oladipo *et al.*, 2024b). Furthermore, the predicted half-life is 4.4 hours in mammalian reticulocytes in vitro, >20 hours in yeast, and 10 hours in *E. coli* in vivo.

3.5. Projection of secondary structure

SOPMA was employed to predict the secondary structure of the constructed vaccine. The server provided additional information on the vaccine construct, revealing an alpha helix content of 25.87%, an extended strand content of 15.55%, a random coil content of 54.04%, and a beta turn structure content of 4.55% (**Fig. 3** and **Table 3**). The high percentage of the random coil suggests a concentrated presence of epitopes at that point (Tahmoorespur *et al.*, 2017). The prediction of the secondary structure of our vaccine indicates stability, good flexibility, and a globular conformation, which aligns with the findings of Oluwagbemi *et al.* (2022).

Table 3. Parameter of the secondary structure prediction.

Parameters	No of Residues	Percentage
Alpha helix	381	25.87%
3_{10} helix	0	0
pi helix	0	0
Beta bridge	0	0
Extended strand	229	15.55%
Beta turn	0	0
Bend region	0	0
Random coil	796	54.04%
Ambiguous states	0	0
Other state	0	0

Source: Elaborated by the authors.

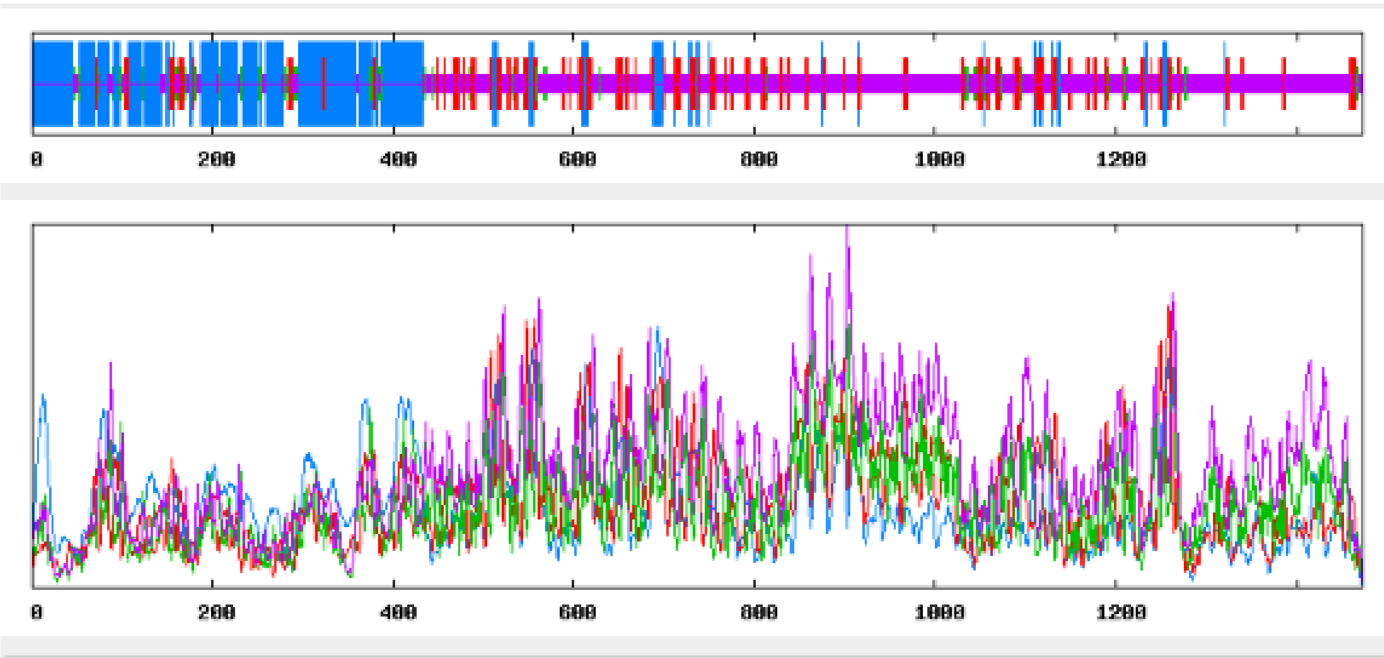

Figure 3. Prediction of the secondary structure of the constructed vaccine.
Source: Elaborated by the authors.

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3.6. Prediction of the tertiary structure of the constructed vaccine

The I-TASSER server was employed to predict the 3D configuration of the multi-epitope vaccine construct. Five models were expected, but model 1 was chosen based on the confidence score of 0.19, the estimated TM-score of 0.74 ± 0.11, and the Root Mean Square Deviation (RMSD) of 9.4 ± 4.6 Å (**Fig. 4**). The B-cell conformational epitope for the vaccine construct was identified using the Ellipro server (**Fig. 5**).

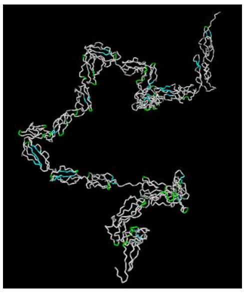

Figure 4. Tertiary structure of vaccine construct.

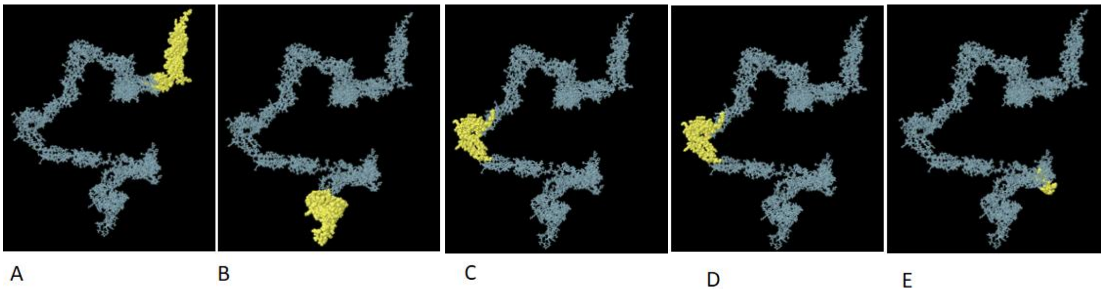

Figure 5. The 3D model of the 5 predicted conformational B-cell epitopes of the final vaccine construct. The yellow region are the conformational B-cell epitopes, while the grey areas are the residue remnant **(a)** 205 residue has a score of 0.852 **(b)** 165 residue has a score of 0.811 **(c)** 125 residue has a score of 0.742 **(d)** 24 residue has a score of 0.667 **(e)** 25 residue has a score of 0.645.

Source: Elaborated by the authors.

3.7. Refinement of the tertiary structure

Refinement of the 3D configuration entails reconstructing protein side chains, molecular dynamics simulation, and repacking to enhance the vaccine's tertiary structure. The Galaxy Refine web server was utilized for refining the vaccine's configuration, resulting in the prediction of five refined models. Model 1 was selected based on specific criteria, including a GDT-HA score of 0.8880, RMSD of 0.597, MolProbity score of 2.712, Clash score of 27.0, Poor rotamers of 0.7, and Rama favored of 75.1.

3.8. Validation of tertiary structure

Refinement provides a refined 3D vaccine model having a higher number of residues in the favored region (67.1.0%), 21.5% in the allowed area and 6.1% found in the disallowed region of Ramachandran plot (**Fig. 6** and **7**). Validation is often carried out to recognize errors within the structure of the final vaccine model. ProSA and PROCHECK servers provided a Z-score value of −4.07 (**Fig. 8**), which indicates the stability of the model. Structural validation scores obtained from ERRAT and ProSA tools proved that the overall quality of the vaccine construct meets the requirement (Oladipo *et al.*, 2022).

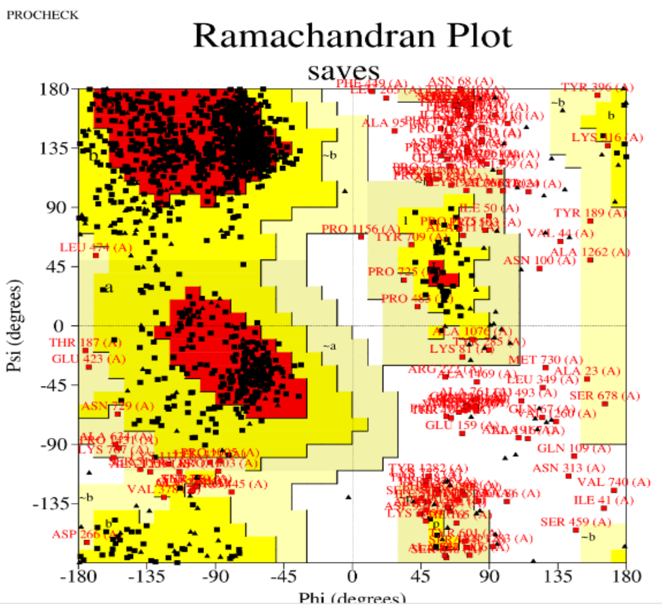

Figure 6. Ramachandran plot showing favored region of the vaccine.

Source: Elaborated by the authors.

Phi (degrees)

Plot statistics

Residues in most favored regions [A, B, L]	720	67.1%
Residues in additional allowed regions [a, b, l, p]	231	21.5%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	57	5.3%
Residues in disallowed regions	65	6.1%
	------	-----
Number of non-glycine and non-proline residues	1073	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	214	
Number of proline residues	184	

Total number of residues	1473	

Based on an analysis of 118 structures of resolution of at 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favored regions.

Figure 7. Showing the favored region and number of residues.

Source: Elaborated by the authors.

 https://doi.org/10.26850/1678-4618.eq.v50.2025.e1555

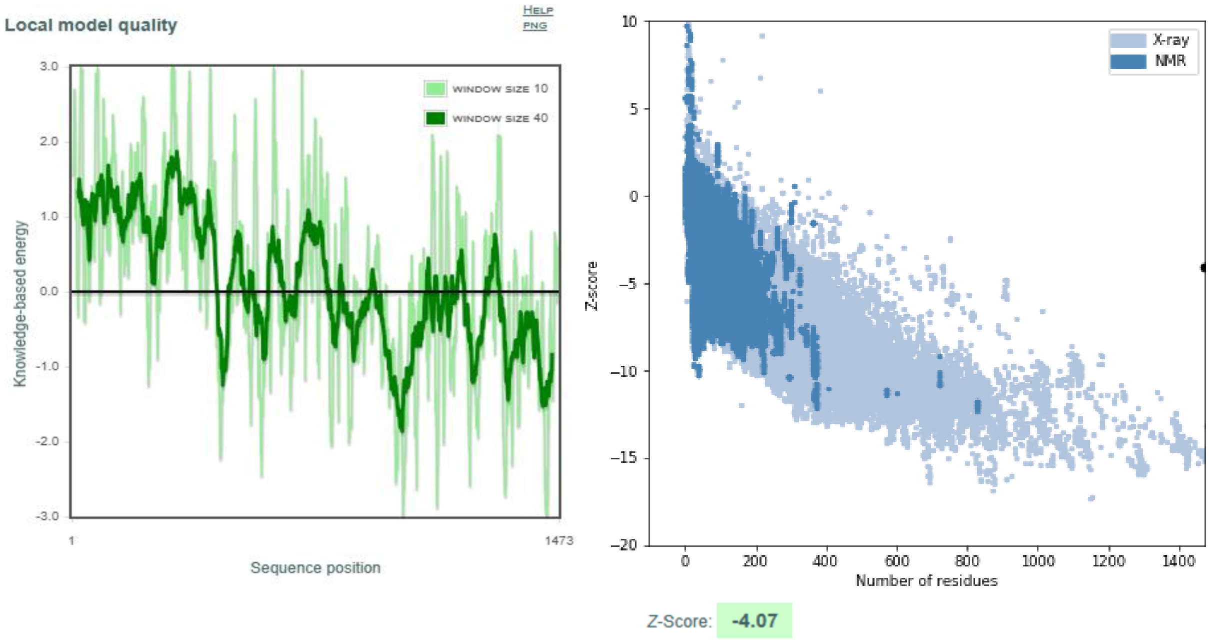

Figure 8. Graph showing model quality of the vaccine.
Source: Elaborated by the authors.

3.9. Molecular docking of the tertiary structure

The Tertiary structure was subjected to molecular docking using HDock server. The binding energy and molecular relationship of the multi-epitope subunit vaccine with TLR-2 (3a7c) and TLR-4 (4g8a) were probed by molecular docking (**Fig. 9**). One model was selected from each of the docked complexes based on their proper receptor interactions, low binding energy and center energy scores (Pandey *et al.,* 2016). The TLR 2 and TLR 4 have binding energies of -305.14 and -303.77, respectively (**Table 4**), which shows that the receptors have a high binding energy with the vaccine construct. This low binding energy score indicates strong affinities between the molecules (Oladipo *et al.,* 2023c).

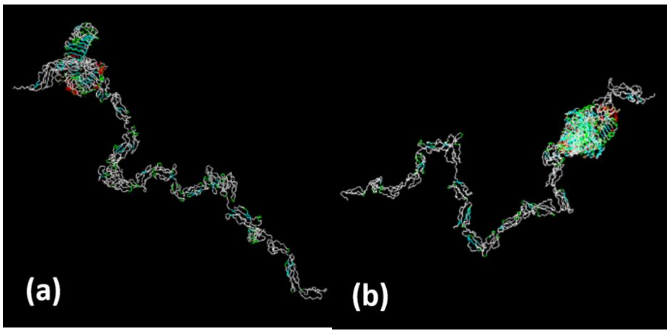

Figure 9. Molecular Docking of vaccine with TLRs **(a)** TLR-2 and vaccine construct **(b)** TLR-4 and vaccine construct.
Source: Elaborated by the authors.

Table 4. Molecular Docking against TLR-2 and TLR-4.

Dock result (rank)	TLR-4	TLR-2
Docking score	-303.77	-305.14
Confidence score	0.9559	0.9578

Source: Elaborated by the authors.

3.10. Molecular dynamic simulation

Standard Mode Analysis (NMA) was performed on the selected docked vaccine-receptor complex to investigate stability and mobility using the iMODs server. The vaccine protein and its receptor were predicted to rotate towards each other, as depicted in **Fig. 10** and **11** for TLR2 and TLR4, respectively. Hinges in regions of high deformability indicate the deformability of the vaccine-receptor complex, as shown in **Fig. 10c** and **11c** for TLR2 and TLR4, respectively. The B-factor is directly proportional to the RMS value inferred through NMA (**Fig. 10d** and **11d**). Eigenvalues for the vaccine-receptor complexes obtained from the iMODs server were 6.01e-09 and 4.75e-09 for TLR2 and TLR4, respectively (**Fig. 10e** and **11e**). Variance is inversely proportional to the Eigenvalue. Residual index graphs indicate correlated, anti-correlated, and uncorrelated pairs of residues in the variance matrix, represented by red, blue, and white colors, respectively (**Fig. 10b** and **11b**). The elastic network model generated by iMODs (**Fig. 10f** and **11f**) illustrates pairs of atoms connected by springs, with stiffer springs represented by dark grey areas. Dynamics results showed positive eigenvalues (2.27e05; 2.06e-06; 2.03e-05; 1.53e-05), which are significant for vaccine stability and rotation, consistent with previous studies (Chauhan *et al.*, 2019).

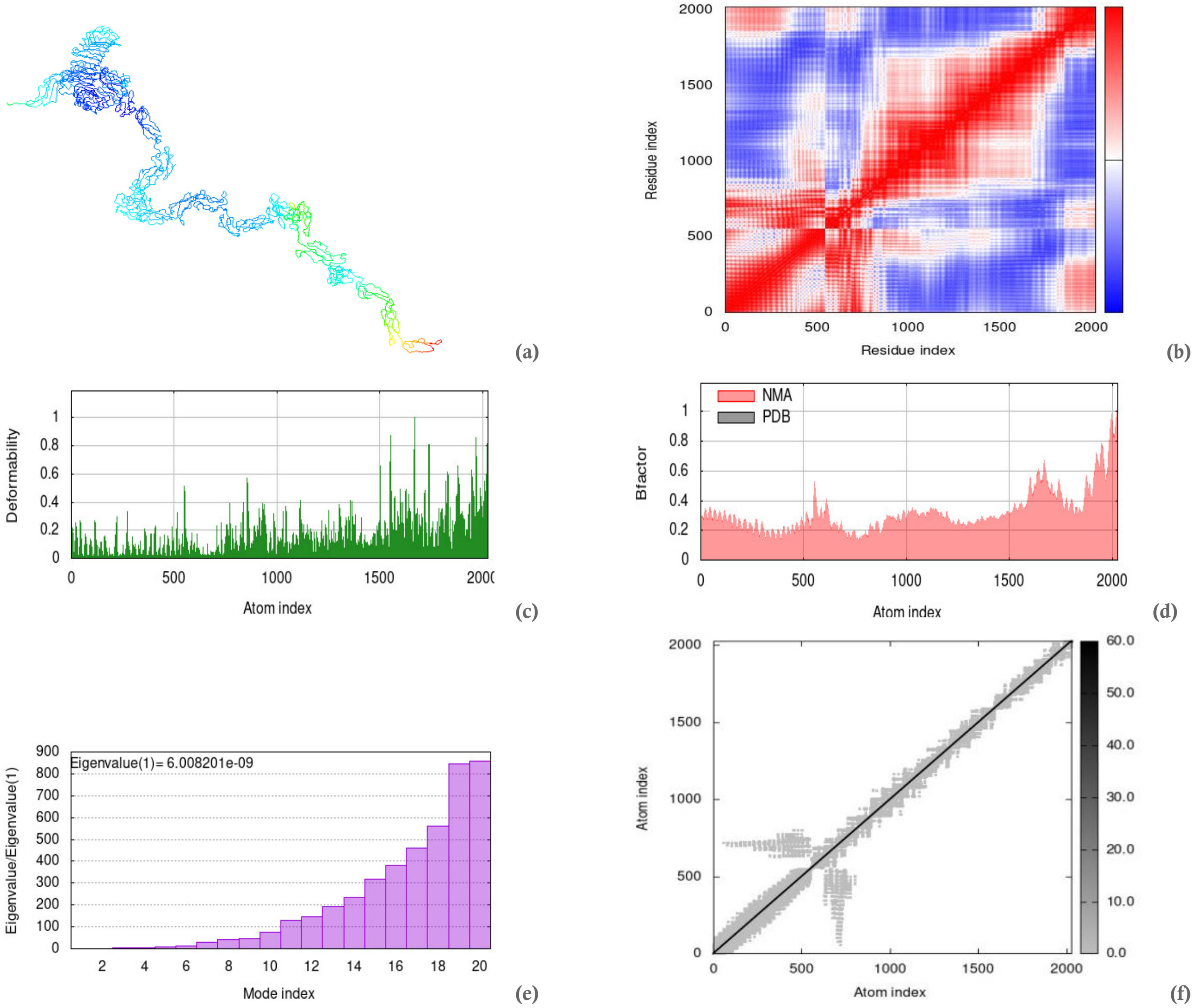

Figure 10. Molecular dynamics simulation for TLR2: **(a)** Spin prediction of the ligand-receptor interaction; **(b)** Covariance map of the ligand-receptor interaction **(c)** Deformability B-factor region of the ligand-protein interaction **(d)** Mobility B-factor of the ligand-protein interaction **(e)** Eigenvalues of the ligand-receptor interaction **(f)** Elastic network of the ligand-protein interaction.

Source: Elaborated by the authors.

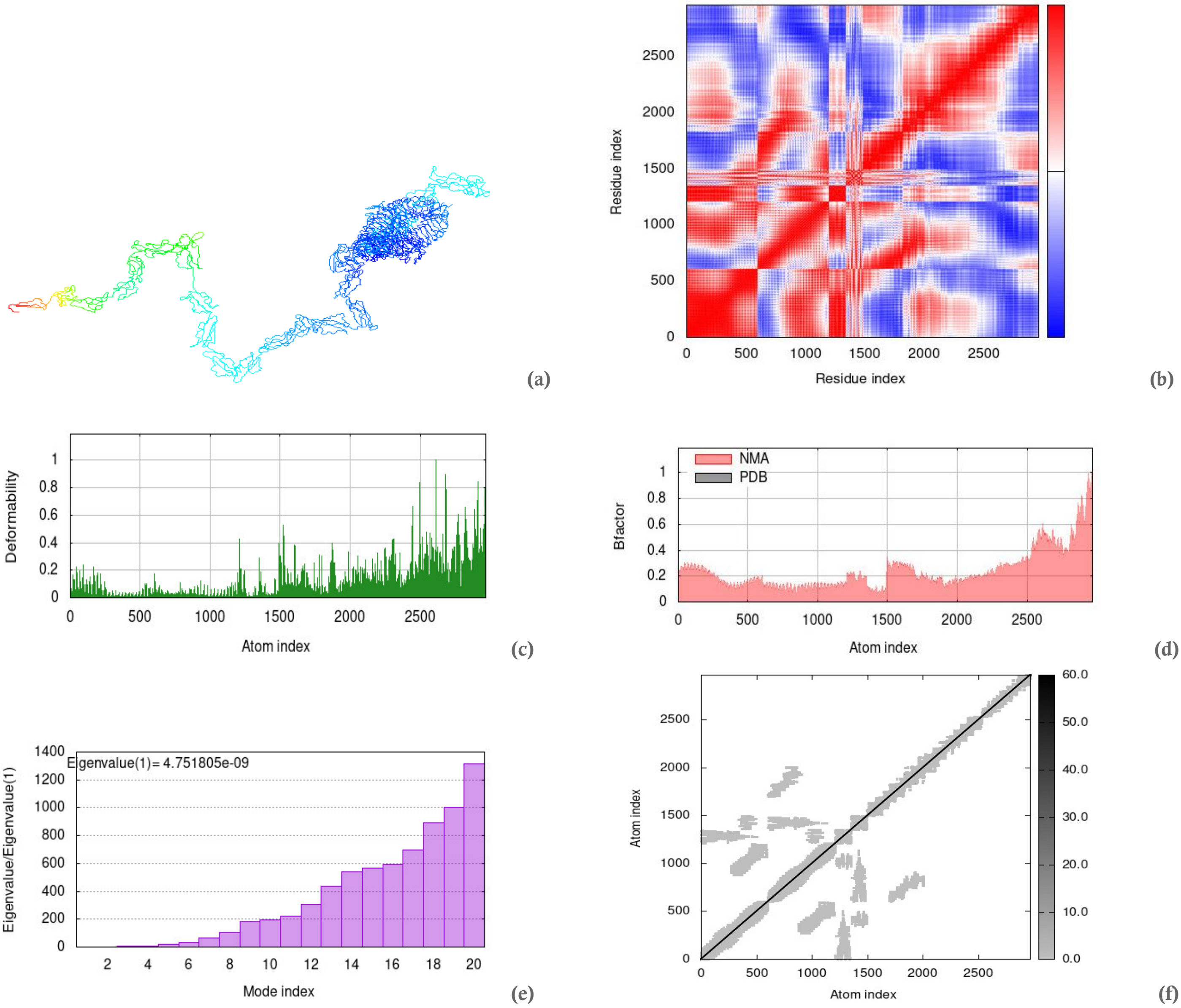

Figure 11. Molecular dynamics simulation for TLR2: **(a)** Spin prediction of the ligand-receptor interaction **(b)** Covariance map of the ligand-receptor interaction **(c)** Deformability B-factor region of the ligand-protein interaction **(d)** Mobility B-factor of the ligand-protein interaction **(e)** Eigenvalues of the ligand-receptor interaction **(f)** Elastic network of the ligand-protein interaction.
Source: Elaborated by the authors.

3.11. Codon adaptation and in silico cloning

Integrating the malaria vaccine construct into the *E. coli* expression system necessitates using JCAT and SnapGene servers. Adapting the vaccine to the *E. coli* K12 strain predicted a GC content of 59.31%, a Codon Adaptation Index (CAI) of 0.91. It translated the protein sequence back to nucleotides compatible with *E. coli* codons. The back-translated nucleotide sequence was incorporated into the *E. coli* expression system using the restriction enzymes ECORI (192) and BAMHI (4106) as cloning sites (**Fig. 12**). The solubility of the overexpressed recombinant protein in the *E. coli* host is crucial for biochemical and functional investigations. Therefore, adapting the vaccine model into an *E. coli* expression system is an essential step in vaccine design, and codon adaptation is a preferred method for achieving efficient expression of foreign genes in a host. This is because when the codons used by the host differ from those of the organism's genes, lower expression rates may occur if the genes are not adapted. Hence, we adapted the final vaccine protein sequences to the *E. coli* strain K12 using the JCAT server and obtained satisfactory results. JCAT also back translated the protein sequences to nucleotides, which were then cloned into the *E. coli* pET28a (+) vector using the ECORI (192) and BAMHI (4106) restriction sites, resulting in a total clone length of 9.2 Kbp. The target sequence was encoded between 6-histidine residues, which would facilitate purification purposes.

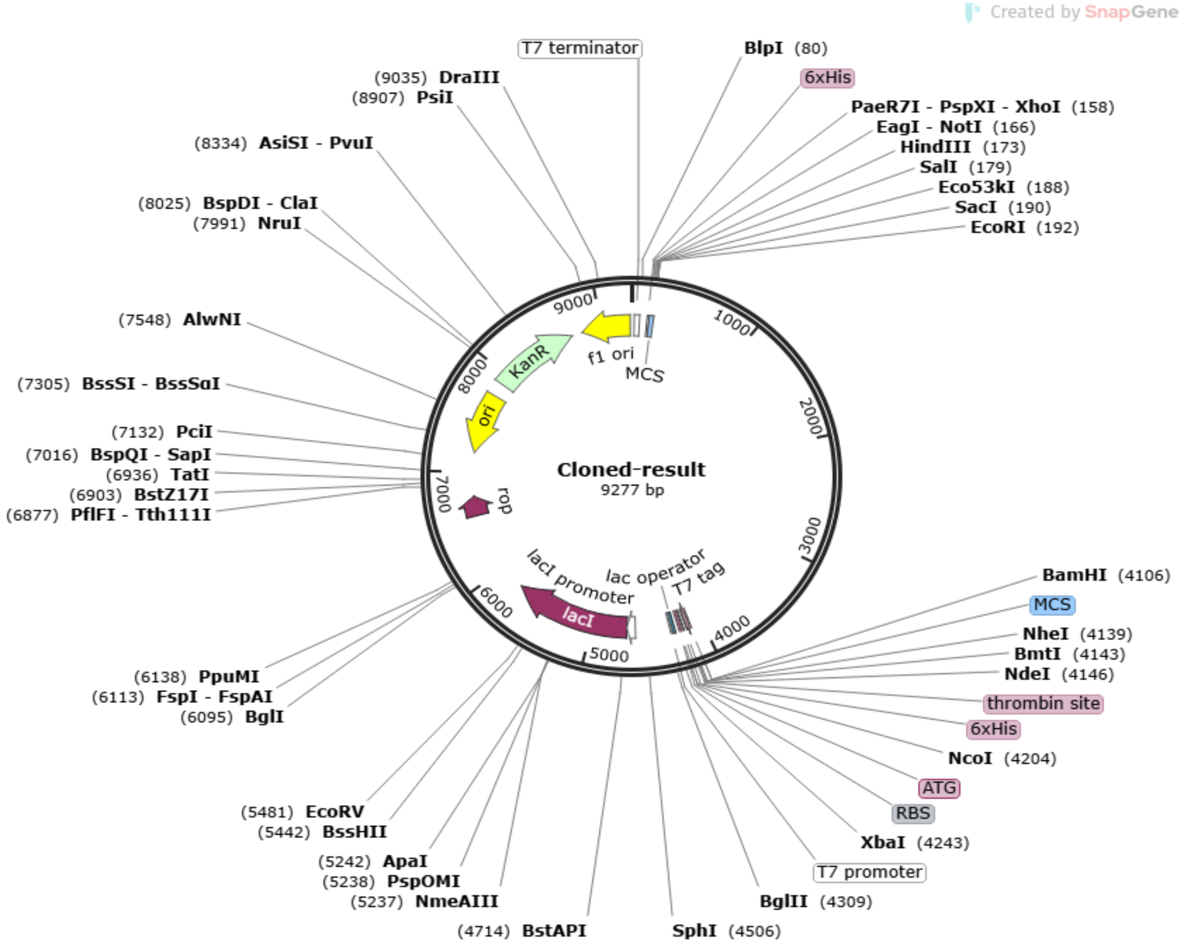

Figure 12. In silico cloning for adapted vaccine into pET28a (+) vector.
Source: Elaborated by the authors.

4. Conclusions

Tropical regions characterized by high temperatures and humidity, particularly in South Asia and Africa, are highly susceptible to malaria. Achieving malaria eradication requires the implementation of innovative control techniques. This study utilized protein sequences eliciting various T-cell (HTL and CTL) and B-cell epitopes in an immunoinformatics approach to develop a potential vaccine. The results of this investigation demonstrate that the final construct has successfully fulfilled the designed criteria for malaria vaccine development. However, this computational work necessitates experimental validation. In vitro and in vivo tests are essential to assess the immunogenicity and safety of the potential vaccine.

Authors' contribution

Conceptualization: Elijah Kolawole Oladipo; **Data curation:** James Akinwumi Ogunniran; **Formal Analysis:** Samuel Nzube Nwosu; **Funding acquisition:** Not applicable; **Investigation:** James Akinwumi Ogunniran; **Methodology:** James Akinwumi Ogunniran; **Project administration:** Not applicable; **Resources:** Not applicable; **Software:** Kehinde Oluyemi Ajayi; Oluseyi Rotimi Taiwo; **Supervision:** Elijah Kolawole Oladipo; **Validation:** Olaoluwa Kehinde Alao; Adeola Christianah Ogunwole; **Visualization:** Kemiki Olalekan Ademola; Michael Asebake Ockiya; **Writing – original draft:** James Akinwumi Ogunniran; Caleb Enejoh Omede; **Writing – review & editing:** James Akinwumi Ogunniran; Anthony Godswill Imolele.

Data availability statement

All datasets analyzed in this research are all publicly available at the National Centre of Biotechnology Information (NCBI) and the Universal Protein Resource (UNIPROT) Server. It could be made available upon request.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

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Molecular modeling and pharmacokinetics studies of sulfamidophosphonate derivatives as potential candidate against *Staphylococcus aureus*

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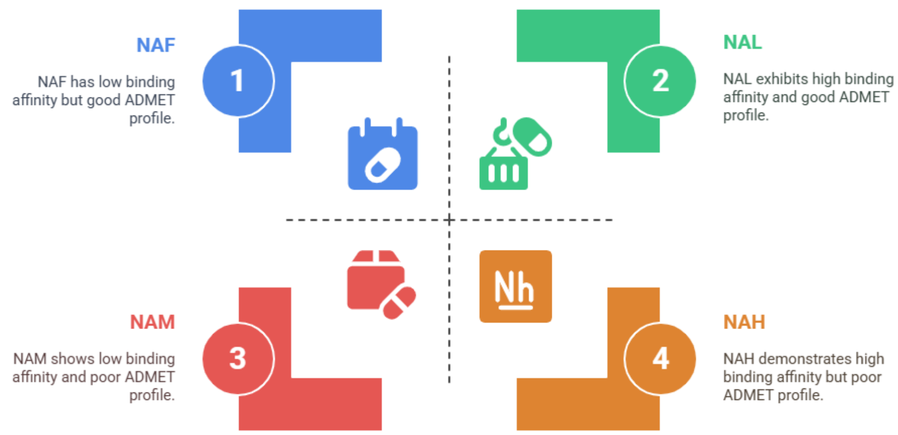
Abstract

In silico methods were used in this paper to assess the anti-bacterial activity of Sulfamidophosphonate derivatives against *Staphylococcus aureus* proteins (1XSD and 4WK3) using molecular docking and ADMET analysis. The results showed that binding affinity (ΔG kJ/mol) ranged from –4.1(NAM) to –7.1 kJ/mol (NAL) for 1XSD, and –5.0 (NAE) to –6.7 kJ/mol (NAM) for 4WK3. Therefore, compounds NAH, NAL, NAN, NAI, NAJ, NAK, 5AD and NAM could be more desirable as inhibitors than Penicillin (–6.0 kJ/mol for 1XSD and –5.4 kJ/mol for 4WK3) in the treatment of *Staphylococcus aureus*; but ADMET profile revealed that compounds NAF, NAI, NAK NAN and 5AC present attractive pharmacokinetic properties. In this study, compounds NAH, NAL, NAI and NAJ exhibited stronger affinities than the standard (penicillin) against BlaI repressor in complex with DNA (PDB ID: 1XSD) suggesting better inhibitory potential than the standard drug.



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4. ADMET.

Section Editors

Assis Vicente Benedetti

Highlights

- The antibacterial activity of sulfamidophosphonate derivatives was evaluated.
- Descriptors found from optimized sulfamidophosphonate derivatives were identified.
- Nonbonding interactions between drugs and the studied targets were observed.

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1. Introduction

Bacterial diseases such as tuberculosis, anthrax, pneumonia and osteomyelitis, among others, are deadly and have a significant impact on public health. *Staphylococcus aureus* is both a commensal bacterium and a human pathogen. About 30% of the human population is colonized with *S. aureus* (Wertheim *et al.,* 2005). In the early 1930s, doctors began to employ a more efficient test to determine the presence of an S. *aureus* infection by means of coagulase testing, which helped to detect the enzyme produced by the bacterium. Before the 1940s, *S. aureus* infections were deadly in most patients until the discovery of penicillin. However, the Meca gene carried by Methicillin-resistant *Staphylococcus Aureus* (MRSA) encodes the protein PBP-2a (penicillin-binding protein 2a) marked the outbreaks of the resistant strain of this bacterium family (Orent, 2006). The PBP-2a is a penicillin-binding protein (PBP), or an essential bacterial cell wall enzyme that catalyzes the production of the peptidoglycan in the bacterial cell wall. PBP-2A has a lower affinity to bind to beta-lactams (and other penicillin-derived antibiotics) when compared to other PBPS. The PBP-2A continues to catalyze the synthesis of the bacterial cell wall even in the presence of many antibiotics (Rasigade, 2014).

The chemistry of heterocyclic molecules plays a huge role in the development of effective drugs for bacterial, fungal and viral diseases. Heterocyclic compounds are the most important organic compounds that present great interest in medicinal chemistry (Arora *et al*., 2012; Winum *et al*., 2006*).* Sulfonamide derivatives, a class of heterocyclic compounds in which many this class compounds have been reported to show biological activities such as anti-mycobacterial, anticonvulsant, anti-hypoglycemic, anticancer, and enzyme inhibition (Farhan *et al.*, 2018; Hu *et al.*, 2008; Supuran, 2017; Zhao *et al.*, 2019). Sulfonamide derivatives are attractive due to their diverse biological targets and practical therapeutic ability with minimal side effects (Hu *et al.*, 2008; Ratchanok, 2021).

Presently, there have been a remarkable advance in the development of new potent antibiotics for combating antimicrobial resistance (Bazine *et al*., 2020a; Masters *et al.,* 2003); however, the microorganisms are getting resistant to the existing antibiotic classes by mutation of membrane permeability and spore formation to the drugs (Forgacs *et al.,* 2009) thereby adapting themselves to withstand the potency of the drug. This has led to continuous research on drug development and discovery to produce new drugs that can fight the resistance caused by *Staphylococcus Aureus*. In recent times, Sulfamidophosphonate, a sulfonamide and cyclosulfamide derivative, have been a focus of chemical and biological researchers for the development of new drugs, due to its wide range of biological and physical properties (Krátký *et al.,* 2012; Waring *et al.*, 2015). In this study, computational methods, including molecular docking and ADMET profiling to evaluate antibacterial activities of some selected Sulfamidophosphonate derivatives based on the work of Bazine *et al*. (2020b). These compounds are 1-(1,1-dioxo-1λ^6,2,5-thiadiazolidin-2-yl)(2-oxo-1,2,3,4-tetrahydroquinolin-3-yl)(1,1-diethoxyphosphonate (**5AD**), 1,1-(3-methylphenyl-sulfonamidyl)(6-methyl-3,4-dihydroquinolin-2(1*H*)-onyl)methyl)(1,1-diethoxy)phosphonate (**NAI**), 1,1-(3-methylphenyl-sulfonamidyl)(3,4-dihydroquinolin-2(1*H*)-onyl)methyl)(1,1-diethoxy)phosphonate (**NAH**), 1,1-(phenyl-sulfonamidyl)(6-methyl-3,4-dihydroquinolin-2(1*H*)-onyl)methyl)(1,1-diethoxy)phosphonate (**NAJ**), 1,1-(2-methylphenyl-sulfonamidyl)(3,4-dihydroquinolin-2(1*H*)-onyl)methyl)(1,1-diethoxy)phosphonate (**NAK**), 1,1-(3-methylphenyl)-sulfonamidyl)(2-methyl-3,4-dihydroquinolin-2(1*H*)-onyl)methyl)(1,1-diethoxy)phosphonate (**NAL**), 1,1-(3-chlorophenyl)-sulfonamidyl)(2-methyl-3,4-dihydroquinolin-2(1*H*)-onyl)methyl)(1,1-diethoxy)phosphonate (**NAM**), and 1,1-(2-hydroxylphenyl)-sulfonamidyl)(2-methyl-3,4-dihydroquinolin-2(1*H*)-onyl)methyl)(1,1-diethoxy)phosphonate (**NAN**).

2. Details of research methods

2.1. Geometry optimization of the ligands

The conformer search was carried out on each sulfamidophosphonate using the DFT method to find the conformer with the lowest energy for each compound, which was then taken as the equilibrium geometry structure of the compound. The equilibrium optimization was performed on the ligands (**Fig. 1**) with DFT in correlation with Becke's three-parameter hybrid functional with a correlation of Lee, Yang and Parr (B3LYP) (Becke, 1993; Hehre *et al.,* 1988; Parr *et al*., 1999; Yang *et al.*, 2005; Lee *et al*., 1988) with 6-31G(d,p) basis set. Quantum chemical reactivity descriptors of the sulfamidophosphonates such as frontier orbital energies, which are the highest occupied molecular orbital energy (E_{HOMO}), the lowest unoccupied molecular orbital energy (E_{LUMO}) and band energy gap ($E_{(HOMO-LUMO)}$); chemical hardness ($\eta = \frac{E_{HOMO}-E_{LUMO}}{2}$); chemical softness ($\sigma = \frac{1}{2\eta}$); chemical potential ($\mu = \left(\frac{E_{HOMO}+E_{LUMO}}{2}\right)$; global electrophilicity ($\omega = \frac{\mu^2}{2\eta} = \frac{(IP+EA)^2}{4(IP-EA)}$); electron donating power ($\omega^- = \frac{(3IP+EA)^2}{16(IP-EA)}$) and electron accepting power ($\omega^+ = \frac{(IP+3EA)^2}{16(IP-EA)}$) were estimated form the optimized structures (Flores-Holguín *et al*., 2019; Manzanilla and Robles, 2020; Ramírez-Martínez *et at*., 2023).

2.2. Molecular docking procedures

The optimized ligands were docked into the receptors by using Autodock Tool 1.5.6., AutoDock Vina 1.1.2, Edupymol version 1.7.4.4 and Discovery studio. The receptors/proteins were cleaned up and repaired with Discovery Studio software, and Edupymol was used to visualize the docking results as described (Adegbola *et al.*, 2021a; Adepoju *et al.*, 2022; Oyebamiji *et al.*, 2021). The binding pocket of the protein, as determined in the 3D structure using Discovery Studio and the Autodock tool, was used to prepare the protein. Polar hydrogens were added to the protein, followed by Kolleman charges, before setting the grid box. Using Auto Grid, the grid box center (x = –5.252, y = 9.613, z = 36.518) and size (x = 58, y = 52, z = 66) for BlaI repressor in complex with DNA (**PDB ID: 1XSD**), and (center: x = 0.043, y = 2.734, z = 85.68) and (size: x = 48, y = 40, z = 40) for **4WK3** receptor were set. The receptor and ligand were then saved as PDBQT files. The Docking simulations were then performed using Autodock Vina (Trott *et al.,* 2010) for the calculations of binding affinity, as well as studying non-bonding interactions (hydrophobic interactions) and hydrogen bonding between the ligand and proteins, which were visualized using Discovery Studio 2019.

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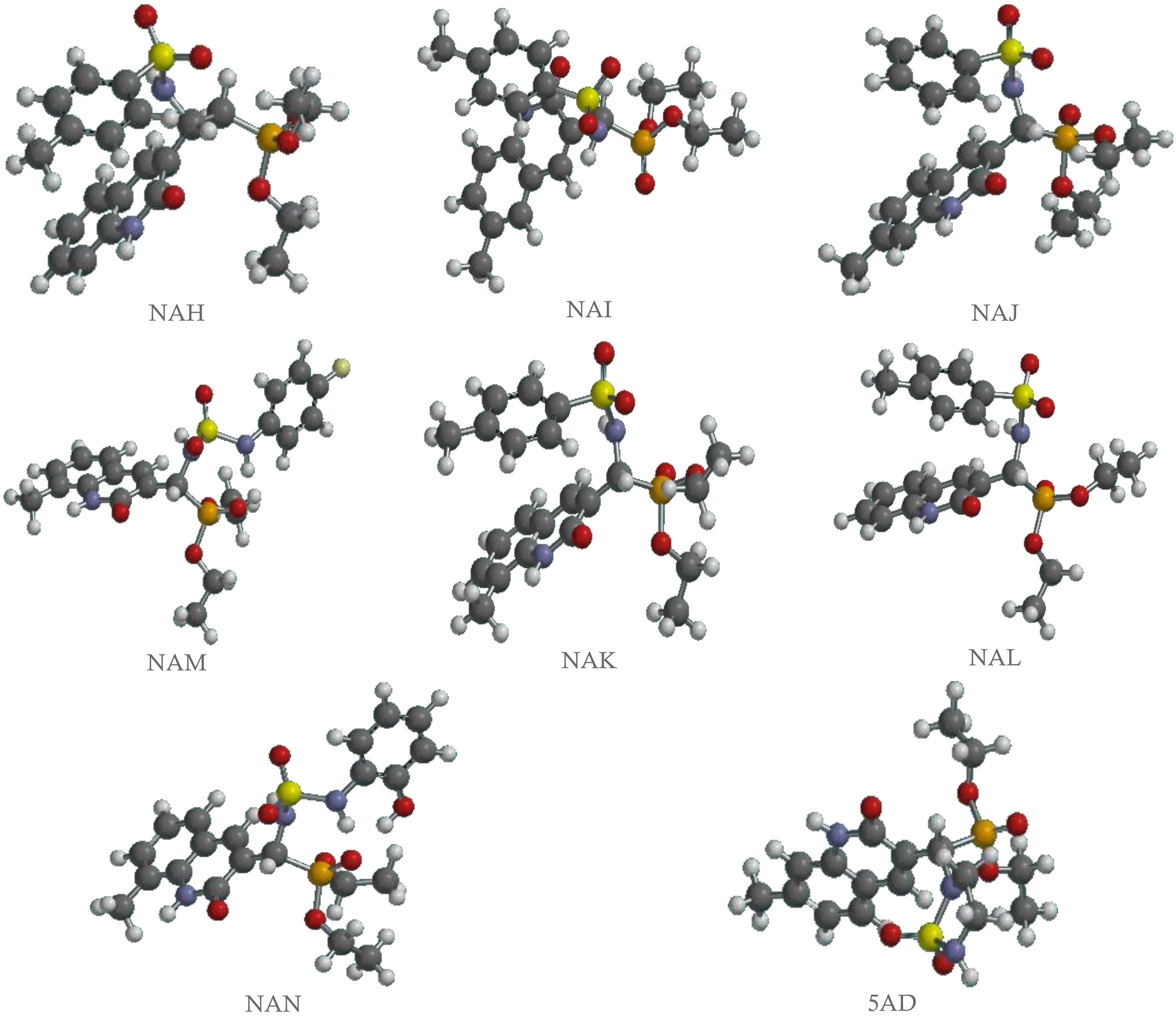

Figure 1. Optimized structures of studied sulfamidophosphonates.
Source: Elaborated by the authors.

2.3. ADME/pharmacokinetic predictions

The smiles of optimized structures of the studied ligands were used for physicochemical and ADMET profiles to assess the qualitative pharmacokinetics properties via; absorption, distribution, metabolism, excretion and toxicity by using ADMET Predictor (http://biosig.unimelb.edu.au/pkcsm/prediction). These early assessments of pharmacokinetic and toxic properties are essential step in drug discovery, although the process of drug discovery and development is a very complex, lengthy and cost-intensive; drug development failures have been attributed to poor pharmacokinetics and bioavailability (Adegbola *et al.*, 2021b; Daina and Zoete, 2016). Therefore, ADMET properties play a crucial role in every stage of drug discovery and development, aiming to reduce likely challenges associated with clinical trial treatments (Darvas *et al.,* 2002).

3. Results and discussion

3.1. Molecular quantum reactivity descriptors

The frontier orbital (the HOMO and LUMO) energies are very essential to the molecular stability and responses to the surrounding molecules, where HOMO and LUMO represent the highest occupied molecular orbital and the lowest unoccupied molecular orbital, respectively. The high HOMO energy (or low ionization energy, IP) typifies the enhancement of a ligand to donate electrons to the neighboring molecule. Lower LUMO energy, on the other hand (or high electron affinity, EA), represents a ligand's ability to accept electrons from the neighboring compounds (Oyebamiji and Semire, 2016). The lower IP values revealed that NAH (4.05 eV), 5AD (5.47 eV), and NAN (5.75 eV) should be able to donate electrons readily to the

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surrounding molecule than Penicillin (9.61 eV) and Sulfamethoxazole (5.93 eV) (Oyeneyin, 2023). Also, all the compounds, especially NAL, NAH, 5AD, NAM, and NAN, should have enhanced electron-accepting tendency from the neighboring molecule (**Table 1**). Low polarizability, low reactivity, stronger stabilization and interactions between a molecule and its receptor favor molecules with a larger energy gap (Eg), whereas a smaller energy gap favors high reactivity, high polarizability, weaker stabilization and interactions of a molecule with neighboring compound. Therefore, higher values, Eg, η and σ, showed that penicillin and Sulfamethoxazole are more stable, which may support strong interactions, although NAJ, NAI, NAK and NAM may also display strong interactions (Thanikaivelan *et al.,* 2020).

The chemical potential (μ) showed that all the sulfamidophosphonate compounds are more likely to interact strongly through electrostatic interactions with the receptor than the standard drugs (penicillin and Sulfamethoxazole). The difference between electron-donating ability (ω–) and electron-accepting ability (ω+) is |Δω|, which measures the total responsiveness of a molecule to its surroundings. Higher values typify a strong response leading to strong interactions between the molecule and its surroundings (Asibor *et al.*, 2024). Therefore, NAL, NAM, NAK, NAJ, and 5AD are likely disposed of stronger interactions with the receptor than penicillin and Sulfamethoxazole.

Table 1. Quantum chemical descriptors of sulfamidophosphonates using B3LYP/6-31G**.

	IP	EA	Eg	η	σ	μ	ω	ω-	ω+	$\lvert\Delta\omega\rvert = (\omega^+ - \omega^-)$
NAH	4.05	2.68	1.37	0.685	0.730	–3.365	8.265	10.033	6.668	3.365
NAI	6.26	1.81	4.45	2.225	0.225	–4.035	3.659	5.954	1.919	4.035
NAJ	6.11	1.55	4.56	2.280	0.219	–3.830	3.217	5.417	1.587	3.830
NAK	6.33	1.80	4.53	2.265	0.221	–4.065	3.648	5.963	1.898	4.065
NAL	6.47	4.01	2.46	1.230	0.407	–5.240	11.162	13.935	8.695	5.240
NAM	6.22	2.03	4.19	2.095	0.239	–4.125	4.061	6.385	2.260	4.125
NAN	5.75	2.03	3.72	1.860	0.269	–3.890	4.068	6.245	2.355	3.890
5AD	5.47	2.54	2.93	1.465	0.341	–4.005	5.474	7.660	3.655	4.005
Penicillin	9.61	–3.34	12.95	6.475	0.077	–3.135	0.759	3.136	0.001	3.135
Sulfamethoxazole	5.93	0.51	5.42	2.710	0.185	–3.220	1.913	3.862	0.642	3.220

Source: Elaborated by the authors.

3.2. Docking orientation and binding affinity

BlaI is a repressor, as well as a beta-lactamase that is responsible for penicillin resistance in *Staphylococcus aureus* and essential for *Staphylococcus aureus* reproduction. The BlaI repressor in complex with DNA (**PDB ID: 1XSD**) was docked with sulfamidophosphonate derivatives. Results were presented in **Table 2** and **Fig. 2**. The binding affinities for all the studied ligands with **1XSD** ranged from –17.2 (NAM) to –29.7 (NAL) kJ/mol, these inferred that inhibitory constants (Ki) of the drugs fall between 6.21 μmol/L to 984.0 μmol/L as compared to –25.1 kJ/mol for penicillin, used as standard (**Table 2**). The H-bond distances between amino acid residues in the binding purse and ligands ranged from 1.9 to 3.50 Å. They exhibited other non-bonding interactions with **1XSD** receptor (**Fig. 2**). Compound/ligand **NAL** with the highest binding affinity (–29.7 kJ/mol) is hydrogen bonded with ARG'60, TYR'69, ARG'46, ARG' 46 and THR'50 and showed interactions with ALA'27, TYR'67, TYR'69, ARG'46, THR'50 and ARG'60. **NAN** with the binding affinity of –23.8 kJ/mol displayed H-bond interactions with ARG'60 and TYR 69, and hydrophobic interactions with ARG 46, TYR'69, LYS'54, ALA'27, ARG'60 and THR'50. Also, **NAI** and **NAK** with binding affinities of –28.5 and –25.1 kJ/mol, respectively, showed only hydrophobic interactions with PHE'86, LEU'98, PHE'102, TRP'13 and ALA'83 for **NAI**, and LYS'69, ALA'83, PHE'86 for **NAK**. Ligand **NAJ** with a binding affinity of –28.0 kJ/mol hydrophobically interacted with LYS'54 ARG'60 ARG'46 TYR'69, and THR'50, as well as H-bonding with ARG'26 and ARG'60**.** Also, **NAH** presented H-Bonding with ARG'26 and ARG'60' and hydrophobic interactions with ARG'60, TYR'67, ILE'66, and LYS'43.

Furthermore, **5AD** with a binding affinity of -26.4 kJ/mol is hydrogen-bonded with **TYR'59 ARG'46** and **TYR'69** and shows hydrophobic interactions with **ARG'60 TYR'69** and **ARG'46**, while **NAM** with a binding affinity of –17.2 kJ/mol displayed hydrophobic interactions with LYS'43 **ARG'46** and **TYR'67**. Penicillin, which was used as the standard drug, has a binding energy of –25.1 kJ/mol, interacting hydrophobically with **ALA'83 ASN'17** and **PHE'86**. The ionization energy (IP = $-E_{HOMO}$) and electron affinity (EA = $-E_{LUMO}$) estimated from the DFT calculations showed that NAL has highest values for both IP (6.47 eV) and EA (4.01 eV) which led to lower energy gap; thus, supported quick electrons transition and donation to the receptor than other compounds. Therefore, NAL, NAH, NAI and NAJ exhibited the most significant inhibitory potential against **1XSD** when compared with the standard drug (penicillin).

Table 2. Binding affinity and non-bonding interactions of **1XSD** and **4WK3 receptors** with ligands.

Ligand	1XSD	4WK3
	Binding Affinity ΔG (kJ/mol)	Binding Affinity ΔG (kJ/mol)
NAH	−26.4	−24.3
NAI	−27.6	−24.7
NAJ	−28.5	−25.1
NAK	−26.8	−25.5
NAL	−27.2	−24.3
NAM	−25.9	−28.0
NAN	−23.8	−28.0
5AD	−24.3	−23.0
Penicillin	−26.4	−22.6
Sulfamethoxazole	−23.4	−22.6

Source: Elaborated by the authors.

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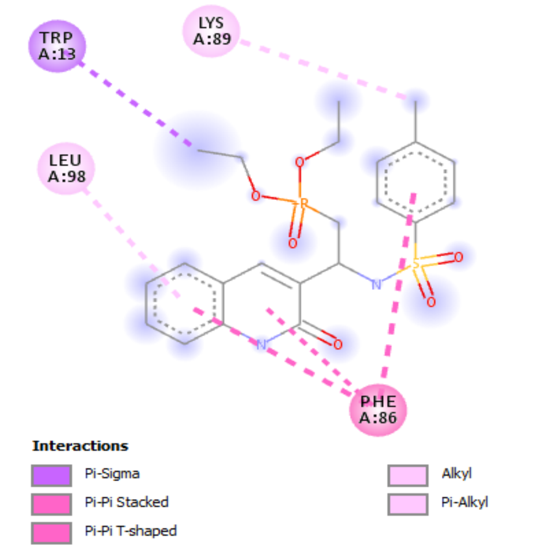
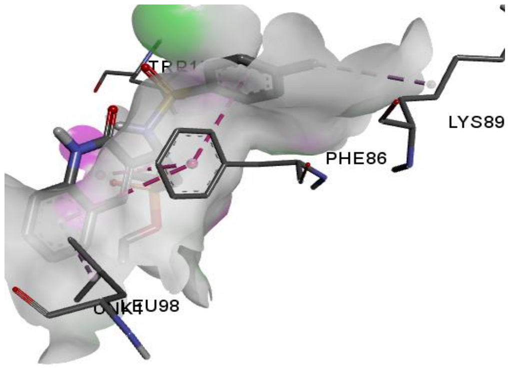
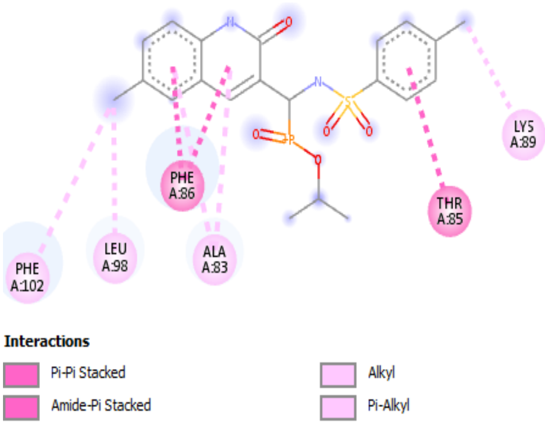
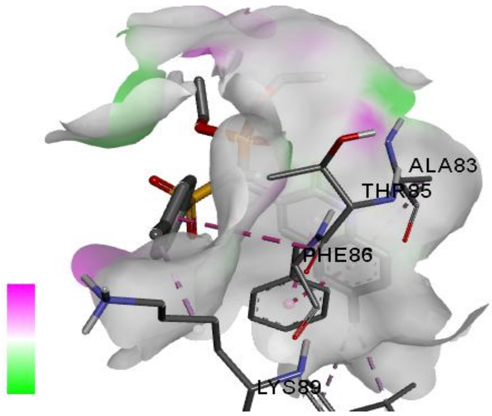
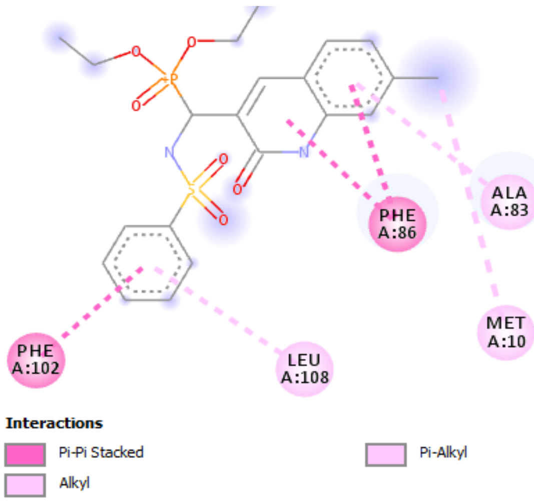
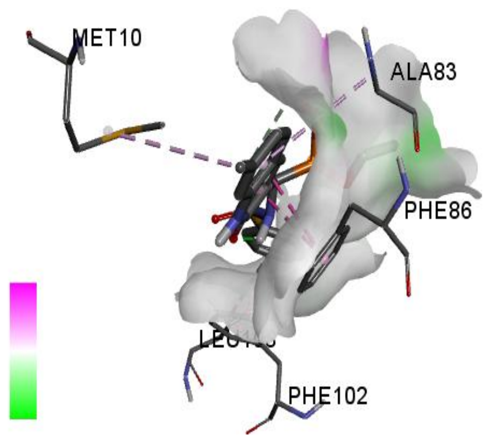
	2D Structure	3D Structure
1XSD + NAH		
1XSD + NAI		
1XSD + NAJ		

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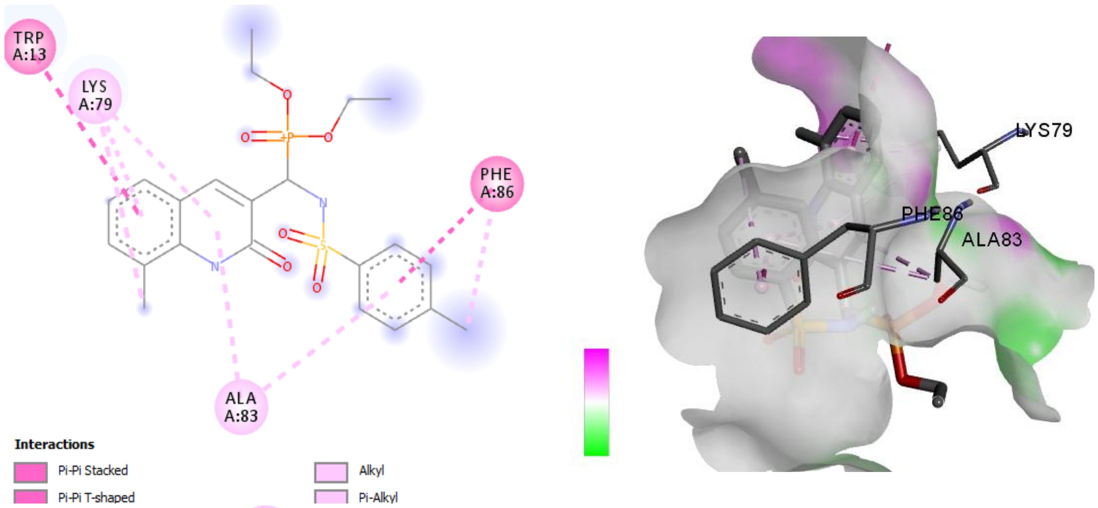
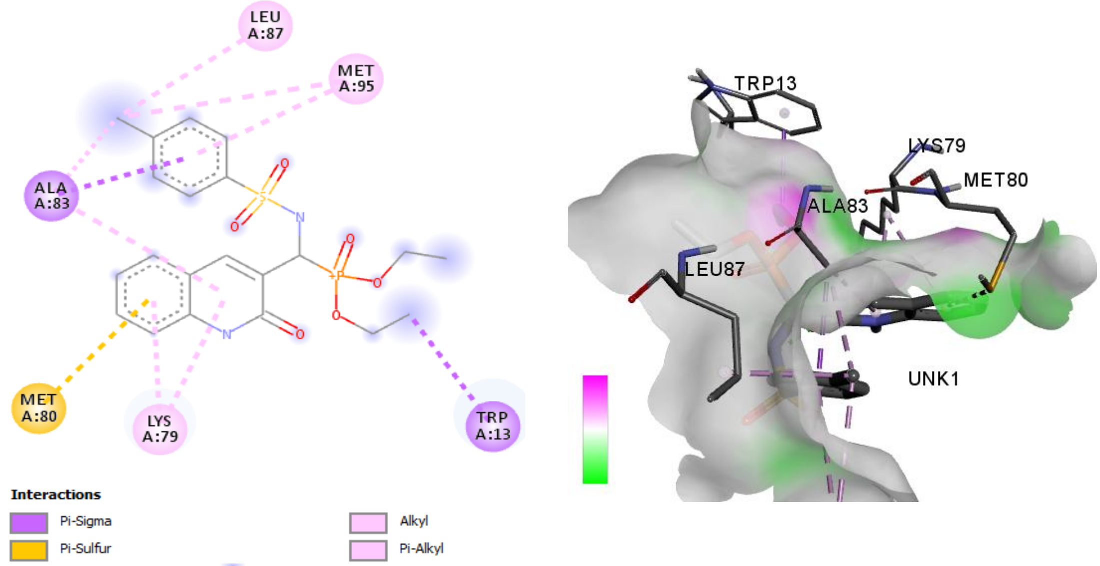
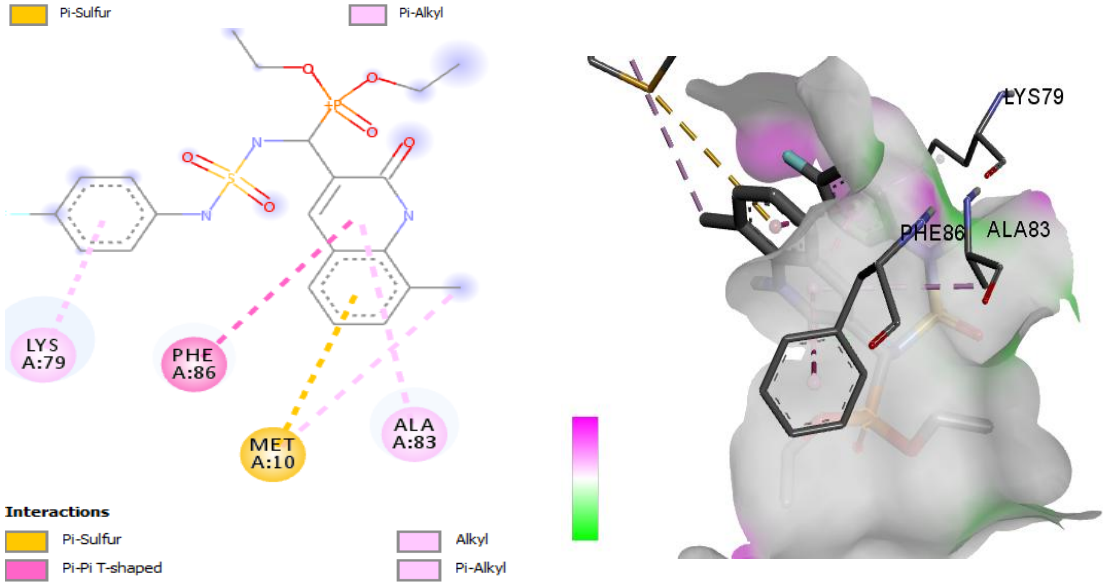
1XSD + NAK



1XSD + NAL



1XSD +NAM



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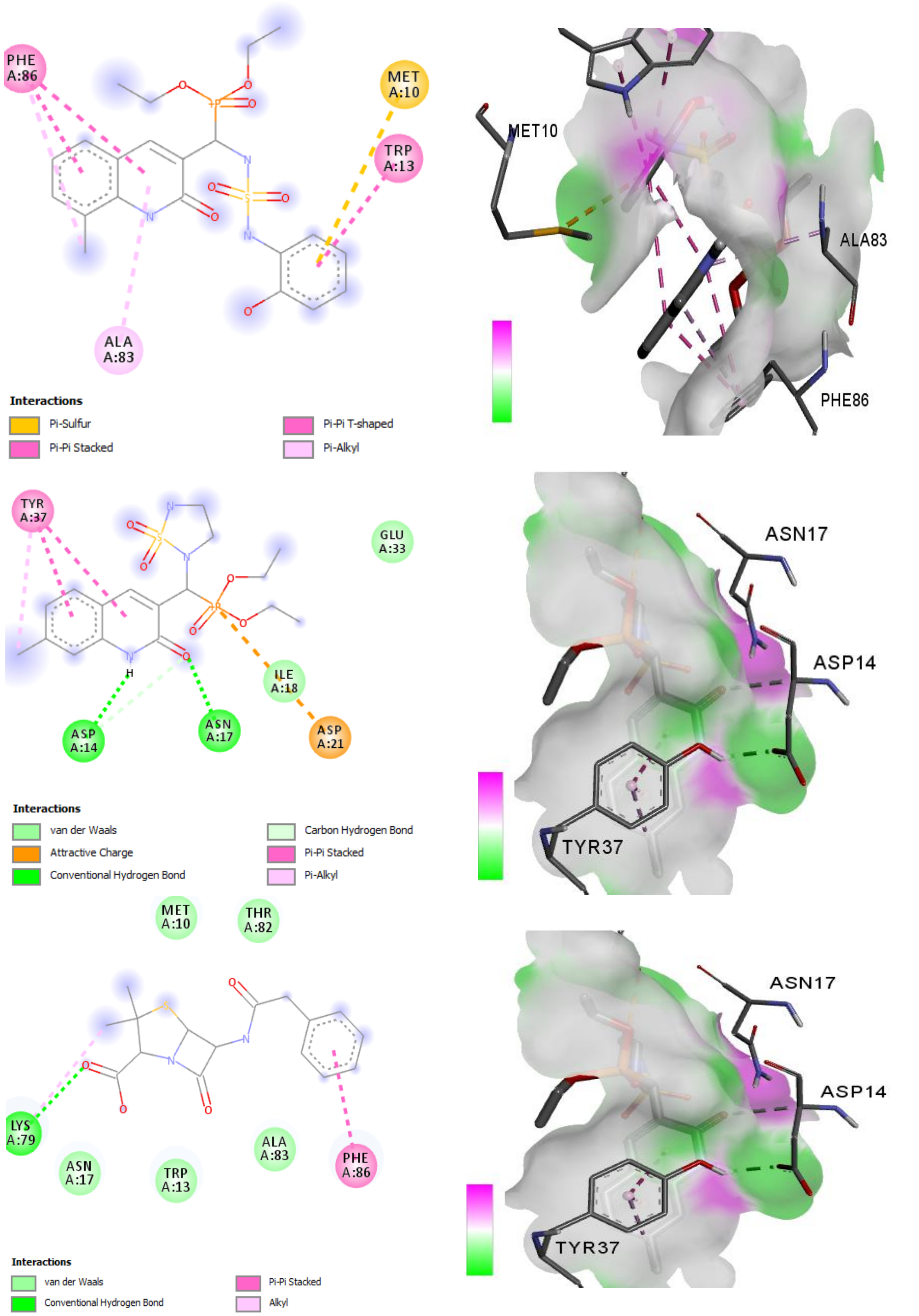
1XSD +NAN

1XSD+ 5AD

1XSD + Penicillin



1XSD + Sulfonamide

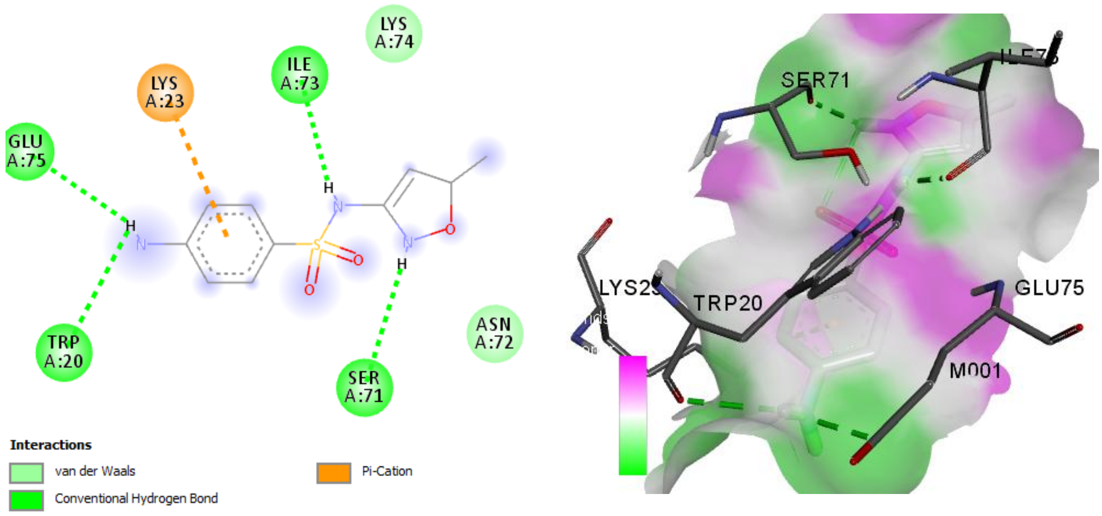
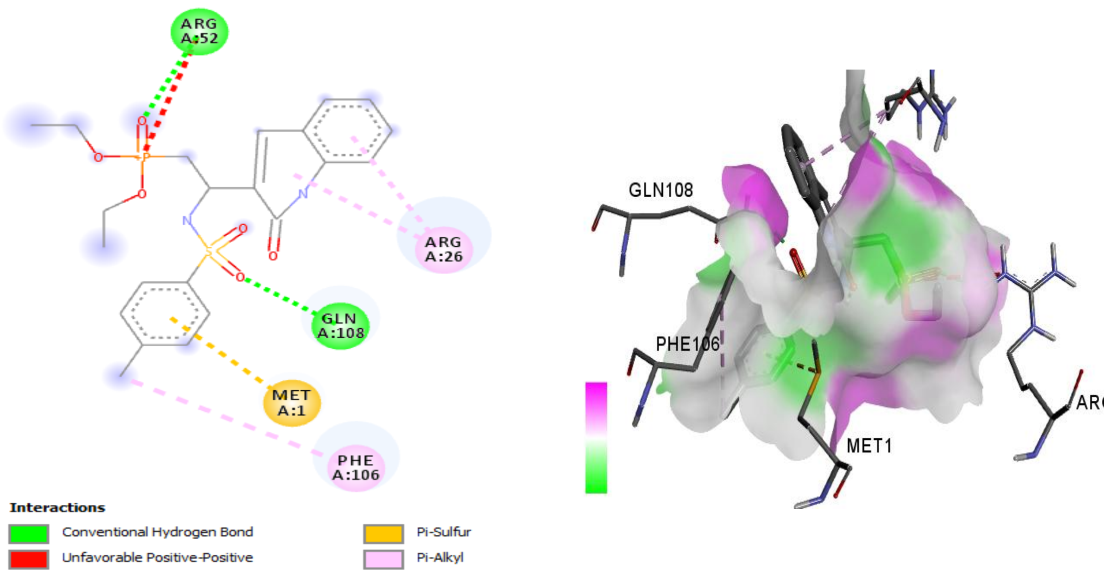


Figure 2. 2D and 3D of **1XSD** with the *s*ulfamidophosphonates showing hydrogen interactions.
Source: Elaborated by the authors.

The calculated binding affinities for the ligands with **4WK3** receptor showed that the drugs interacted with **4WK3,** the bacterial secondary messenger involved in sensing integrity, cell wall metabolism and potassium transport. The binding affinities from the docking results showed that **NAK**, **NAN**, **5AD**, and **NAH** presented binding affinities of –23.0, –24.3, –25.1, and –25.5 kJ/mol, respectively. Additionally, NAL, NAJ, NAI, and NAM bind to 4wk3 receptors with affinities of –25.5, –25.9, –27.6, and –28.0 kJ/mol, respectively (**Table 2**). The H-bond formed between **4WK3** receptor amino acid residues and the drugs is within 3.5 Å. Other hydrophobic interactions of the medications with **4WK3** are displayed in **Fig. 3**. Compound **NAK** is H-bonded with LYS'29 and also has hydrophobic interactions with ALA'17, VAL'21, LYS'29 and THR'42; NAJ is hydrogen bonded with ASN'24, GLY'47, GLN'108, ARG'26, and formed hydrophobic interactions with ARG'26, GLN'108, GLY'47, VAL'48, ARG'26 and ARG'52; **NAN** showed H-bond interactions with ASP'10 THR'42 and **LYS'29**, hydrophobic interactions with SER'13 LYS'29 and THR'42; **5AD** is H-bonded with ARG'26 GLY'47 ARG'26 ASN'24 ARG'26 and ARG'52 and also has hydrophobic interactions with CYS'46 ARG'26 ARG'52, GLN'47 and ARG'26; **NAH** showed hydrophobic interactions with GLN'14 LYS'29 SER'13 and THR'42; **NAL** ARG'26 MET'1 PHE'106 GLN'108 GLY'47 ASN'49 and VAL'48; **NAI** showed hydrophobic interactions with THR'42 GLN'14 SER'13 and LYS'29; **NAM** showed hydrophobic interactions with VAL'103 PHE'106 THR'28 GLY'47 and ARG'26; whereas Penicillin is H-bonded to ASN'61 ASN'66 and ALA'96, and hydrophobically interacted with ASN'61 ASN'66 and ALA'96 residues (**Fig. 4**). Although no direct relationship is observed between the frontier energies of the ligands and the binding affinities, those ligands with higher IP presented good binding affinities. The results revealed that compounds NAH, NAL, NAN, NAI, NAJ, 5AD and NAM exhibited significant inhibitions against **4WK3** when compared with the standard drug (penicillin).

Complex	2D	3D

4WK3 +NAH



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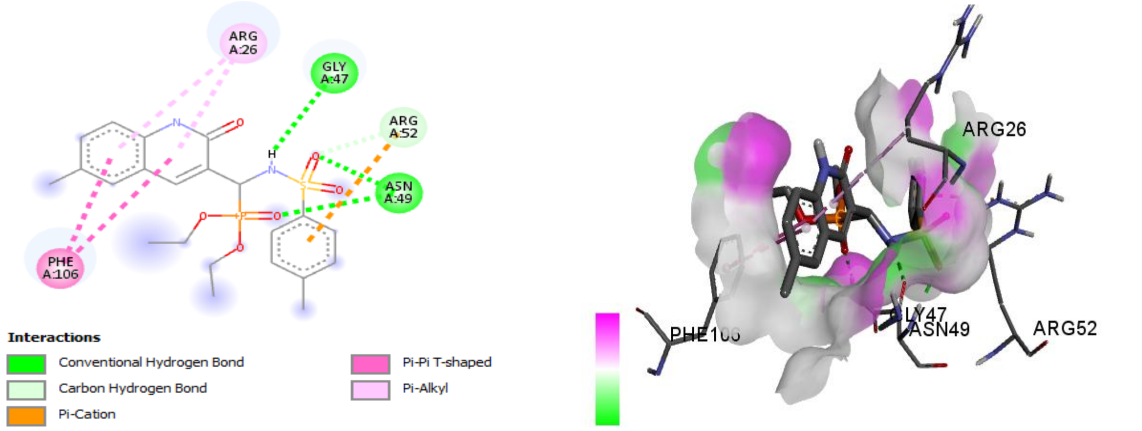
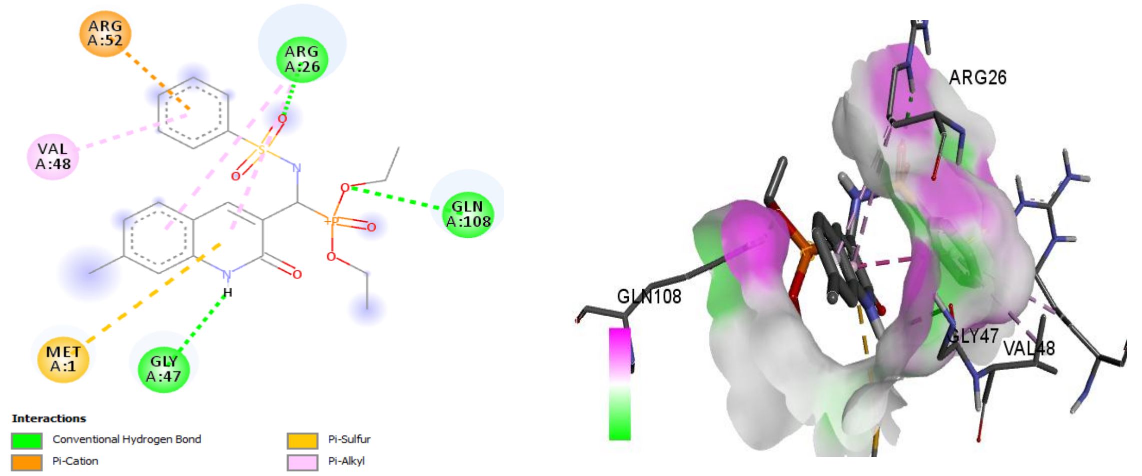
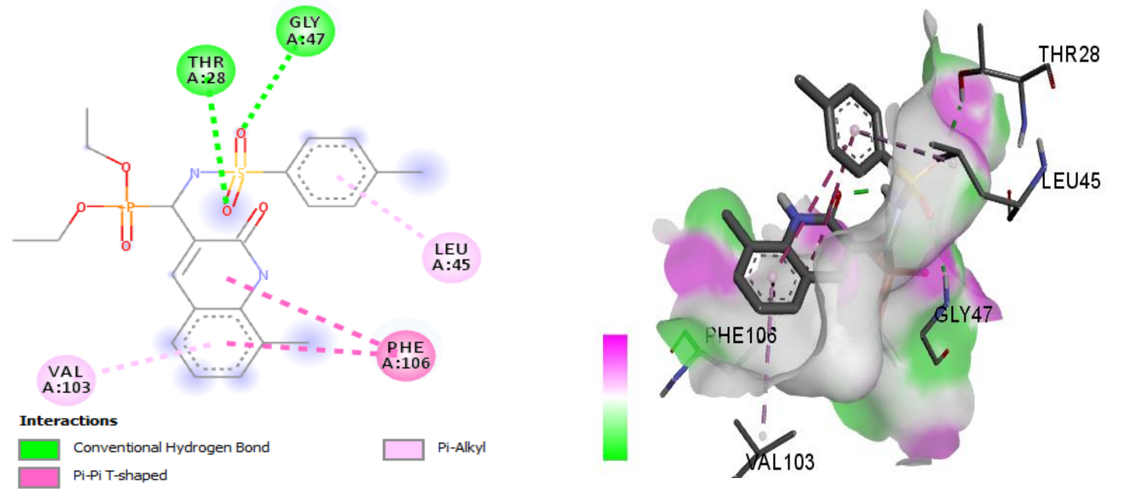
4WK3 +NAI



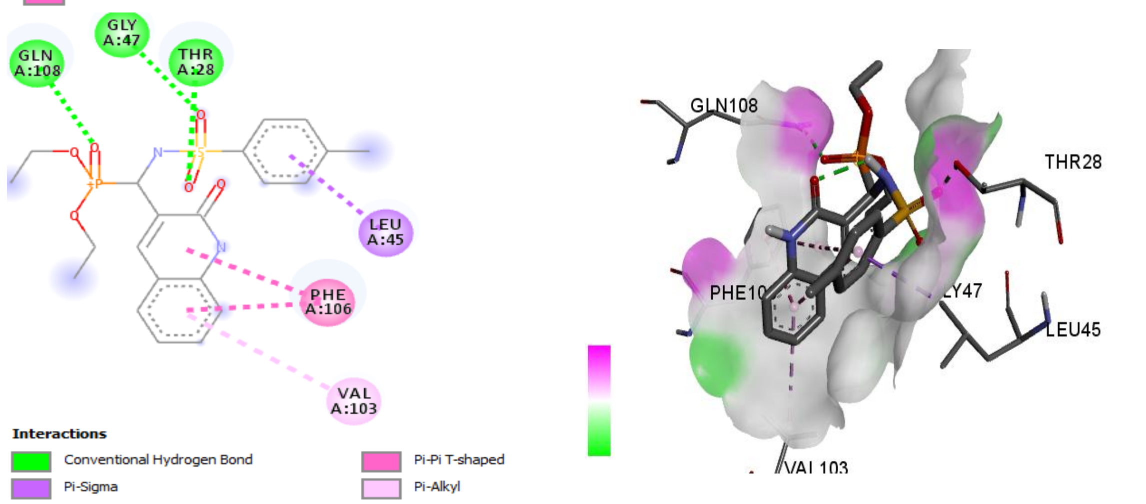
4WK3 +NAJ



4WK3 +NAK



4WK3 +NAL



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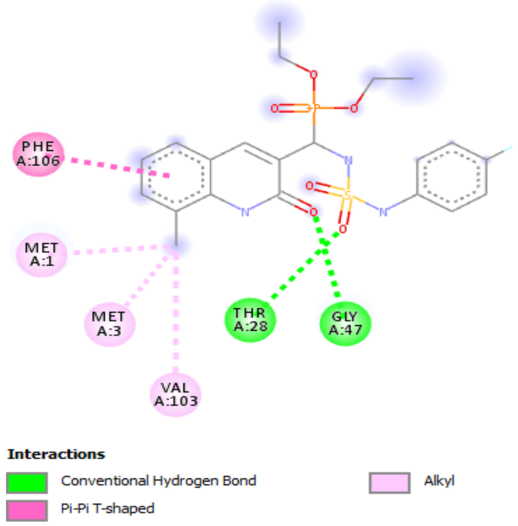
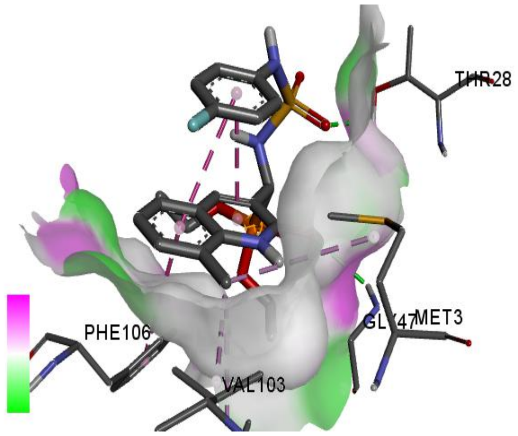
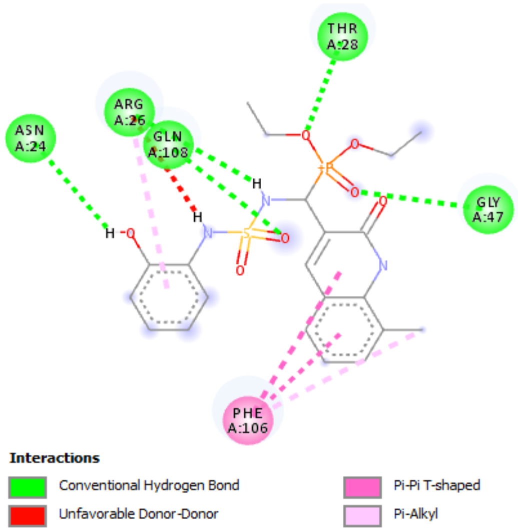
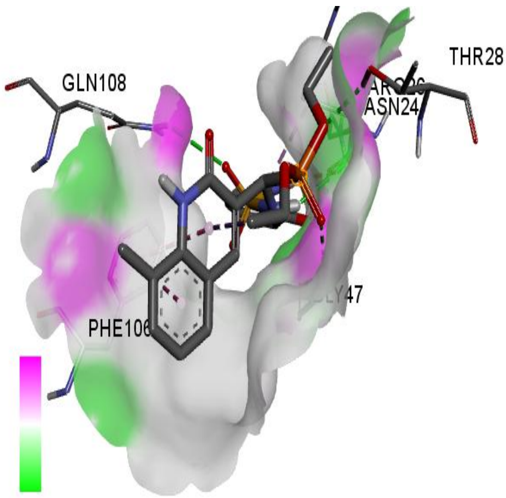
4WK3 +NAM



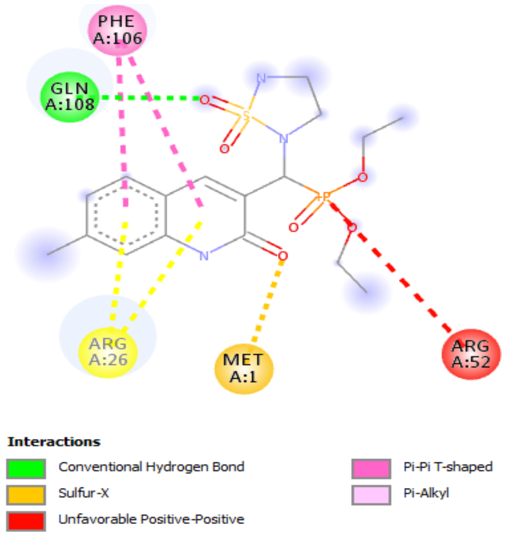


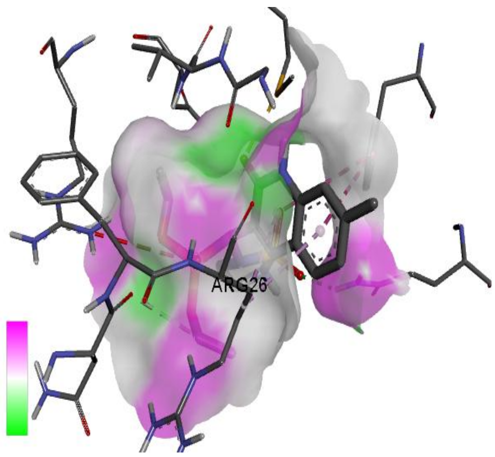
4WK3 +NAN





4WK3 +5AD





4WK3 + PENCILLIN

4WK3 + Sulfameth oxazole

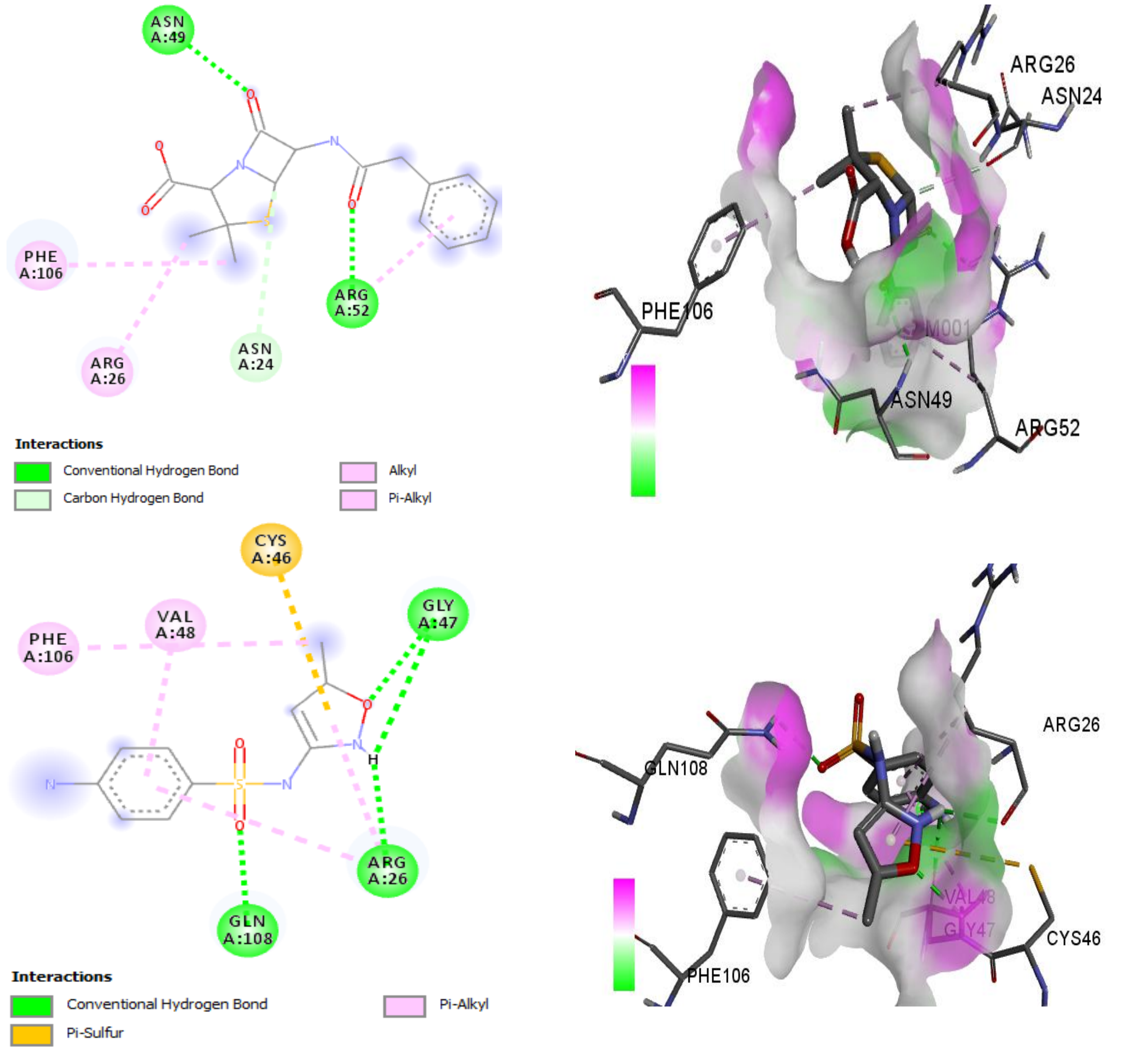


Figure 3. 2D and 3D of **4WK3** with sulfamidophosphonates showing hydrogen interactions.
Source: Elaborated by the authors.

3.3. Molecular dynamics simulation

In this study, the root mean square deviation (RMSD), root mean square fluctuation (RMSF), and actual binding energies for NAJ, NAN, and NAM, as well as the studied drug (Penicillin), were investigated and reported. As shown in **Table 3**, it was observed that NAJ proved to have the highest strength in inhibiting BlaI repressor (**1XSD**) than the studied reference compound. Additionally, the report in **Table 3** revealed that NAM has the lowest inhibitory activity compared to NAN and the reference compounds. More so, NAN with 2.3 kJ/mol as binding energy is expected to have the highest capacity to inhibit *Staphyloccus aureus* PstA (**PDB ID: 4WK3**) than NAM and the reference compound. In this work, the RMSD for NAJ-1XSD and NAN-4WK3 is presented in **Figs. 4** and **5**. As shown in the exact figures, the extent of deviation of the studied complex from its initial configuration bound to the studied receptor was examined in the calculated RMSD. The simulated system proved stable in the last five ps for the two selected compounds in **Table 3**.

Table 3. Calculated binding Energies for NAJ-1XSD, **NAM-4WK3** and **NAN-4WK3** with Penicillin-receptor complexes.

Complexes	Binding Energy Components (kJ/mol)				
	ΔE_{vdw}	ΔE_{ele}	ΔG_{gas}	ΔG_{sol}	ΔG_{bind}
NAJ-1XSD	−2.6±0.4	8±1	5±1	1.00±0.04	6.0 ± 1
Penicillin-1XSD	−29±2	−32±8	−61±6	79±9	18±3
NAM-4WK3	−2.2±0.5	−0.38±0.08	−0.38±0.08	3.5±0.3	3.1±0.4
NAN-4WK3	−2.1±0.4	0.1±0.1	1.0 ± 0.1	0.9±0.3	2.3±0.3
Penicillin-4WK3	−2.0±0.4	−0.3±0.2	−0.3±0.2	3±5	2.3±0.3

Source: Elaborated by the authors.

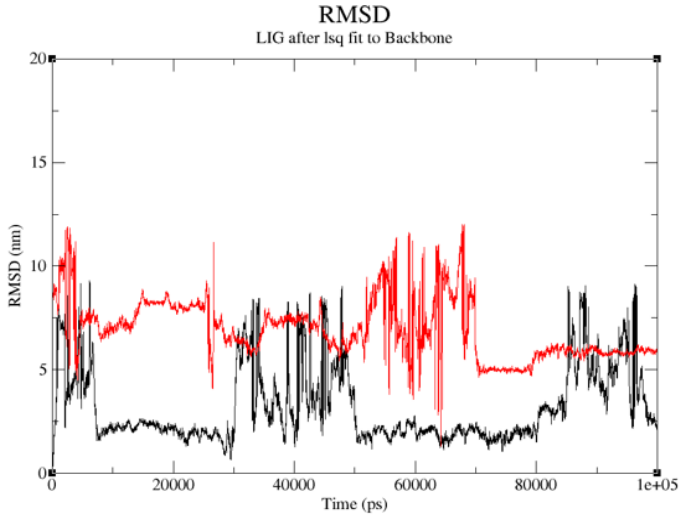


Figure 4. RMSD for **NAJ-1XSD** and **Penicillin-1XSD** complexes.
Source: Elaborated by the authors.

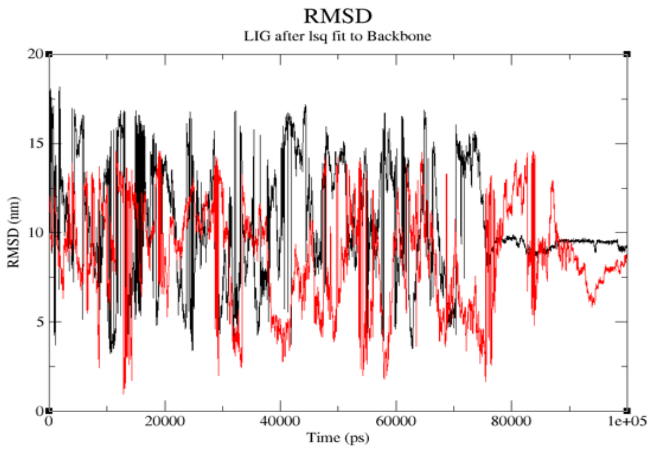


Figure 5. RMSD for **NAN-4WK3** and **Penicillin-4WK3** complexes.
Source: Elaborated by the authors.

3.3. ADME/pharmacokinetic predictions

Safety is paramount in drug usage; in this research, the physicochemical and ADMET properties of the ligands were assessed. However, there are lots of drug candidates that are not drug-like. Lipinski's rule of five (Ro5) was used to access the physicochemical properties of the ligands, which include molecular weight (150 ≤ MW ≤ 500), lipophilicity (log P ≤ 5), number of rotatable bonds (ROTBs ≤ 10), hydrogen bond donor (HBD ≤ 5) and hydrogen bond acceptor (HBA ≤ 10) (Daina *et al.*, 2017; Lipinski *et al.*, 2004). All the compounds and the standard drugs obeyed Lipinski's rule for hydrogen bond donors, hydrogen bond acceptors and molecular weight and WLogP. They are moderately soluble in water, whereas standard medicines are highly soluble. However, all the ligands have TPSA values between 132.75 and 165.01 Å, which are higher than 131.6 Å as the upper limit (**Table 4**).

Lack of efficacy and safety are the two major causes leading to drug failure (Sun *et al.*, 2022), which means the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of chemicals play vital roles in every stage of drug discovery and development. *Hence, using ADMET profiling is considered* one way to reduce likely challenges associated with clinical trial treatments (Daina and Zoete, 2016; Darvas *et al.*, 2002). Compounds NAH, NAI, NAJ, NAK, NAL, NAM, NAN and 5AD were inhibitors to CYP3A4; therefore, they may cause an elevation in the concentration of the corresponding compounds, leading to drug overdose (Conrad *et al.*, 2024). Additionally, all the compounds except compound NAN exhibit high GI absorption, and none of the compounds possess blood–brain barrier BBB penetration. NAC, NAD, NAE, NAG, NAN, NAF, NAK, NAI, and NAM were identified as Pgp substrates (i.e. they can be transported out of the cell. Additionally, compounds NAD, NAE, and NAF could be inhibitors of Cyp2C19, while compounds NAC, NAG, NAN, NAK, 5AC and NAM could be substrates for Cyp2C19. None of the compounds could be inhibitor of Cyp2C9, and NAC was an inhibitor of Cyp2D6 (**Table 5**). The ADMET results revealed that NAF, NAI, NAK NAN and 5AC have attractive pharmacokinetic properties (**Table 5**).

Table 4. Physicochemical properties of sulfamidophosphonates.

Drugs	Mw g/mol	Heavy Atoms	Aromatic Heavy Atoms	Fraction Csp3	Rotatable Bonds	HBA	HBD	Molar Ref	TPSA	Lipinski violation	Log P (o/w)	Bio Score	H_2O solubility
NAH	478.50	32	16	0.32	10	7	2	124.79	132.75	0	3.03	0.55	Mod soluble
NAI	478.50	32	16	0.32	9	7	2	124.94	132.75	0	3.29	0.55	Mod soluble
NAJ	464.47	31	16	0.29	9	7	2	119.98	132.75	0	2.94	0.55	Mod soluble
NAK	478.5	32	16	0.32	9	7	2	124.94	132.75	0	3.33	0.55	Mod soluble
NAL	478.48	32	16	0.24	9	6	3	126.71	146.72	0	2.84	0.55	Mod soluble
NAM	497.48	33	16	0.29	10	8	3	125.11	144.78	0	3.23	0.55	Mod soluble
NAN	495.49	33	16	0.29	10	8	4	127.18	165.01	0		0.55	Mod soluble
5AD	429.43	28	10	0.47	7	8	2	114.55	135.96	0	1.49	0.55	Soluble
Penicillin	334.39	23	6	0.44	5	4	2	90.54	121.13	0	1.39	0.55	Soluble
Sulfameth-oxazole	253.28	17	11	0.1	3	4	2	62.99	106.6	0	-0.15	0.55	Soluble

Source: Elaborated by the authors.

Table 5. Pharmacokinetics properties of sulfamidophosphonates.

Drugs	GI Absorption	BBB Permeant	p-gp Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log K_p (skin Permeation) cm/s
NAH	Low	No	Yes	No	Yes	Yes	No	Yes	−7.28
NAI	Low	No	No	No	Yes	Yes	No	Yes	−6.95
NAJ	Low	No	No	No	Yes	Yes	No	Yes	−7.12
NAK	Low	No	No	No	Yes	Yes	No	Yes	−6.95
NAL	Low	No	Yes	No	No	No	No	Yes	−7.25
NAM	Low	No	Yes	Yes	No	No	No	Yes	−7.45
NAN	Low	No	Yes	No	No	No	No	Yes	−7.76
5AD	High	No	Yes	No	No	No	No	No	−8.63
Penicillin	High	No	No	No	No	No	No	No	−6.75
Sulfameth oxazole	High	No	No	No	No	No	No	No	−6.93

Source: Elaborated by the authors.

4. Conclusions

This study emphasized an *in-silico* assessment of anti-bacterial activities of sulfamidophosponate derivatives as compared with standard drugs (Penicillin and Sulfamethoxazole) against target proteins of *Staphylococcus aureus* via molecular docking and ADMET prediction. In this study, compounds NAH, NAL, NAI and NAJ exhibited stronger affinities than the standard (penicillin) against BlaI repressor in complex with DNA (PDB ID: **1XSD**), suggesting a better inhibitory potential than the standard drug. Furthermore, compounds NAH, NAI, NAJ, NAL, NAM, NAN and 5AD potentially inhibit **4WK3** than the standard drug (penicillin). These compounds could be inhibitors with potential for treating bacterial infection. Additionally, the ADMET results revealed that compounds NAG, NAE, NAH, and NAK have attractive pharmacokinetic properties. In general, this study demonstrated that compounds NAF, NAI, NAK and NAN could be potential inhibitors against *Staphylococcus aureus,* and they possess good pharmacokinetic properties, which are probable for drug discovery.

Authors' contribution

Conceptualization: Abimbola Modupe Olatunde, Kehinde Gabriel Obiyenwa; **Data curation**: Nathaniel Oladoye Olatunji; Kehinde Gabriel Obiyenwa; **Formal Analysis**: Nathaniel Oladoye Olatunji; Kehinde Gabriel Obiyenwa; **Funding acquisition**: Abimbola Modupe Olatunde, Kehinde Gabriel Obiyenwa; **Investigation**: Tofunmi Emmanuel Oladuji, Dayo Felix Latona; **Methodology**: Abimbola Modupe Olatunde, Kehinde Gabriel Obiyenwa; **Project administration**: Abel Kolawole Oyebamiji; Banjo Semire; **Resources**: Tofunmi Emmanuel Oladuji, Dayo Felix Latona; **Software**: Abel Kolawole Oyebamiji; Banjo Semire; **Supervision**: Abel Kolawole Oyebamiji; Banjo Semire; **Validation**: Tofunmi Emmanuel Oladuji, Dayo Felix Latona; **Visualization**: Abel Kolawole Oyebamiji; Banjo Semire; **Writing – original draft**: Abel Kolawole Oyebamiji; Banjo Semire; **Writing – review & editing**: Abel Kolawole Oyebamiji; Banjo Semire.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Overview of difficulties and material identification of chemical bonds based on multiple representations: Teacher's view

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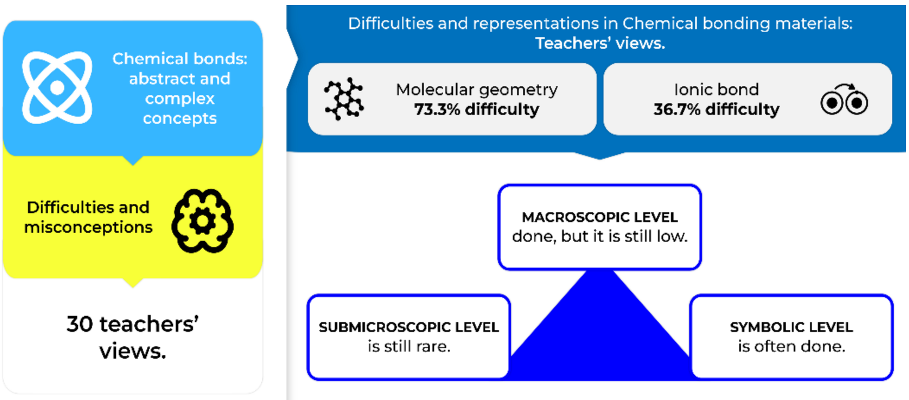
Abstract

Chemical bonding involves three levels of representation, namely macroscopic, submicroscopic, and symbolic, which are often difficult for students to understand due to their abstract and complex concepts. In this case, teachers play an important role as facilitators. However, teachers still experience various challenges in applying a multiple representation approach. This study aims to identify and analyze the difficulties in multi-representation-based chemical bonds based on the views of several chemistry teachers. A total of 14 open and closed question items via Google Forms were distributed online. Based on a survey of several chemistry teachers, the most difficult sub-material in chemical bonds is molecular geometry, while the easiest is ionic bonds. Chemistry teachers participating in this study tend to focus more on symbolic, macroscopic, and submicroscopic representations in teaching chemical bonds.



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1. chemical bonding representations;
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Highlights

- The method used was a survey method with 30 open and closed-question items.
- The molecular geometry sub-material is considered quite difficult for respondents.
- Explanations linking chemical bonding material with multiple representations are few.
- The findings are an identification of multi-representation-based chemical bonding.

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1. Introduction

Chemistry is a scientific discipline that focuses on studying the characteristics, structure, and changes in matter and energy (Widarti, 2021; Widarti *et al.*, 2025). Several concepts in chemistry create difficulties and different perceptions for students because abstract concepts dominate chemistry (Widarti *et al.*, 2018). In addition, there are several difficulties in learning chemistry based on its characteristics, namely: **a)** Chemistry simplifies the truth, resulting in a gap between understanding and applying concepts, **b)** Chemistry has a dynamic and rapidly evolving nature, making the materials studied in chemistry very complex, **c)** Chemistry can be applied in everyday life (Ahmar *et al.*, 2020).

Chemical bonds are one of the chemical materials studied in high school. Generally, chemical bonding is grouped into several sub-materials, including ionic bonds, covalent bonds, metal bonds, molecular geometry, and intermolecular forces (Dawati *et al.*, 2019). The concept of chemical bonding material is abstract and complex, so students have relative difficulty understanding the sub-subjects in chemical bonds (Widarti *et al.*, 2018). The research results of Sari *et al.* (2020). It shows that students have difficulty understanding the concept of chemical bonding. The percentages of each subconcept are as follows: stable electron configuration at 35.8%; valence electrons at 35.1%; ionic bonds at 55.4%; covalent bonds at 58.7%; and metal bonds at 54.3%. Yakina *et al.* (2017) also, show other research results on student learning difficulties in chemical bonding. The study shows that students had trouble in the term category of 48.99%, the 41.32% concept category, and the 70.97% calculation category. Based on this description, it can be concluded that students have not fully understood chemical bonding. Students' difficulties can stem from the threshold concept that students do not understand. If the concepts in chemistry are not well understood, students will experience learning difficulties, one of the impacts of which is the occurrence of conceptual errors or commonly called misconceptions (Meltafina *et al.*, 2019).

Misconceptions are one of the barriers to mastering concepts that need to be minimized (Hasanah *et al.*, 2024). Misconceptions are still a problem in the learning process because they can reduce the effectiveness of student learning and hinder students from mastering further concepts. If misconceptions are not addressed immediately, it will result in students' difficulties in understanding more complex concepts (Meltafina *et al.*, 2019). Based on the results of the research conducted by Setiawan and Ilahi (2022) that chemical bonding causes quite many misconceptions, namely the subconcepts of stability of electrons, the Lewis structure, duplet rules, octets and their exceptions, metallic bonds and metallic properties, VSEPR theory, van der Walls, ionic and covalent compounds. A deep understanding of chemical bonding is needed to minimize the percentage of misconceptions among students (Widarti *et al.*, 2018). Using appropriate chemical representations in the learning process can reduce students' misconceptions and help students understand chemical concepts as a whole (Hasanah *et al.*, 2024).

Chemistry deals with three levels of representation, namely the macroscopic level, which refers to what can be observed, the submicroscopic level, which relates to events at the molecular level, and the symbolic level, which refers to how the phenomenon is symbolized (Danin and Kamaludin, 2023; Widarti, 2021). Therefore, chemistry will be easier to understand if students can represent it in three levels of representation Hasanah *et al.*, 2024; Meltafina *et al.*, 2019; Siregar and Wiyarsi, 2023). In this case, teachers play an important role as facilitators to help students integrate the three levels of representation (Head *et al.*, 2017). The role of the teacher is vital in helping students see the relationship between these three levels so that students can form a deeper and more comprehensive understanding. However, teachers still experience various challenges in applying a multiple representation approach. Limited time and resources in the classroom, which often do not allow for an optimal variety of representations, are also a challenge for teachers (Li and Arshad, 2014).

Based on the background described the ability to use multiple representations must be developed in chemistry learning, especially chemical bonds. This study aims to analyze the difficulties and identify multiple representations of chemical bonds from the teacher's point of view. It is expected to provide insight into research needs and opportunities to assist chemistry teachers in overcoming difficulties and identifying chemical materials.

2. Experimental

This research uses a survey method. The research instrument contains 14 items of open and closed questions about learning chemical bonding material in schools conducted by chemistry teachers. The instrument that has been developed is then disseminated online through Google Forms. The survey instrument grid used is presented in **Table 1**.

Table 1. Survey instrument grid.

Main component	Indicator	Number and Type of Questions
Chemical bonding material	Easy and difficult chapters	– 3 closed questions with a scaled answer – 2 closed questions with more than 1 answer choice
Multiple representation approach	The use of representation in learning	– 4 closed questions with a scaled answer – 1 closed question with more than 1 answer choice
	Media and learning resources that support the multiple representation approach	– 3 closed questions with a scaled answer – 1 closed question with more than 1 answer choice
Total		**14 Questions**

Source: Elaborated by the authors.

Respondents who filled out the survey were 30 chemistry teachers. The data obtained were analyzed using quantitative data analysis techniques. Quantitative data was obtained from the results of questionnaires distributed to respondents. The data that has been collected is then analyzed in several stages. First, the data collected from respondents was downloaded in spreadsheet format to facilitate further processing and analysis. Next, the data was analyzed using descriptive statistics to get an overview, such as the percentage and average of each answer. For quantitative questions, percentages were used to understand respondents' tendency patterns. The results of each analysis are displayed in graphs and tables to facilitate interpretation and draw conclusions relevant to the research objectives. The teacher demographic data is shown in **Table 2**.

Table 2. Teacher demographic data.

Demographics	Amount		%
Gender	Male	10	33.3
	Female	20	66.7
School Location	Java	29	96.7
	Outside Java	1	3.3

Source: Elaborated by the authors.

3. Results and Discussion

3.1. Difficulty in chemical bonding material

Students' difficulties in learning chemistry are in line with the characteristics of chemistry itself, including that most of chemistry is abstract, sequential and rapidly developing, chemistry is a simplification of the actual material, and the material studied is very complex (Halim *et al.*, 2013; Mindayula and Sutrisno, 2021; Siregar and Wiyarsi, 2023; Widarti, 2021). In studying chemistry, students need to have several abilities, including the ability to think formally, memorize, perform mathematical operations, and have spatial intelligence (Rahmawati *et al.*, 2021).

Several students expressed their difficulties in learning chemistry, including the influence of the teacher, materials, media, and learning methods applied by the teacher. In addition, students' challenges in chemistry learning are due to the abstract nature of chemical concepts, which teachers also recognize (Halim *et al.*, 2013; Hasanah *et al.*, 2024; Widarti *et al.*, 2018). This is in line with the results of a survey conducted by researchers, which found that 56.7% of chemistry teachers stated that chemical bonding material was tricky. The researcher also surveyed the level of difficulties experienced by chemistry teachers in several sub-materials of chemical bonds, namely ionic bonds, covalent bonds, metal bonds, physical properties of chemical bonds, molecular geometry, and intermolecular forces. The survey data on the chemistry teacher's difficulty level with the sub-materials of the chemical bonds is shown in Fig. 1 and 2.

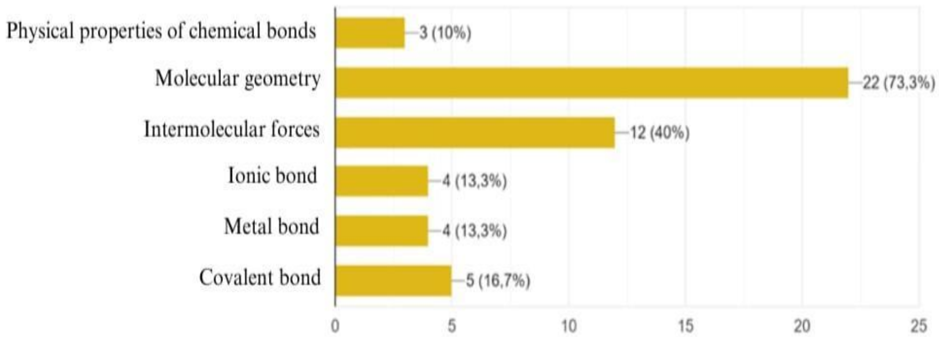

Figure 1. The most difficult sub-material of chemical bonds according to chemistry teachers.
Source: Elaborated by the authors.

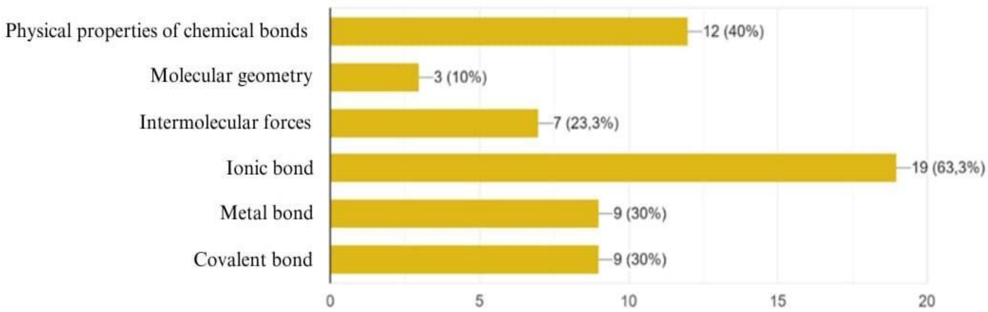

Figure 2. The easiest sub-material of chemical bonds, according to chemistry teachers.
Source: Elaborated by the authors.

Based on **Fig. 1**, as many as 73.3% of chemistry teachers consider molecular geometry the most difficult sub-material concerning chemical bonds. Molecular geometry is an essential sub-material in chemical bonds because it has a role in determining the physical and chemical properties of the molecule (Nugraha *et al.*, 2019). Molecular geometry is a challenging topic in terms of conceptual understanding and teaching. The relevance of this data can also reflect the challenges chemistry teachers face in delivering this material to students. In the molecular geometry sub-material, if students only understand through Lewis structure, they will have difficulty distinguishing the shapes of molecules in a compound. For example, the compounds CO_2, SO_2, and H_2O have identical bond pairs but have different molecular shapes. If students are only shown the Lewis structure, they may assume that the three compounds have the same shape. This is in line with the research of Siregar and Wiyarsi (2023), which states that teaching abstract concepts such as molecular geometry requires representational skills, including the ability to visualize things that cannot be seen directly and to create 3D visualizations of molecular geometry.

Molecular geometry is considered difficult by chemistry teachers because the material is more complex in linking other sub-subjects (Nugraha *et al.*, 2019; Siregar and Wiyarsi, 2023). In addition, chemistry teachers often experience difficulties when teaching molecular geometry due to the limitations of media to explain the shape of molecules in 3D versions, so students will have trouble imagining the shape of molecules if they are only depicted in 2D form on a whiteboard or image in PowerPoint. This will have an impact on students' understanding to understand the sub-material of molecular geometry. Some students do not understand the Lewis structure and valence electrons, so it is difficult to imagine the shape of a molecule. Then, the molecular geometry material requires imagination, which must be expressed visually, but the learning media used by the teacher are inadequate.

The difficulty of the molecular geometry sub-material can be observed, for example, when determining the molecular geometry of PF_5. The first thing to do is identify the number of valence electrons of each atom involved. In PF_5, there are 5 valence electrons from the P atom and 7 valence electrons from each F atom, so the total number of valence electrons is 40. Then, the central atom is determined by looking at the more electropositive atom than the F atom; the P atom becomes the central atom. **Figure 3** shows the Lewis structure of PF_5.

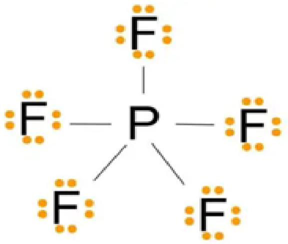

Figure 3. Lewis structure of PF_5.
Source: Elaborated by the authors.

From the Lewis structure of PF_5 above, it can be observed that all the valence electrons of the P atom are paired in the F atom so that there are no lone pairs of electrons in the P atom. Three Fluorine atoms are in the equatorial position, while the other two are in the axial position (Effendy, 2017). This arrangement of atoms forms the trigonal bipyramidal geometry of the PF5 molecule. Thus, in determining the molecular geometry of PF5, it is necessary to understand interrelated concepts such as valence electrons and Lewis structure, so molecular geometry becomes a difficult sub-subject matter.

Meanwhile, when reviewed based on **Fig. 2**, which is the result of a survey of the level of ease of chemistry teachers in teaching sub-material on the topic of chemical bonds, it is known that the most significant percentage, namely 63.3% of chemistry teachers stated that the ionic bonding sub-material is easier to teach to students than other sub-materials. This can be based on the concept of ionic bonds, which is quite simple compared to other sub-matter. Ionic bonds can be easily explained to students by looking at and distinguishing the constituent elements that are bonded to each other, and students can also easily determine which ion is charged.

Ionic bonds, if taught to students, will be easier to understand because examples of compounds and material cores of ionic sub-bonds can be reviewed directly by students in daily life, such as table salt (NaCl). In the NaCl compound, the Na atom has 1 valence electron, so it tends to give up electrons while the Cl atom has 7 valence electrons so it tends to accept 1 electron to fulfill the octet rule (Effendy, 2020). **Figure 4** shows the Lewis structure of NaCl.

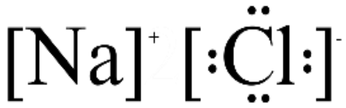

Figure 4. Lewis structure NaCl.
Source: Elaborated by the authors.

3.2. Identification of chemical bonds based on multiple representations

The multiple representation approach in chemistry learning utilizes various forms of representation to help students understand abstract chemical concepts (Danin and Kamaludin, 2023; Hasanah *et al.*, 2024; Widarti, 2023). A comprehensive understanding of chemistry is directly linked to the comprehension of macroscopic, submicroscopic, and symbolic representations, as well as the relationships between these three forms (Widarti, 2021). Multiple representations are needed in every chemical material, especially in chemical bonds.

3.2.1. Macroscopic level

Macroscopic is a chemical representation that looks real using the sense of sight (Widarti, 2021). **Figure 5** shows the frequency of chemistry teachers' explanations at the macroscopic level, where the order of the Likert scale answers is: (1) never, (2) occasionally, (3) sometimes, (4) often, and (5) always. Based on **Fig. 5**, the data shows that most chemistry teachers are neutral with a slightly more positive response (46.7% choosing scales 4 and 5), and only a small proportion (3.3% on scale 2). This shows that most chemistry teachers have used macroscopic representations in teaching chemical bonding material. For example, is the change in table salt (NaCl) when heated or cooled? This is because table salt (NaCl) is a standard material that is easily found daily. In addition, NaCl has physical properties that are easily observed at the macroscopic level, such as solubility in water and a high melting point. Teachers can easily demonstrate these properties through simple experiments, such as dissolving salt in water. This is in line with the research of Hasanah *et al.* (2024), which states that chemistry teachers usually use NaCl as an example for macroscopic representation. This is because general chemistry books often use experimental images of reactions forming NaCl ionic compounds to illustrate phenomena that occur at the macroscopic level.

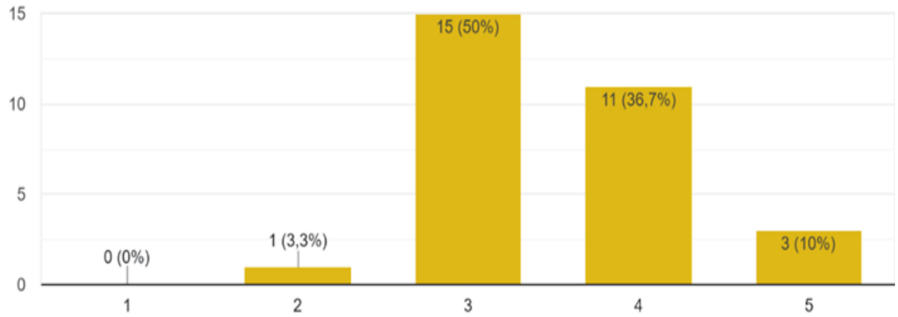

Figure 5. Frequency of chemistry teachers' explanations at the macroscopic level.
Source: Elaborated by the authors.

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3.2.2. Submicroscopic level

Submicroscopic are descriptions of phenomena that can't be seen with the naked eye or even with a microscope, such as atoms, ions, and molecules. The submicroscopic level is a level that describes the structure of chemical substances and their phenomena, reaction mechanisms that occur, atomic or molecular interactions, and chemical changes that underlie a phenomenon (Widarti, 2021). **Figure 6** shows the frequency of chemistry teachers' explanations at the submicroscopic level, where the order of the Likert scale answers is: (1) never, (2) occasionally, (3) sometimes, (4) often, and (5) always. Based on **Fig. 6**, the data shows that most chemistry teachers are neutral, with a slightly more positive response (26.7% choosing scales 4 and 5), and only a small proportion never (10% on scale 2). This is due to the submicroscopic level, which involves understanding particles such as atoms, ions, and electrons that cannot be seen directly, making it an abstract concept for students. For example, chemistry teachers stated that students have difficulty understanding the concept of molecular geometry without in-depth visualization, so students will find it difficult when learning the theory more realistically.

The limitations of learning media, such as animations or 3D models, are also a factor. Some general chemistry books often do not provide submicroscopic representations for molecular geometry concepts (Hasanah *et al.*, 2024). Chemistry teachers often focus more on macroscopic and symbolic representations because they are easier to explain and more relevant in the context of laboratory experiments or everyday learning (Mindayula and Sutrisno, 2021; Siregar and Wiyarsi, 2023). Submicroscopic processes are considered an extension of this basic understanding. Widarti (2021) stated that chemistry teachers rarely use learning media that can help students understand concepts through submicroscopic representations. However, submicroscopic representations play a crucial role in illustrating the nature of material particles such as atoms, ions, and molecules, which cannot be observed directly and are the basis for interpreting chemical concepts or phenomena.

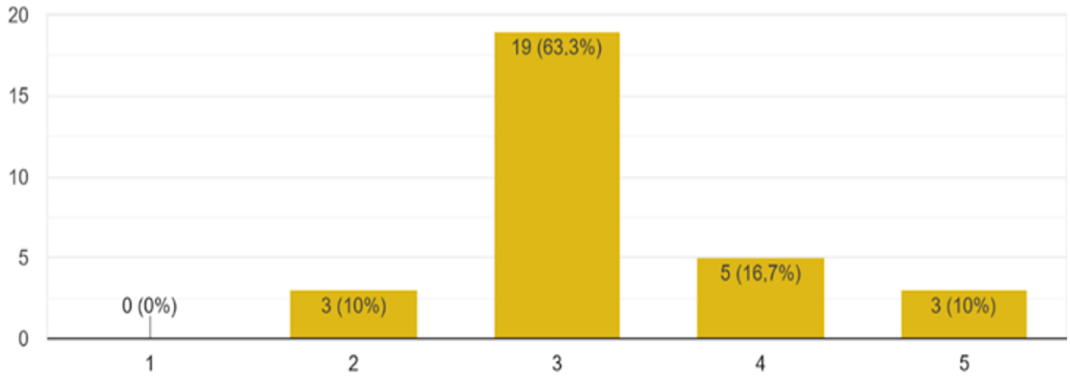

Figure 6. Frequency of chemistry teachers' explanations at the submicroscopic level.
Source: Elaborated by the authors.

3.2.3. Symbolic level

Symbols in chemistry can be pictures, reaction equations, chemical formulas, diagrams, stoichiometry, and mathematical calculations. In addition, symbols in chemistry also show the form of a substance and the number of atoms in an ion or molecule (Widarti, 2021). **Figure 7** shows the frequency of chemistry teachers' explanations at the symbolic level, where the order of the Likert scale answers is: (1) never, (2) occasionally, (3) sometimes, (4) often, and (5) always. Based on **Fig. 7**, most chemistry teachers gave neutral to positive responses (66.6% choosing scales 4 and 5). This shows that most chemistry teachers have used symbolic representations in teaching chemical bonding material. For example, there are the concepts of writing the electron configuration of an element and the concept of describing the Lewis structure of a compound. Both concepts use symbols to present complex and abstract information in a more straightforward and easily understandable form. This is in line with the research of Hasanah *et al.* (2024), which states that concept of describing Lewis structures using symbolic representation.

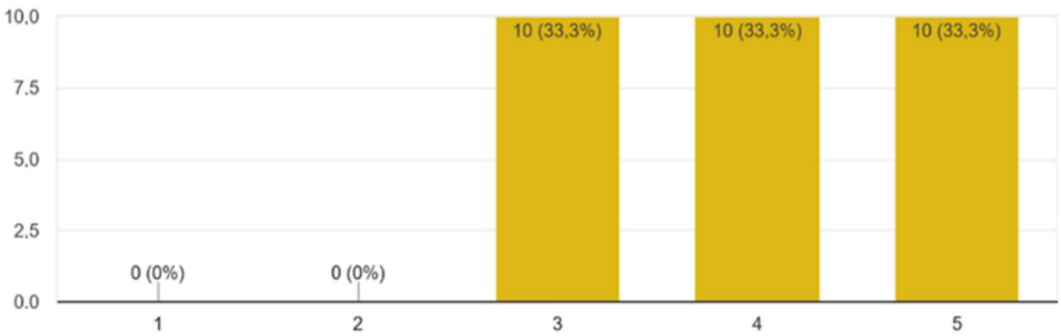

Figure 7. Frequency of chemistry teachers' explanations at the symbolic level.
Source: Elaborated by the authors.

4. Conclusions

Based on the results of the discussion above, it was found that chemistry teachers find molecular geometry the most difficult sub-material of chemical bonds and ionic bonds the easiest. Chemistry teachers participating in this study tend to focus more on symbolic, macroscopic, and submicroscopic representations in teaching chemical bonds. This study recommends developing learning media for chemical bonds that integrate the three types of representations to facilitate understanding of abstract concepts.

Authors' contribution

Conceptualization: Hayuni Retno Widarti; Antuni Wiyarsi; Sri Yamtinah; Ari Syahidul Shidiq; **Data curation:** Meyga Evi Ferama Sari; Putri Nanda Fauziah; Shella Natasya; Cahya Aulia Khandi; Deni Ainur Rokhim; **Formal analysis:** Ari Syahidul Shidiq; Cahya Aulia Khandi; Deni Ainur Rokhim; **Funding acquisition:** Hayuni Retno Widarti; **Investigation:** Meyga Evi Ferama Sari; Putri Nanda Fauziah; Shella Natasya; Cahya Aulia Khandi; Deni Ainur Rokhim; **Methodology:** Hayuni Retno Widarti; Antuni Wiyarsi; Sri Yamtinah; **Project administration:** Hayuni Retno Widarti; **Resources:** Not applicable; **Software:** Deni Ainur Rokhim; **Supervision:** Antuni Wiyarsi; Sri Yamtinah; **Validation:** Hayuni Retno Widarti; Ari Syahidul Shidiq; **Visualization:** Shella Natasya; Putri Nanda Fauziah; **Writing – original draft:** Putri Nanda Fauziah; Shella Natasya; **Writing – review & editing:** Hayuni Retno Widarti; Antuni Wiyarsi; Sri Yamtinah.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

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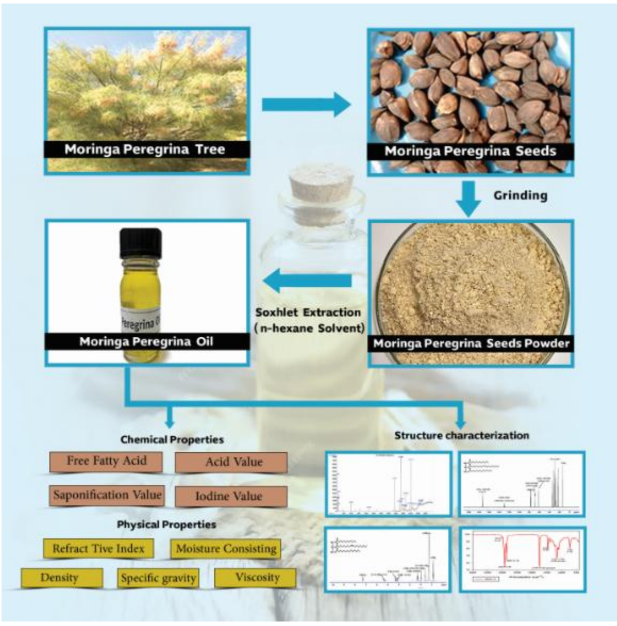
Physicochemical characteristics of Hadhramaut *Moringa peregrina* seeds oil

Maher Ail **Al-Maqtari**[1], Hussen Manaa **Al-Maydama**[1], Murad Awadh **Bahadi**[2,3+], Hani Mahfoodh **Barfed**[1]

Abstract

The Hadhramaut *Moringa peregrina* seeds oil (HMPSO) composition was characterized by Fourier transform infrared, hydrogen and carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopy. The oil was extracted from the Hadhramaut *Moringa peregrina* seeds using the Soxhlet method with hexane as the solvent, which reached 34.0±0.2%. The physicochemical properties showed the free fatty acid consisted of 3.18±0.5%, an acid value of 7.0±0.5 mg NaOH/g, an iodine value of 69.4±0.2 g I_2/100g, saponification value of 185.1±0.1 mg KOH/g, refractive index of 1.47±0.01 at 25 °C, the moisture consisted of 0.48±0.03%, density of 0.92±0.03 g mL^{-1} at 25 °C, and a viscosity of 48±0.1 cP at 25 °C. The gas chromatography showed oleic acid (78.2±0.1%), palmitic acid (9.80±0.05%), stearic acid (3.6±0.1%), behenic acid (2.52±0.07%) and arachidic acid (1.83±0.05%). The major triacylglycerols of HMPSO, estimated by using high-performance liquid chromatography, were OOO (39.43%), POO (24.54%), SOO (8.18%), and AOO (6.74%). These findings provide important insights into the physicochemical properties of HMPSO; they could have significant implications for its utilization in various industries.



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Keywords

1. *Moringa peregrina* seeds oil;
2. extraction oil;
3. triacylglycerols composition;
4. fatty acid composition.

Section Editors

Michelle Jakeline Cunha Rezende

Highlights

- To extract oil from Hadhramaut Moringa peregrina seeds using a Soxhlet method.
- To analyze the physicochemical properties of *Moringa peregrina* seed oil.
- To assess the oil's stability and nutritional value for potential applications.
- To compare Moringa oil with other edible oils in terms of quality and benefits.
- To explore the oil's potential as a biodiesel feedstock and its viability.

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1. Introduction

Moringa peregrina is a fast-growing and highly valuable tree with many beneficial applications in food, medicine, and industry. *Moringa peregrina* is a small genus comprising more than 13 species of trees (Elsorady *et al.*, 2023; Padayacheea and Baijnath, 2012). *Moringa peregrina* is spread in many areas in Yemen; however, in Hadhramaut, it is rarely scattered (Bataher, 2009). *Moringa peregrina* is a species of flowering plant in the family *Moringaceae* that is native to the Horn of Africa, Sudan, Egypt, the Arabian Peninsula, and as far north as Syria (Hegazy and Doust, 2016). It grows on rocky wadis and cliffs in drier areas (Shahin *et al.,* 2021). *Moringa peregrina* can attain heights between 3 to 10 meters within 10 months of planting. It has a grayish–green bark and long leaves; it is also distinguished by bisexual yellowish-white to pink showy, fragrant flowers. Its fruits are elongated and capsule-like, with a glabrous beak slightly narrowed amongst the seeds. The seeds have an orbicular to ovoid or trigonous shape (Al Khateeb *et al.,* 2013; Zaghloul *et al.,* 2010). In addition, *Moringa peregrina* seed oil is bright yellow with high nutritional value, comparable to olive oil, and it is a significant source of vitamins, minerals, proteins, and carbohydrates. This versatile oil can be used in many applications, including cosmetics, pharmacology, lubricants, biodiesel, and many industries (Al-Owaisi *et al.,* 2014).

The seeds of the *Moringa* plant contain a wide spectrum of fatty acids, esters, amides, and vitamins. The proportion of fatty acids was the highest, reaching 29.52%, followed by alcohol, which reached 3.57%. Then the hydrocarbon compounds 1.84% as well as the ketones and stearate, adenines, and amides, reached successive levels: 1.71, 0.13, 0.04, and 0.15%, respectively, plus vitamin E, which amounted to 0.27% (Senthilkumar *et al.,* 2020). So, the oil extracted from *Moringa peregrina* is recognized as Ben Oil; it is confirmed to comprise 70% oleic acid, a long-chain monounsaturated fatty acid with 18 carbons. Compared to polyunsaturated fatty acids, oleic acid exhibits better oxidative stability, which enables it to be used for extended storage and high-temperature frying in the food industry (Mariod and Salaheldeen, 2017). In a study in Saudi Arabia, the chemical composition, antioxidant activity, tannic acid content, mineral, fatty acid, and amino acid profiles of oil-extracted *M. peregrina* seed meal (OEMPSM) were determined where the neutral detergent fiber, acid detergent fiber, and hemicellulose were 40.2, 29.7, and 10.5%, respectively. About 15.41% of total FFAs were saturated and 84.57% unsaturated (Al-Harthi *et al*., 2022). In a study conducted in Egypt, the extraction of Moringa peregrina oil was investigated using a Soxhlet apparatus with a 1:1 (v/v) mixture of dichloromethane and methanol. The result showed that the yield of *Moringa peregrina* oil was 42.23% (Hanaa and Gamal, 2013). In another study conducted in the United Arab Emirates, researchers employed response surface methodology to optimize the extraction of oil from *Moringa peregrina* seeds. The study aimed to identify the optimal conditions for maximizing oil yield by examining several variables. The optimal ranges determined for these variables were as follows: a liquid-to-solid ratio of 5–20 mL/g, an extraction time of 5–30 minutes, an ultrasound power of 348 W, and an extraction temperature of 30 °C. The findings indicated that response surface methodology is a robust approach for optimizing oil extraction processes from *M. peregrina* seeds, demonstrating that these optimal conditions can yield high-quality oil efficiently (Mohammadpour *et al*., 2019). A study was conducted in Iran to analyze the physicochemical properties of *Moringa peregrina* seeds. The oil was extracted from the ground kernel using cold pressing after subjecting it to steam exposure and pressing with a screw pressing machine. The extracted oil was collected and stored at 4 °C. The physicochemical properties of the extracted oil showed that the acid value (0.06%), iodine value (91.7±1 gI_2/100 g oil), peroxide value (0.66 meq O_2/kg oil), saponification value (179.3±0.3 mg KOH/g oil) and unsaponifiable matter (0.32±0.01 g/kg), the oil refractive index, viscosity, and density were 1.4621, 52.05 mPa·s, and 0.9092 g/cm^3, respectively (Gharibzahedi *et al.,* 2013). In a study conducted by Mariod *et al.* (2022), the cold-pressing extraction of *Moringa peregrina* oil led to the following physicochemical characteristics: acid value 0.481, saponification value 206.4, free fatty acid 0.242, and peroxide value 0.394. Therefore, this study aims to extract oil from Hadhramaut *Moringa peregrina* seeds using the n-hexane solvent (Soxhlet) method and study the physicochemical characteristics of Hadhramaut *Moringa peregrina* oil.

2. Materials and methods

2.1. Sample collection

Hadhramaut *Moringa peregrina* seeds were selected from the Agricultural Research Station in Wadi Hadhramout-Al-Suwairi (Yemen). The sample was manually collected at room temperature. **Figure 1** illustrates the *M. peregrina* tree, its seeds, and the extracted oil.

(a) (b) (c)

Figure 1. Photos illustrate the *Moringa peregrina* tree **(a)**, seeds **(b)**, and seeds extracted oil **(c)**.
Source: Elaborated by the authors.

2.2. Chemicals and reagents

All chemicals, as well as the solvents, were used as received, and they included ethanol (99.8%), isopropanol (99.7%), hydrochloric acid (36.5%), potassium hydroxide (99%), potassium iodide (99.5%) and sodium sulfate (99%). The Wij's solutions, including cyclohexane (99.7%), sodium thiosulfate (99%), hexane (99%), and phenolphthalein, were purchased from Sigma-Aldrich.

2.3. Oil extraction

The HMPSO was extracted using the Soxhlet extraction method with hexane as a solvent, and a mild extraction temperature was chosen to avoid thermal degradation. The crushed seeds were placed in the drying oven at 100 °C for 30 min before extraction. 10 g of *Moringa peregrina* seeds were placed in an extraction thimble, which was placed in a Soxhlet extractor. The seeds were extracted with 200 mL of hexane for 4 h at 60 °C. The solvent was evaporated using a rotary evaporator, and the residual material was dried in an open-air, dark area. The oil yield was calculated, stored in hermetically closed dark bottles, and kept in a refrigerator for further physicochemical study (Azhari, 2020).

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2.4. Physicochemical characteristics

2.4.1. Determination of free fatty acids and acid value (AV)

The free fatty acid content (FFA) was estimated by titrimetry as described in the AOCS Ca 5a-40 standard (Bahadi *et al.*, 2016a; Özcan *et al.*, 2019). First, 50 mL of isopropanol and 0.5 mL of the indicator phenolphthalein solution were put into a flask and neutralized with sodium hydroxide (NaOH, 0.1 mol/L) until a permanent pink color. The neutralized isopropanol was added to 5 g of *Moringa peregrina* oil in an Erlenmeyer flask. Until being dissolved at 40 °C, the mixture was first heated and then titrated with (NaOH, 0.1 mol/L) solution, forming a light pink color through 1 mL of phenolphthalein solution as the indicator. The FFAs percentage was calculated using **Eq. 1**.

$$\% \text{ FFA as oleic acid} = \frac{28.2 \times N \times V}{W} \quad (1)$$

where V is the volume of NaOH solution used in (mL); N is normality of NaOH solution in equivalent per liter (Eq/L); W is the weight of the sample in grams.

$$\text{Acid value} = \% \text{ FFA as oleic acid} \times 1.99 \quad (2)$$

where 1.99 is the conversion factor for oleic acid.

2.4.2. Determination of iodine value (IV)

The iodine value of HMPSO was determined using two methods, namely, the AOCS (1989) method Cd 1-25 and the method recommended by Al-Maqtari *et al.*, (2024) and Balaji (2022). In the first method, approximately 0.5 g of HMPSO was poured into a 500 mL flask. After that, 15 mL of cyclohexane was added to the flask. Next, 25 mL of Wijs solution was added; the flask was corked with a stopper. Later, the flask containing the mixture was gently shaken and left in the dark for 60 minutes. After incubating, 20 mL of 10% potassium iodide (KI) solution and 150 mL of distilled water were added to the mixture. Then the mixture was titrated with sodium thiosulfate (0.05 eq/L $Na_2S_2O_3$ solution or 0.025mol/L) until a yellow color was observed, indicating that the iodine almost disappeared. Subsequently, starch solution (1%) was added to the flask, and the titration continued until the blue color disappeared after shaking the flask. The blank was treated under the same conditions. The iodine value was determined using **Eq. 3**.

$$I.V = \frac{12.69 \times N(V_b - V_s)}{W} \quad (3)$$

where N is the normality of 0.05 N $Na_2S_2O_3$ solution (0.025 mol/L); V_b is the volume in mL of $Na_2S_2O_3$ solution used for the blank test; V_s is the volume in mL of $Na_2S_2O_3$ solution used to determine the sample; W is the weight in grams of the sample test portion.

2.4.3. Determination of saponification value

The HMPSO saponification value was calculated based on the methods suggested by Garba *et al.* (2023) and Derawi *et al.* (2014). Initially, 2 g of HMPSO were placed in a flask containing 25 mL of ethanolic potassium hydroxide solution (0.5 mol/L KOH solution) and some boiling stones. The boiling flask was connected to a condenser, and the mixture was gently boiled for one hour. Later, 1 mL of phenolphthalein solution (1%) was added to the mixture. Then, the mixture was titrated with 0.5 mol/L HCl solution until it became colorless, which indicated the endpoint. The saponification value was measured through **Eq. 4**.

$$S.V = \frac{56.1 \times N(V_b - V_s)}{W} \quad (4)$$

where V_b = mL of blank; Vs = mL of titrant; *W* = weight (g) of the sample; *N* = normality of the KOH (Eq/L).

2.4.4. Refractive index

The refractive index was determined as reported by Abdelwanis *et al.* (2023) and Salimon and Ahmed (2012). The HMPSO refractive index was determined in conjunction with the AOCS Official Methods (Cc7-25) using a refractometer (TAGO Co. Ltd. Series No.1211) linked to a Digital Thermometer (DTM-1T) at 25 °C.

2.4.5. Moisture content

The moisture content of HMPSO was identified using a Moisture Analyzer model and MX-50. About 5 g of the sample was weighed and put into a moisture dish. Then, it was dried in the moisture analyzer for 30 min at 101 °C, as Wiltshire et al. (2022) and Bahadi *et al.* (2016a) reported.

2.4.6. Viscosity

The viscosity of HMPSO was determined using a Brookfield viscometer model RV DV-I+ with a spindle size of 5. According to Tunit *et al*. (2022) and Bahadi *et al*. (2016a), after the sample was kept at 100 rpm/L/min at 25 °C, the viscosity was directly measured in centipoises (cP) using the viscometer.

2.4.7. Density

The density of HMPSO was measured using a balance. The weight of one milliliter of HMPSO placed on a balance was recorded at room temperature (Bahadi *et al*., 2019; Lema, 2022).

2.4.8. Analysis of fatty acid composition in *Moringa peregrina* oil

The fatty acid composition of HMPSO was determined using a gas chromatography (Shimadzu GC-17A) equipped with a capillary column BPX 70 (30 m × 0.25 mm × 0.25 μm) and a flame ionization detector. The column temperature was programmed to 120 °C and was gradually raised as much as 3 °C every 1 min for 57 min. The injector and detector temperatures were set at 260 and 280 °C, respectively. The carrier gas was helium, with a flow rate of 0.3 mL min^{-1}. The parameters of GC were carried out according to Bahadi *et al.* (2016 b). Fatty acids methyl esters (FAME) were prepared with base-catalyzed transesterification using the method of Japir *et al.* (2018). One mL of hexane was added to 0.1 mL of HMPSO. One mL of sodium methoxide (1.55 g of NaOH in 50 mL methanol) solution was added to the oil mixture. The solution was stirred vigorously for 10 s using a vortex stirrer and then left for 10 min for phase separation of the clear FAME solution and the cloudy aqueous layer. The upper FAME layer was carefully decanted and dried with anhydrous sodium sulfate. The fatty acid content of HMPSO composition was determined using their respective FAME and then injected into gas chromatography for analysis. The peak identifications were through the retention time, i.e., by comparing them with genuine standards analyzed under the same conditions.

2.4.9. Triacylglycerols (TAGs) profile

Triacylglycerols of HMPSO were determined using high-performance liquid chromatography (HPLC Ultimate 3000 DIONEX) equipped with an evaporative light scattering (ELS) detector and an auto-injection. The separation of TAGs of HMPSO was carried out using a commercially packed C18 column 5 µm × 120 Å (4.6 × 250 mm) at room temperature. The parameters of HPLC were evaluated in line with Japir *et al*. (2017). The mobile phase consists of a mixture of acetone and acetonitrile (63.5%:36.5%, respectively), with a flow rate of 1 mL min^{-1}. The sample preparation entailed diluting 0.1 mL of the sample with 1.5 mL of an acetone-to-acetonitrile (63.5:36.5) mixture. The HPLC system was then auto-injected with this mixture, and the analysis was conducted over a total run time of 40 min. The TAG peaks were identified using the retention times of commercially obtainable TAG standards. The relative percentages of TAG peaks were evaluated from all peaks that emerged after 10 min.

2.4.10. Fourier transform infrared spectroscopy analysis of HMPSO

Fourier transform infrared spectroscopy (FTIR) was performed according to the methods described by Noor *et al*. (2022) and Mariod *et al*. (2022). The HMPSO FTIR spectrum was recorded using a Perkin Elmer Spectrum GX Spectrophotometer from 4000 to 500 cm^{-1}. The functional groups of HMPSO were measured, and a very thin film sample was covered on NaCl cells (25 mm ID × 4 mm thickness) and was used for analysis.

2.4.11. NMR analysis of HMPSO

Nuclear magnetic resonance (NMR) spectroscopy was employed to determine the molecular structure of triacylglycerols, using the methods adopted by Awang *et al*. (2007) and Aigbodion and Bakare (2005). ^{1}H and ^{13}C NMR analyses were conducted using a Joel FCP model at 400 MHz, with the solvent $CDCl_3$ from Sigma-Aldrich, which had a purity of 99.8%. After that, ^{13}C and ^{1}H NMR spectra of the products were recorded on a Bruker 400 NMR Spectrophotometer, where 10 mg of HMPSO was dissolved in 560 µL of $CDCl_3$. The sample was inserted into a glass tube before being introduced into the NMR tube.

3. Results and discussion

3.1. Oil extraction and physicochemical characteristics of *Moringa peregrina* seeds oil

Table 1 shows the physicochemical properties of the HMPSO studied. The yield of oil extracted was 34%, obtained from Hadhramaut Moringa peregrina seeds using the Soxhlet method with hexane as the solvent. The results of the extracted oil were like those of other studies (Lema *et al*., 2022). The color is an important factor that indicates product composition, purity, and degree of deterioration. Thus, it can be used to verify oil degradation, stability and suitability for a specific use (Rossi *et al*., 2001). The color test of HMPSO was light yellow and had a favorable fragrance. The percentage of FFAs in oil indicates their level of degeneration and quality. Also, the seeds' duration and storage conditions may affect the value of free fatty acids (Ibrahim, 2013). Accordingly, the result shows that the average value of FFA for HMPSO is 3.2±0.5%. Based on Ngozi *et al*. (2013) and Bale *et al*. (2015), FFA is one of the most significant quality parameters in the HMPSO as it specifies the level of oil deterioration. As shown in Table 1, the acid value of HMPSO was determined to be 6.3±0.5 mg NaOH/g. The acid value of the extracted oil (HMPSO) is higher, indicating lower quality, possibly due to an active lipase present in the seed oil, which is responsible for the hydrolysis of triacylglycerols to free fatty acids, diacylglycerol and monoacylglycerol (Albert *et al*., 2011). The iodine value of the HMPSO measured in this study was 69.4±0.2 g I_2/100g, as shown in Table 1. Chemical attributes like iodine value indicate the presence of unsaturated fatty acids, and a lower value marks the lower quantity of unsaturated fats and vice versa. Similarly, the saponification value of HMPSO was 185.1±0.1 mg KOH/g. The saponification value indicates the proportion of lower fatty acids in the oil. Therefore, the saponification value controlled the oil quality. The refractive index of HMPSO was determined to be 1.47±0.01, signifying a high concentration of carbon atoms in the fatty acid composition. The HMPSO moisture content was identified to be 0.48±0.03 at 101 °C, as illustrated in Table 1. The sample density highlighted a slight variation, with HMPSO falling within the range of 0.92±0.03 g/mL. This result is identical to the study performed in Saudi Arabia and Egypt. In Saudi Arabia and Egypt, the results for *Moringa peregrina* seed oil were, respectively, 0.30 and 0.01 equivalent to mg KOH/g for acid value, 69.6 and 67.9 g of I_2/100 g of oil for iodine value, 185 and 179 mg of KOH/g of oil for saponification value, 1.46 and 1.43 for refractive index, and 0.91 and 0.82 g/cm^3 for density (Hanaa and Gamal, 2013; Tsaknis, 1998). Thus, Ibrahim (2013) and Bahadi *et al*. (2019) emphasized that the data for viscosity indicated that HMPSO showed the highest resistance to flow with a viscosity of 48.0±0.1 cp.

Table 1. Physicochemical properties of HMPSO.

Components	Units	Values
Oil content	%	34.0±0.2
FFA (as oleic acid)	%	3.2±0.5
Acid value (AV)	mg NaOH/g	6.3±0.5
Iodine value (IV)	g I2/100 g	69.4±0.2
Saponification value (SV)	mg KOH/g	185.1±0.1
Refractive index	-	1.47±0.01
Moisture content	%	0.48±0.03
Density	g/mL	0.92±0.03
Viscosity	cp	48±0.1

Source: Elaborated by the authors.

3.2. Fatty acid composition of HMPSO

Moringa peregrina seeds have a novel fatty acid composition compared to other plant oils. In lipid science, fatty acid composition analysis is a widely used and common analytical technique. Nevertheless, the fatty acid composition is important for studying the characteristics of *M. peregrina* seed oil. Methylation is a method for preparing fatty acid methyl esters (FAMEs) from glycerolipids. Conventionally, FAMEs are prepared from base-catalyzed transesterification (Carvalho and Malcata, 2005). Base-catalyzed methanolysis proceeds much more rapidly under room temperature in 2 min. In this study, the fatty acid composition of *Moringa peregrina* seed oil is listed in Table 2 as a percentage of total FAME. Fatty acid methyl ester peaks were classified and quantified by comparing their peak area and retention times with standard methyl esters. There are three main types of fatty acid in *Moringa peregrina* seeds oil, which comprises saturated (Cn:0), monounsaturated with one double bond (Cn:1), and polyunsaturated with two double bonds (Cn:2). *Moringa peregrina* seeds oil consists of 82.25% unsaturated fatty acids and

17.75% saturated fatty acids as shown in **Table 2**. The results were like studies in Egypt, Saudi Arabia, and elsewhere (Robiansyah *et al.*, 2014; Tsaknis, 1998).

Table 2. Fatty acid composition of HMPSO using GC-FID analysis.

No	Fatty acids	Molecular formula	Percentage (%)
1	Palmitic Acid	$C_{16}H_{32}O_2$	9.80±0.05
2	Palmetoleic Acid	$C_{16}H_{30}O_2$	1.8±0.1
3	Stearic Acid	$C_{18}H_{36}O_2$	3.6±0.1
4	Oleic Acid	$C_{18}H_{34}O_2$	78.2±0.1
5	Linoleic Acid	$C_{18}H_{32}O_2$	0.43±0.02
6	Arachidic Acid	$C_{20}H_{32}O_2$	1.83±0.05
7	Eicosenoic Acid	$C_{20}H_{38}O_2$	1.4±0.1
8	Behenic Acid	$C_{22}H_{44}O_2$	2.52±0.07
9	Erucic Acid	$C_{22}H_{42}O_2$	0.42±0.09
	Total unsaturated fatty acids		82.25
	Total saturated fatty acids		17.75

Source: Elaborated by the authors.

3.3. Triacylglycerols composition of HMPSO

The triacylglycerol (TAG) profile of HMPSO was identified using high-performance liquid chromatography (HPLC). The result from the reversed-phase HPLC shows that HMPSO was composed of at least nineteen important TAGS. **Table 3** shows the TAG composition of HMPSO. The main TAG of HMPSO contained a wide range of TAG species such as 1, 2,3-trioleoyl-glycerol, 1-stearoyl-2,3-dioleoyl-glycerol, 1-arachidoyl-2,3-dioleoyl-glycerol, 1,3-dioleoyl-2-linoleoyl-glycerol. The primary TAG composition of HMPSO was tri-unsaturated (45.31%), followed by di-unsaturated (42.73%), mono-unsaturated (6.87%) and tri-saturated (1.26%), as shown in **Table 3**. These results reflect the high unsaturation of HMPSO content and show good agreement with the fatty acids found in this study. It was expected that HMPSO would exhibit a notable content of FFAS, monoacylglycerols (MAG), and diacylglycerols (DAG), as shown in **Table 3**.

3.4. FTIR analysis of HMPSO

FTIR spectroscopy enables the deduction of molecular structures of substances by analyzing absorption bands associated with distinct functional groups, which are manifested as peak spectra (Lema *et al.*, 2022). In the case of HMPSO, the FTIR spectrum commonly exhibits prominent bands corresponding to triacylglycerol as shown in **Fig. 2**. **Table 4** displays the distinctive bands of the principal functional groups found in HMPSO. **Figure 3** represents an infrared spectrum of HMPSO spanning the range of 500 to 4000 cm^{-1}. The strong absorption band of HMPSO at 1750 cm^{-1} is probably due to the esterified carbonyl function, which is also accountable for the band at 1168 cm^{-1}. The band at 3012 cm^{-1} indicates =C-H stretch for Sp^2 (aliphatic), while the bands at 2925 cm^{-1} and 2863 cm^{-1} indicate C-H stretching vibration for Sp^3 (aliphatic) in HMPSO. The band at 1469 cm^{-1} is assigned to -CH deformation. The 1243-1168 cm^{-1} band denote -C-O-C stretching vibration (ester). The absorption band at 725 cm^{-1} indicates the presence of $-(CH_2)-n$, where n is greater than 3, representing an open-chain structure. Most of the remaining bands are absorption frequencies of the hydrocarbon chain.

Table 3. Triacylglycerol composition of HMPSO.

Triacylglycerol species	Composition (%)
Tri-unsaturated	
1,2,3-trioleoyl-glycerol (OOO)	39.43
1,3-dioleoyl-2-linoleoyl-glycerol (OLO)	4.94
1-oleoyl-2,3 dilinoleoyl- glycerol (OLL)	0.37
1,2,3-trilinoleoyl-glycerol (LLL)	0.34
1-eicosenoyl-2,3-dioleoyl-glycerol (EiOO)	0.23
Σ Tri-unsaturated	45.31
Di-unsaturated	
1-palmitoyl-2,3-dioleoyl-glycerol (POO)	24.54
1-stearoyl-2,3-dioleoyl-glycerol (SOO)	8.18
1-arachedoyl-2,3-dioleoyl-glycerol (AOO)	6.74
1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol (POL)	1.28
1-palmitoyl-2,3 dilinoleoyl-glycerol (PLL)	0.58
1-palmitoyl-2-palmetoleoyl-3-linoleoyl-glycerol (PPL)	1.08
1-oleoyl-2-linoleoyl-3-arachedoyl-glycerol (OLA)	0.33
Σ Di-unsaturated	42.73
Mono-unsaturated	
1,3-dipalmitoyl-2-oleoyl-glycerol (POP)	4.12
1-palmitoyl-2-oleoyl-3-behenoyl-glycerol (POB)	1.89
1-palmitoyl-2-linoleoyl-3-stearoyl-glycerol (PLS)	0.42
1,2-dipalmitoyl-3-linoleoyl-glycerol (PPL)	0.23
1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS)	0.21
Σ Mono-unsaturated	6.87
Tri-saturated	
2,3-tripalmitoyl-glycerol (PPP)	1.26
Σ Tri-saturated	1.26
Total Triacylglycerol (TAG %)	96.19
Total Diacylglycerol (DAG %)	1.62
Mono Diacylglycerol (MAG %)	1.16
Free Fatty Acid (FFA%)	1.08

Source: Elaborated by the authors.

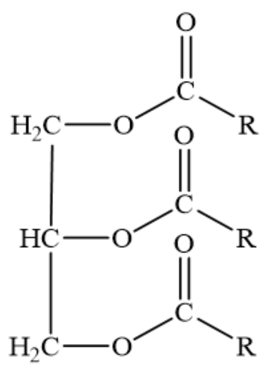

Figure 2. Structure of triacylglycerol.
Source: Elaborated by the authors.

Table 4. The functional groups of HMPSO in terms of the main wavenumber in the FTIR.

Functional Group	Wavenumber (cm^{-1})
C=C bending vibration (aliphatic)	3012
C-H stretching vibration (aliphatic)	2925, 2863
C=O stretching vibration (ester)	1750
C-H scissoring and bending for methylene	1469
=C–H (cis) unsaturated	1415
-CH3 sym deformation	1374
-C-O- stretching vibration (ester)	1243−1168
C-H group vibration (aliphatic)	725

Source: Elaborated by the authors.

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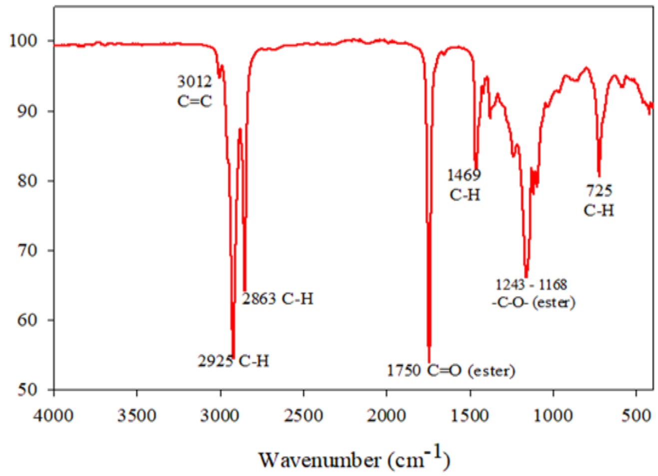

Figure 3. FTIR spectrum of HMPSO.
Source: Elaborated by the authors.

3.5. NMR analysis of HMPSO

Nuclear Magnetic Resonance (NMR) spectroscopy is useful in determining the chemical structure of HMPSO. In this study, the analysis of HMPSO was conducted through 1H NMR and 13C NMR. The findings were discussed in detail.

3.5.1. 1H NMR spectrum

The ^{1}H NMR results for HMPSO can be shown in **Table 5** and **Fig. 4**. The spectrum of ^{1}H NMR indicates the existence of proton peaks at a chemical shift of 5.32 ppm demonstrating protons attached to a double bond (vinyl protons) (-CH=CH), and the peaks at chemical shifts of 1.99-2.03 ppm signifying the presence of allylic protons attached to a carbon adjacent to a double bond (-CH_2-C=C-), where both represent the top of the double bond in the HMPSO. The chemical shift displacement of these two double bond peaks was close to the range of chemical shift displacement reported by Pavia *et al*. (2015); the chemical shift range of group -CH=CH is 4.5-6.5 ppm, and the chemical shift range of group -CH_2-C=C- is 1.6-2.6 ppm. For the molecular structure of an ester, two distinct types of protons exist. Those on the carbon atom attached to the oxygen atom in the alcohol part of the ester (-CH_2-O) have a chemical shift range of 3.5-4.8 ppm. The protons from the alpha carbon in the acid part of the ester (-CH_2-C=O) have a chemical shift of 2.1-2.5 ppm. **Figure 4** illustrates alcohol proton (glyceryl-CH_2-O-CO- (α–esterified glycerol)) identified at a chemical shift of 4.27-4.31 ppm and acid proton (-CH_2C=O) detected at chemical shift 2.28-2.32 ppm for HMPSO. The β-esterified glycerol (-CH–OCOR) is indicated by a chemical shift at 4.27-4.31 ppm for HMPSO. The chemical shift at 0.88-0.87 ppm corresponded to -CH_3 (terminal methyl in the alkyl chain) next to the terminal methyl -CH_2 at 1.25-1.29 ppm. The chemical shift at 7.28 ppm represented the solvent $CDCl_3$.

3.5.2. 13C NMR spectrum

The primary signal assignments in the ^{13}C NMR spectrum of the HMPSO are shown in **Table 6** and shown in **Fig. 5**. This spectrum was a feature of triacylglycerols and contains resonances arising from the methyl, methylene, glycerol backbone, and olefinic carbons of polyunsaturated fatty acids and monounsaturated fatty acids as well as carbonyl carbons. The ^{13}C NMR spectrum was divided into four groups of signals according to their chemical shifts. These four groups contain carbonyl carbons that resonate between 172 and 174 ppm, unsaturated carbons that resonate from 124 to 134 ppm, glycerol backbone carbons that resonate from 60 to 72 ppm, and aliphatic carbons that resonate between 10 and 35 ppm. The ^{13}C NMR of the triacylglycerols' acyl chains was assigned according to the extensive chemical shift data tabulated by Gunstone (2009).

The peak at 14.07 ppm corresponds to the carbon atoms of the methyl groups attached to the end of the acyl chains. The peaks ranged between 22.67 and 34.18 ppm, symbolizing the methylene carbon atoms in fatty acid moieties of HMPSO. Glycerol carbons in triacylglycerols ranged from 60 to 70 ppm. The glycerol carbons (β) were found at 68.87 ppm, and glycerol carbons (α) and (ά) were at 62.06 ppm. The olefinic carbons of unsaturated fatty acids of HMPSO triacylglycerols resonated between 127.86 and 130.14 ppm. The signals at 172.79 ppm and 173.21 ppm were observed in the carbon atom of the carbonyl group.

Table 5. The main signals present in the ^{1}H NMR spectrum of HMPSO.

Assignment	HMPSO δ(ppm)
–CH_3 (terminal methyl)	0.88–0.87
–(CH_2)n–(saturated alkyl chain)	1.25–1.29
–CH_2–CH=CH–CH_2-	1.99–2.03
–CH=CH–	5.32
-CH_2-COOR, acyl methylene	2.28–2.32
-CH_2-O-CO-R (α–esterified glycerol)	4.11–4.16
CH–OCOR (β-esterified glycerol)	4.27–4.31
-C=C-CH_2-C=C-	5.24–5.36
$CDCl_3$	7.28

Source: Elaborated by the authors.

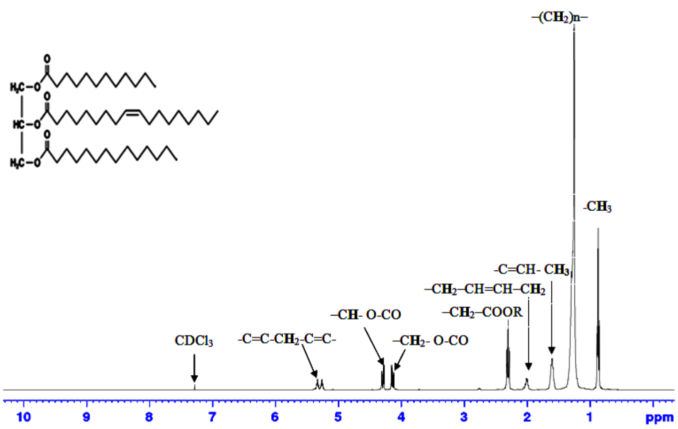

Figure 4. ^{1}H NMR spectrum of HMPSO.
Source: Elaborated by the authors.

Table 6. The main signals present in the ^{13}C NMR spectrum of HMPSO.

Assignment	HMPSO δ(ppm)
–CH_3	14.07
–(CH_2) n-	22.67–34.18
–CH_2-O-CO-R glycerol carbons (α) & (ά)	62.06
CH–OCOR glycerol carbons (β)	68.87
–HC=CH- (Olefinic Carbone)	127.86–130.14
CH_2–OCOR (carboxylic ester)	172.79–173.21
–CH_3	14.07
–(CH_2) n-	22.67–34.18
–CH_2-O-CO-R glycerol carbons (α) & (ά)	62.06

Source: Elaborated by the authors.

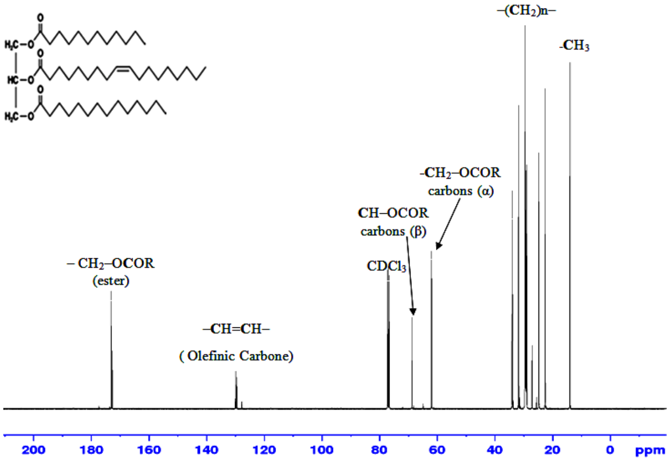

Figure 5. ^{13}C NMR spectrum of HMPSO.
Source: Elaborated by the authors.

4. Conclusions

This study demonstrated that the physicochemical characteristics of HMPSO were comparable to those of *Moringa peregrina* seeds oil from other countries. The GC-FID analysis revealed that the dominant fatty acids present in HMPSO were oleic acid, palmitic acid, stearic acid, behenic acid, and arachidic acid. The triacylglycerol composition of HMPSO contained a high concentration of unsaturated TAG and a low concentration of saturated TAG. The ^{1}H NMR, ^{13}C NMR and FTIR analyses performed on HMPSO confirmed the molecular structure of its components. Understanding the pattern of interactions in TAG can also have implications in numerous industries and medical treatments.

Authors' contribution

Conceptualization: Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Data curation:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; **Formal Analysis:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Funding acquisition:** Not applicable; **Investigation:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; **Methodology:** Murad Awadh Bahadi; **Project administration:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Resources:** Not applicable; **Software:** Murad Awadh Bahadi; **Supervision:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; Murad Awadh Bahadi; **Validation:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; **Visualization:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; Maher Ail Al-Maqtari; **Writing – original draft:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Writing – review & editing:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama.

Data availability statement

Data sharing is not applicable.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Enhancing higher-order thinking skills in chemical equilibrium through a game-based learning module

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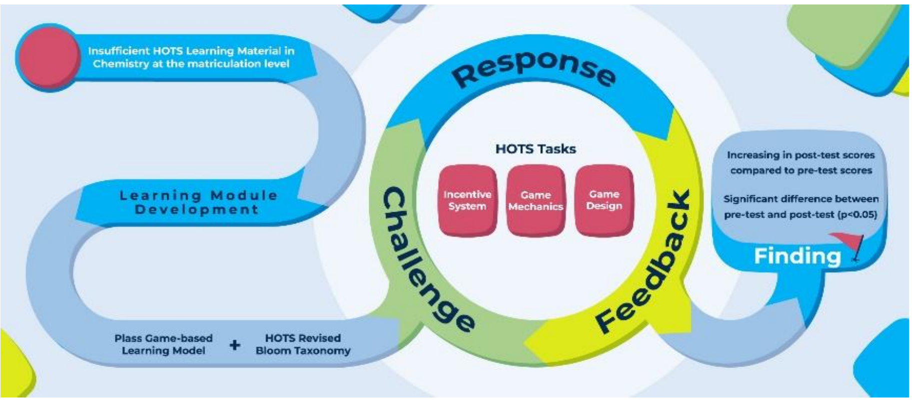
Abstract

In 21st-century learning, higher-order thinking skills (HOTS) are essential for matriculation students. However, lacking HOTS materials gives students less opportunity to practice HOTS in chemistry. This research paper developed a game-based learning module based on the Revised Bloom's taxonomy and a game-based learning model. Pre-experimental design with a one-group pre-test and post-test was employed to measure the effects of the module on students' HOTS namely analyzing, evaluating, and creating thinking skills, involving 30 students at matriculation college. The result shows an increase in mean scores in post-test compared to pre-test scores and paired sample T-test shows a significant difference between pre-test and post-test scores with $p<0.05$. The findings contributed to the potential of game-based learning for promoting HOTS at the matriculation level and providing learners with a quality education.



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Highlights

- Lacking HOTS materials limits students' opportunities to practice HOTS in chemistry.
- Game-based learning has potential for promoting HOTS at the matriculation level.
- Game-based learning module applying HOTS for quality 21st-century learning.
- Matriculation needs more HOTS materials that are aligned with the syllabus.
- A Game-based model with Revised Bloom's Taxonomy offers HOTS module for learning.

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1. Introduction

Along with the effort to make effective 21st-century learning, the Sustainable Development Goal (SDG) is primarily focused on assuring wellness, economic prosperity, environmental laws, and academic advancement to promote sustainable growth. The education system has played a role in the fourth goal of SDG, which is to ensure inclusive and equitable quality education and promote lifelong learning opportunities for all. Since 2013, the Malaysian government, through the Ministry of Education (MOE) has introduced a strategic plan called Malaysian Education Blueprint (MEB) that has a specialized focus on improving lifelong learning in 21st-century learning within the years 2013-2025. One of the focuses is to strengthen the skills of higher-order thinking, and Malaysia aims to be among the top one-third of countries in the Programme for International Student Assessment (PISA) and Trends in International Mathematics and Science Study (TIMSS). The efforts to strengthen higher-order thinking skills (HOTS) are gaining continuous attention among teachers and educators (Saban, 2021). Much research also proved that HOTS helps students articulate their ideas clearly and effectively (Ichsan *et al.*, 2019) and is essential for global sustainable growth and future industry.

Previous research has reported that one of the challenges in implementing HOTS in chemistry subjects is that students' mastery of HOTS in chemistry is very weak (Azid and Md-Ali, 2020; Misrom *et al.*, 2020). Generally, HOTS involves the skills of analyzing, evaluating, and creating according to the Revised Bloom Taxonomy by Anderson and Krathwohl (2001). In chemistry, analyzing needs skills of separating chemical concepts into their parts and relating to one another; Evaluating acquired students to make a judgment about the value of information in chemistry-related phenomena; and creating allows students to generate new ideas by applying chemistry concepts in problem solutions. Mastery in analyzing, evaluating, and creating in the chemistry subject will make chemistry learning more meaningful and lifelong benefited students.

However, students are weak mastering these skills due to insufficient opportunities to develop HOTS at the matriculation level (Jamal *et al.*, 2020; Khamhaengpol *et al.*, 2021; Sasson *et al.*, 2022; Wan *et al.*, 2022). Therefore, educators should create more opportunities for students to practice HOTS skills throughout their education. This could include activities such as group projects, problem-solving, and critical-thinking exercises. Additionally, educators should provide students with feedback on their work to help them refine their skills and improve their understanding of chemistry. These types of activities help students to better understand the concepts they are learning and to develop the skills they need to apply their knowledge in real-world scenarios. Additionally, providing feedback helps to ensure that students are engaging with the material in a meaningful way and that they are making progress toward mastering the content.

In learning Chemical Equilibrium, students need HOTS to construct the concept by making the relation between one topic and another (Aini *et al.*, 2019). HOTS is needed to learn various factors and effects on chemical equilibrium (Rokhim and Septiani, 2021). For example, analyzing skills are needed to distinguish information using appropriate formulas. Evaluating skills helps students to examine the provided conditions to relate to the dynamic equilibrium. Creating thinking skills enables students to innovate and optimize conditions or products of chemical reactions. Additionally, learning activities can help students build ideas and gain a more in-depth understanding of the material. Game-based learning strategies can be used to engage students and help to develop problem-solving and critical thinking skills, as well as allow them to practice and apply what they are learning.

Game-based learning can provide an interactive learning environment to upskill their 21st-century skills with HOTS (Noh and Karim, 2021; Patiño *et al.*, 2023). An effective engagement can provide a comprehensive understanding beyond chemistry by using the game setting (Chen *et al.*, 2021). Game-based learning requires active learning so that students learn chemistry directly, and not put chemistry as an isolated subject (Tangkui and Keong, 2020; Tong *et al.*, 2022). Through game-based learning, students can apply the knowledge they have acquired in the game setting, which allows them to gain a more holistic and in-depth understanding of the subject. Based on Plass *et al.* (2020), game-based learning promotes active learning, as students are encouraged to think critically and interact with the game environment. This helps them to view chemistry as an interconnected subject, rather than an isolated one. By engaging in game-based learning, students develop higher-order thinking skills such as problem-solving, critical thinking, and creative thinking. Bello *et al.* (2016) also reported that there was a significant difference in students' achievement after the game-based learning was applied as the learning approach, as students had improved their critical and evaluative thinking. Previous research studies have proven that game-based learning module helps to create an enjoyable learning experience that connects the learning content with the real world (Farihah *et al.*, 2021), and this research focused on the effects of HOTS after the implementation of the game-based learning module among the matriculation students in chemical equilibrium concepts.

This study aimed:

1. To develop a game-based learning module on the Chemical Equilibrium topic at the matriculation level.
2. To evaluate the effects of the game-based learning module on students' higher-order thinking skills, namely analyzing, evaluating, and creating.

The research questions are:

1. What are the key features of an effective game-based learning module for chemical equilibrium that can promote higher-order thinking skills?
2. What are the changes in the higher-order thinking skills of students after using the Equi-Brilliant Module?

2. Research method

2.1. Research design

This study uses an experimental research design with a one-group pre-test and post-test. The one-group pre-test and post-test were chosen to obtain a carefully controlled situation (Johnson and Christensen, 2019) while performing the intervention using the module that had been developed. It is also widely accepted that the one-group pre-test and post-test were measured while testing the effectiveness of new teaching and learning approaches or curricular innovations (Cohen *et al.*, 2017). In this research, the game-based learning module is a new approach to be implemented to improve a conventional method at the matriculation institution.

2.2. Research sample

Two types of samples were used in this study. A random sample was used to answer RQ1, which involved thirty-seven chemistry lecturers from a population of 371 chemistry lecturers at matriculation colleges in Malaysia. Purposive sampling was used to answer RQ2, which involved thirty students of a one-year science program from a matriculation college located in south Malaysia.

2.3. Research instrument

The instruments involved are achievement tests in the form of pre-test and post-test questions, the game-based learning module during the intervention, and survey forms for the need analysis on key features of the game-based learning module, as well as module reflection to see the effect of the game-based learning module on students' HOTS. The achievement tests were built by the researcher and the questions prepared were ten HOTS items, involving analyzing (C4), evaluating (C5), and creating (C6) thinking skills in the form of multiple-choice questions for C4 and C5 questions and open-ended questions for C6 questions. For the survey purpose, twenty-one 5-Likert scale questions were constructed, to obtain the key features of the game-based learning module. For the module reflection, open-ended questions were prepared to get feedback from the users regarding this module. The instruments were validated by the chemistry content experts and were tested for reliability.

2.4. Module development

This study used the ADDIE model for module development, which involves the analysis, design, development, implementation, and evaluation of the module.

2.4.1. Need analysis phase

This phase is to find the key features of a game-based learning module to enhance students' HOTS in the Chemical Equilibrium topic. The analysis involves 37 chemistry lecturers, who were selected randomly from 370 chemistry lecturers from matriculation colleges in Malaysia. Survey forms on key features of the game-based learning module on the Chemical Equilibrium topic were distributed through Google Forms to reach the chemistry lecturers from all matriculation colleges in Malaysia.

2.4.2. Design and development phase

The game-based learning module was designed based on the combination of the Revised Bloom Taxonomy (Anderson and Krathwohl, 2001) and the game-based learning model (Plass *et al.*, 2015). The analyzing, evaluating, and creating items are implemented in a game setting adapted from Plass *et al.* (2015) model that includes the elements of Challenge, Response, and Feedback. The combination of both HOTS and Plass *et al.* (2015) model integrates a learning process with learning objectives that prepare students with HOTS tasks, game design, an incentive system, and game mechanics in a continuous game loop as an interactive learning environment. The game-based learning design used a game board as a platform for the learning process to occur. During the development phase, this module was validated by chemistry experts to ensure that it is relevant and valid for implementation among matriculation students.

2.4.3. Implementation and evaluation phase

The implementation of this game-based learning module is run for 30 science students at Matriculation College. The one-group pre-test and post-test were used during the implementation. Students sit for the pre-test before the intervention and sit for the post-test after the intervention to see the effect of this module on students' HOTS in the chemical equilibrium topic. The reflection form is assigned for each sample to see the evaluation from the students after using the game-based learning module.

3. Results

To answer RQ1, the descriptive analysis using percentage and mode determines the suitable key features of the game-based learning module to be developed in the topic. **Table 1** shows the survey results.

The key features chosen by the chemistry lecturers at the Matriculation College include the 11 items listed in **Table 1**. Items 1, 4, 5, 8, and 11 got 100% scores, showing that all agreed to include these features in the game-based learning module. Items 2,3,6,7,9, and 10 obtained the highest per cent of "totally agree" and "agree," showing that the items were also preferred as the key features for the module.

To answer RQ2, the pre-test and post-test results were compared. The significant difference in the scores toward HOTS among students in their pre-test and post-test performances was determined using a paired-sample t-test. The hypothesis was formulated as:

H_o: There is no significant difference between the pre-test and the post-test

H1: There is a substantial difference between the pre-test and the post-test

Table 1. Findings of key features of the game-based learning module survey based on 5-Likert scale form.

No	Key Features	Mode	Percentage (%)	Description
1	Clear teaching objectives	5	100	Totally agree
2	Questions like a real exam	5	51.4	Totally agree
3	Integrate with technology	4	54.1	Agree
4	Cater to students' response	5	100	Totally agree
5	Quick Feedback	5	100	Totally agree
6	Incentive system (points, scores, drawbacks)	5	56.8	Totally agree
7	HOTS elements are integrated into each activity	5	40.5	Totally agree
8	Real-life problems on the Chemical Equilibrium topic	5	100	Totally agree
9	A clear guide to running the activities	5	51.4	Totally agree
10	Align with the matriculation syllabus	4	54.1	Agree
11	Consider the timeline of the matriculation system	5	100	Totally agree

Source: Elaborated by the authors.

The analysis's result is shown in **Table 2**;. The pre-test mean score was 49.61, while the post-test mean score was 73.06. From the average point of view, the value between the pre-test and post-test increased after the intervention using the GBL model.

Table 2. Pre-test and post-test statistics.

Test	Mean	N	Std. Deviation	Std. Error Mean
Pre-test	49.61	30	8.273	1.486
post-test	73.06	30	9.740	1.749

Source: Elaborated by the authors.

Based on **Table 3**, the result of the paired sample T-Test was significantly different in that the significance level (P) was 0.000 < 0.05. The result of the statistical analysis rejects the null hypothesis (H_o) and confirms the alternative hypothesis (H_1). Therefore, there was a significant difference between HOTS Test (pre) scores and HOTS Test (Post) scores.

Table 3. Paired samples test.

Test	Mean	*df*	Sig. (2-tailed)
Pre-test - post-test	-23.452	30	0.000

Source: Elaborated by the authors.

4. Conclusions

In conclusion, the game-based learning module has proven to be an effective strategy to enhance students' higher-order thinking skills. The new generation needs new ways of learning while enhancing their 21st-century skills like analyzing, evaluating, and creating thinking skills to be applied in solving real-world problems. For this study that focuses on the matriculation level, this module helps to develop students' thinking and prepares students for a higher level of education after completing the pre-university level.

Suggestions for the next researchers to conduct the game-based learning module in a wide range of samples and can study deeper effects of game-based learning for each HOTS category like analyzing, evaluating, and creating so that more significant findings according to each HOTS category can be measured. The empowerment of HOTS among matriculation students can produce quality graduates for 21st–century labor demands and contribute to the sustainable development growth goals by 2030.

Authors' contribution

Conceptualization: Nurul Hanani Rusli; Nor Hasniza Ibrahim; **Data curation**: Norliyana Md Aris; **Formal Analysis**: Nurul Hanani Rusli; **Funding acquisition**: Not applicable; **Investigation**: Nurul Hanani Rusli; Nor Hasniza Ibrahim; **Methodology**: Nurul Hanani Rusli; **Project administration**: Nurul Hanani Rusli; **Resources**: Norliyana Md Aris; **Software**: Not applicable; **Supervision**: Nor Hasniza Ibrahim; **Validation**: Nurul Hanani Rusli; Norliyana Md Aris; **Visualization**: Nurul Hanani Rusli; **Writing – original draft**: Nurul Hanani Rusli; **Writing – review & editing**: Nor Hasniza Ibrahim.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Review Article, Celebrating the centenary of the 20th century Acid-Base Theory

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Teaching acid-base theories in the era of disinformation: A systematic review with proposals for content integration

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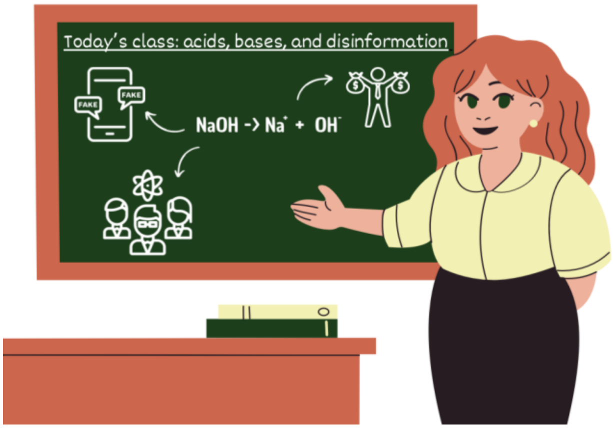
Abstract

This paper is rooted in the premise that addressing scientific disinformation and misinformation about acid-base theories requires a multidisciplinary approach, approximating acid-base knowledge with content typically not addressed in chemistry classes. Stemming from this principle, this paper analyzes how peer-reviewed publications for chemistry teaching approach the representation of acid-base theories in the context of informational disorders (disinformation and misinformation) through a literature review and textual analysis. The papers analyzed address cases of informational disorders through a disciplinary lens, with a pronounced emphasis on resorting to acid-base contents to debunk erroneous messages, even though the contexts explored reference socioscientific issues. As a response, this paper broadens the conservation, by proposing other contents that could convey the nature of science as well, the functioning of traditional and modern media, and values and ideologies. By doing so, this paper provides more elements for critical and broader readings of acid-base theories in informational disorders, elevating chemistry teaching to better address contemporary challenges arising from the interplay between science and media.



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Highlights

- Disinformation on pH and diets calls for new approaches in acid-base teaching.
- A systematic review and thematic analysis guide the study's methodology.
- The review exposes limits of disciplinary acid-base teaching.
- Media, and communication studies enrich acid-base teaching in this paper.

CONTENTS

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1. Introduction

The 100th anniversary of acid-base theories offers an opportunity to reflect on how that knowledge is represented beyond textbooks and Chemistry classes, and how educators might enhance their effectiveness in teaching those theories in more coherent, relevant and transformative manners. Over a century of academic knowledge production on acids and bases, alongside the "scholarization" of acid-base theories, has revealed numerous challenges in teaching this topic. These challenges include a lack of conceptual and classificatory accuracy in textbooks (e.g. Campos and Silva, 1999; Lima and Moradillo, 2019), an insufficient or deficient approach regarding the phenomenological and particle levels during instructional processes (e.g. Drechsler and Van Direl, 2007; Furió-Más *et al.*, 2005), and a limited alignment of epistemological and historical approaches with core concepts (e.g. Souza and Silva, 2018). Indeed, teaching acids and bases highlights a central issue in chemistry education: establishing effective and meaningful connections between the macroscopic, microscopic, and symbolic levels and other domains, such as science, technology, and society (STS), history of science, and epistemology.

This paper addresses some of those challenges, especially regarding the portrayal of acids and bases in the media. Paying attention to this specific context is deemed relevant in current times marked by social media and an influential load of inaccurate scientific information.

To provide a glance at the inaccuracies, a message highly shared on WhatsApp during the pandemic offered a misleading treatment for COVID-19 based on an alkaline diet, alleging that SARS-CoV-2 possesses an acidic nature (Locatelli, 2021). The message recommended consuming lemon (with a supposed pH of 9.9) and avocado (with an alleged pH of 15.6). In another case of WhatsApp's content, a post falsely attributed to specialists from the clinical hospital of São Paulo, Brazil, erroneously compared the alkaline property of herbal teas to the efficiency of antiviral drugs as an alternative way to treat the flu (Ghinea *et al.*, 2020). Claims like the previous ones concerning health have particularly severe consequences regarding people's quality of life (Swire-Thompson and Lazer, 2022).

Considering messages that contest core scientific principles, it is crucial to confront them through the lens of misinformation and disinformation, terms that are part of the wider concept of information disorders (UNESCO, 2019). According to Swire-Thompson and Lazer (2022), misinformation is information contrary to the current scientific consensus; disinformation has the added attribute of being spread deliberately to gain money, power, or reputation. Both are problems for society, but disinformation is particularly dangerous because it is frequently organized, well-resourced, and reinforced by automated technology (UNESCO, 2019).

In the new scenario of social media, where the production and spread of scientific disinformation have reached unprecedented levels, it is reasonable to redouble efforts at teaching critical thinking, integrating critical discussion about media and science, aiming for civic and critical thinking training for everyone (Arroio, 2017). It would not be different for chemistry teaching, mainly when socioscientific problems (such as the COVID-19 pandemic) are intertwined with media, information, power, along with knowledge about science practices and contents (Siqueira and Arroio, 2022).

1.1. Teaching science in times of digital media and disinformation

In today's global communication ecosystem, scientific knowledge, reliable information, and even facts often wield less influence than emotional appeals and personal opinions in shaping individual and collective decisions (Arroio, 2017). This is particularly evident in matters such as vaccination, elections, and climate crisis policymaking (Feinstein and Waddington, 2020). Some even name this historical moment the post-truth era, denoting "circumstances in which objective facts are less influential in shaping public opinion than appeals to emotion and personal belief" (Oxford Languages, 2016). Valladares (2022) points out two factors that might explain post-truth: (1) socio-technical advancements of digital societies, which enable the rapid spread of vast amounts of information on social media; (2) and individuals with political agendas and corporate economic interests who further distort information to serve partisan goals or profit-driven projects. Notably, digital media plays a pivotal role in both factors.

In this scenario, a compelling argument for science educators is that they should go beyond teaching traditional scientific knowledge in the form of concepts and theories. Osborne and Pimentel (2023) argue that nowadays it is essential to prepare students as "competent outsiders" –individuals who can critically evaluate scientific claims and determine the credibility of sources– rather than "competent insiders", who possess a broad but surface-level understanding of scientific concepts gained through formal education. To interact critically with scientific information, Höttecke and Allchin (2020) argue that students need a holistic understanding of the epistemic structure and provenance of scientific claims encountered in everyday life. This involves understanding the nature of science communication, including the full trajectory of these claims – the mechanisms, mediations, and potential distortions – as they move from experts to the public sphere through digital media (Allchin, 2021; Miller *et al.,* 2021; Reid and Norris, 2015). In summary, this approach highlights the urgency of examining the pathways through which knowledge is mediated –from "test tubes to YouTube" and from "lab book to Facebook" (Höttecke and Allchin, 2020, p. 644)– to assess how knowledge maintains its integrity and reliability and to explore the social architecture that sustains and justifies trust in science.

In the context of disinformation and the digital media landscape, science educators must recognize the enduring importance of teaching scientific concepts and theories. Valladares (2021) highlights that a crucial yet often-overlooked dimension of contemporary science literacy is the ability to read and write using the specific codes, norms, and language of science. Science educators bear a historical and cultural responsibility to equip students with the skills needed to effectively engage in scientific language. Because scientific practices are inherently textual, proficiency in reading, writing, and communicating scientific information is essential for a deeper understanding of science itself (Santos, 2007; Silva and Sasseron, 2021; Valladares, 2021). Furthermore, it is well understood that this fundamental aspect of scientific literacy, when integrated with suitable educational goals, approaches, and other knowledge, offers essential and transformative potential in an era dominated by digital media and disinformation (Siqueira and Arroio, 2022). After all, concepts like pH and acid-base theories are key to unveiling disinformation, such as that surrounding so-called alkaline diets.

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In summary, a prominent approach to teaching science in the current era is to foster the ability to read and write in the language of science, promote an understanding of the nature of science communication –including its norms, social actors, and epistemological practices– and integrate critical, technical knowledge of media and digital communication, such as its languages, values, interests, and ideologies (Belova and Eilks, 2015; Cardoso and Gurgel, 2019; Silva and Arroio, 2025; Siqueira and Arroio, 2022).

This paper examines how peer-reviewed publications for chemistry teaching approach the representation of acid-base theories in the context of informational disorders, and expands, when appropriate, their contributions by proposing additional content that could be integrated into chemistry classes alongside the analyzed cases. Ultimately, it is expected to provide more elements for critical and broader readings of acid-base theories in traditional and modern media, particularly in the context of disinformation and misinformation.

2. Experimental

The experimental approach begins with a systematic review (Aromataris and Pearson, 2014; Shaheen *et al*., 2023) to select peer-reviewed papers in both English and Portuguese that examine the representation of acid and base content within information disorders across various media genres. Furthermore, this analysis specifically focuses on publications of Chemistry teaching. As a following step, the experimental procedure also included reanalyzing, when necessary, some of the media and messages conveyed in the papers through a critical lens, suggesting other contents that could be addressed in chemistry classes. In this sense, the experimental procedure consists of four procedures as follows.

2.1. Procedure 1

Through a systematic review, the first step included consulting six academic databases (ERIC, Scielo, Latina, Scopus, Web of Science, and CAPES journals) whose indexed papers contained in the title, summary, or keywords the following terms: fake news AND chemistry; disinformation AND chemistry; misinformation AND chemistry; conspiracy theory AND chemistry; post-truth AND chemistry[1]. The keywords were systematically selected to capture meanings related to informational disorders, aligning with Shaheen *et al*. (2023)'s recommendations on identifying key concepts, search terms, and establishing appropriate inclusion and exclusion criteria. Considering the narrow context of the search, no time constraints were applied. With the search results, this first procedure entailed reading all the resulting papers' titles and summaries to select those related to chemistry teaching. This first curation process resulted in ten peer-reviewed publications.

2.2. Procedure 2

The second step involved reading all ten papers from procedure one, with the aim of selecting those that partially or integrally address acid-base theories. This second curation procedure resulted in four peer-reviewed papers (**Table 1**).

Table 1. Papers that address information disorders related to acid-base theories in media for chemistry teaching.

Papers	Authors	Title	How acid-base-related contents are explored
I	Cunha (2021)	[Chemistry in Fake Science] A Química "mal dita" em *Fake Science*	The paper analyzes fake science messages (according to the authors, fake news with scientific content) in written and video formats spread on WhatsApp during the first two years of the COVID-19 pandemic. The publication aims to provide teachers with classroom material to problematize and discuss chemistry and the nature of science knowledge present in fake science messages. The paper presents and discusses chemistry-related content from seven messages. In five of them, acid and base theories are conveyed.
II	Ribeiro *et al*., (2022)	[Discussing fake news related to chemistry during the COVID-19 pandemic: what was its influence on students?] Discutindo fake news sobre química durante a pandemia da COVID-19: como elas têm influenciado os alunos?	The paper analyzes and discusses students' responses to twelve affirmations related to cases of disinformation broadly spread online during the first two years of the COVID-19 pandemic. One hundred twenty-two high school students participated in the research. The twelve affirmations are further contextualized and corrected from the perspective of the related chemical concepts. Among the affirmations, three convey acid-base theories: 1) Vinegar is a more efficient alternative to protect against the virus than alcohol gel; 2) Lemon is an alkaline and efficient fruit to protect against the SARS-Cov-2 virus; 3) Gargling with water and baking soda helps to protect against the virus.
III	Toby (1997)	Chemistry in the Public Domain: A Plethora of Misinformation—or Don't Believe Everything You Read in the Newspapers!	The paper presents examples of various kinds of chemical information disorders culled from newspapers and magazines. The media's statements are debated, considering the (in)accuracy of the associated chemical concepts. The paper shows and discusses twelve excerpts, some with conceptual errors and others with quantitative mistakes. Two of them are related to acid-base theories.
IV	Algarra *et al.* (2008)	Magnetized Water: Science or Fraud?	The paper presents the results of a laboratory experiment measuring drinking water's total hardness, surface tension, and pH after filtration through a commercial device. It also problematizes how chemistry concepts are portrayed in the device's advertisement.

Source: Elaborated by the authors.

[1]For each database, a second round of searches was conducted using the equivalent terms in Portuguese: *Fake news* AND *química*; *desinformação* AND *química*; *informação incorreta* AND *química*; *teoria da conspiração* AND *química*; *pós-verdade* AND *química*.

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2.3. Procedure 3

The third step included identifying patterns and trends, resorting to the principles of thematic analyses, a method of qualitative data analysis to identify, analyze, interpret, and report patterns (themes) from qualitative data, such as textual documents (Souza, 2019). Thematic analyses were crucial for identifying trends in how acid-base contents are conveyed in the publications and for highlighting gaps to recommend contents that could be addressed from the media and contexts under study (following procedure).

2.4. Procedure 4

Stemming from the patterns and gaps identified in the previous step, the final phase involved selecting and adapting additional content that could be incorporated into chemistry classes. This selection considered the media and contexts analyzed in the reviewed publications and was grounded in contemporary science education literature, particularly debates surrounding science, media, and informational disorders.

3. Results and discussion

First, it is essential to highlight that systematic reviews do not require a minimum number of resulting studies, as they can vary significantly depending on the research topic, investigative focus, and available evidence (Aromataris and Pearson, 2014). This paper argues that in the context under study, four papers are insufficient to establish patterns among data, primarily due to the narrow scope of investigation: academic peer review focused on acid-base content within information disorders across various media genres. However, there is value in this limited dataset. As Shaheen *et al.* (2023) point out, in cases where available or reliable data is lacking, it is imperative to explicitly state limitations and provide a sound rationale for the absence or unreliability of data. Shaheen *et al*. (2023) further note that while statistical tests typically require larger sample sizes to ensure representative distributions and enable generalizations, the relevance of sample size in qualitative research is generally reduced. Therefore, despite the limited results, this paper focuses on reinforcing or proposing perspectives for teaching acid-base theories in the context of information disorders, relying on the current review findings without applying quantitative treatments.

The four publications explore acid-base contents through socio-scientific contexts: the COVID-19 pandemic (papers I and II), chemistry-related information disorders in newspapers and magazines (paper III), and water-filtering health claims (paper IV). Through these studies, the authors underscore the crucial role of natural science and chemistry teaching in understanding chemical content in various media (social media, TV, magazines, and newspapers). More importantly, all of them stress the significance of contextual learning in enhancing the relevance of chemistry teaching.

Besides, it is highlighted that knowing chemical contents (papers I, II, III) and processes (paper IV) – in other words, having conceptual and procedural understanding – is a legitimate way to not fall for information disorders in traditional and digital media. As paper IV's authors state, "Chemistry applied to magnetic treatments of water provides a good opportunity to help university and secondary students develop educated skepticism against claims made about some products" (Algarra *et al*., 2008, p. 1416). Paper IV is the only article that goes beyond a conceptual approach, demonstrating how advertisement claims can be verified by experimentation and conceptual discussion, such as pH scale.

The acid-base contents are addressed in various forms and include pH scale (papers I, II, and III), acid-base reactions (paper I and III), and buffer solutions (paper I). The authors use contents to debunk the information disorder, as seen in paper I, which discredits a message claiming that tea with lemon and sodium bicarbonate can alkalize the immune system and cure COVID-19:

> Sodium bicarbonate ($NaHCO_3$), is classified as an acidic salt [....] If it is mixed with any acid, it releases carbon dioxide and water. Its most well-known use is as a stomach antacid, neutralizing the excess HCl present in gastric juice. The mixture of sodium bicarbonate and lemon acts as a buffer in the body, and there is no evidence that it works in viral conditions (Cunha, 2021, p. 9).

It is worth highlighting that the paper I stands out as the only publication concerned with preliminary aspects of media literacy and the nature of science. It suggests that discussions should include: how scientific authority is used to legitimize disinformation; how scientific knowledge is produced; and how social media platforms influence the circulation and reach of false scientific claims. However, in paper I, these elements are only briefly suggested, focusing predominantly on a conceptual analysis aimed at debunking acid-base-related information disorders.

Acknowledging the significance of these conceptual approaches, this paper advocates for expanded discussions on media and messages, considering contemporary challenges and what science education can be in service for nowadays. For example, papers II, III, and IV do not go further and discuss elements both fundamental for media literacy and science literacy, such as: how the messages were created and distributed; how nature of science is portrayed; what values and ideologies are present or absent; how and why the platforms that distribute the messages affect them; etc. In the next section, this paper amplifies the debate, suggesting contents that go beyond the text (or the acid-base contents) and bringing up contents that compose the messages' broader context. Those contents are usually absent in traditional chemistry classes but are recognized as fundamentals to make them more contextual and meaningful in contemporary times.

3.1. Nature of science

One disinformation analyzed by Paper I refers to a video message through which an unidentified person defends a highly efficient COVID-19 treatment assigned by Professor Chen Durrine, an alleged medical researcher in the Military Hospital of Pequin, China. The narrator claims that hot lemon can inhibit the proliferation of the SARS-Cov-2 virus. For this, the lemon must be cut into three parts and mixed with hot water, which would transform the blend into alkaline water. Moreover, the person claims that the carboxylic acids from the lemon tea could yet regulate hypertension, blood circulation, and reduce blood clotting.

Paper I's author debunks the claim of "alkaline water" made of lemon tea, using the chemical concepts of acids and bases, pH scale, and buffer solution. They also briefly commented that there was not enough evidence about the claimed efficiency of vitamin C (ascorbic acid) against COVID-19. Departing from the cited disinformation, those elements are suggested to be addressed in the chemistry classroom, but with more emphasis on the conceptual domain. Taking into consideration the paper's findings about the disinformation mentioned, it is argued that the nature of science is one aspect that can be covered with similar emphasis.

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To better understand how science-related information is conveyed in media, specialists advocate fostering in science classes a deeper understanding of how scientific knowledge is created and communicated (Allchin, 2021; Osborne and Pimentel, 2023; Reid and Norris, 2015). For example, it is not desirable to solely question the existence or non-existence of the cited Military Hospital of Pequin and its researcher, Professor Chen Durrine, but also debate whether one researcher or one research center is enough to represent scientific knowledge. Science is a social enterprise based on the consensus of specialists (Allchin, 2012; Höttecke and Allchin, 2020; Oreskes, 2019). Moreover, understanding scientific knowledge as the result of rigorous consensus among experts from diverse backgrounds and experiences is a crucial factor that makes science more reliable than other forms of knowledge (Valladares, 2022).

It is also necessary to recognize that science requires the contribution of those with relevant background knowledge (Oreskes, 2019). Thus, a statement made in the name of science by someone without expertise in the relevant scientific disciplines—such as a physician promoting an alkaline diet—is practically meaningless if it does not align with and reinforce the consensus established by scientific specialists. Under this approach, the focus is to investigate the author, not the (dis)information content, since science is a social endeavor, and epistemic trust emerges as a critical aspect (Oreskes, 2019). This is evident in the reliance on the work and findings of other specialists, their critiques, and so forth. However, trust is not assumed, but earned based on a track record of credentials, such as quality of educational background, mentors, coauthors, and home institutions (Höttecke and Allchin, 2020; Reid and Norris, 2015; Valladares, 2022). In this sense, Paper II's authors discuss a disinformation video through which a worker of the chemistry industry in Brazil, proclaiming himself as a self-educated chemist, criticizes the use of alcohol 70% (w/v) to combat SARS-Cov-2. According to him, the hygiene protocols with alcohol 70% were recommended solely and exclusively for the financial gain of the pharmaceutical industry, suggesting the use of vinegar for hand sanitation instead. Conceptual and procedural mistakes can be easily pointed out in the self-educated chemist's statement. The attempt to speak on behalf of science, by self-assigning credentials as a chemist, presents an intriguing case for discussing consensus, expertise, and epistemological trust. This is particularly relevant since, despite lacking a formal degree in chemistry, not all chemists may be considered experts in the specific matter addressed.

In summary, consensus, expertise, and epistemological trust are key attributes underpinning science's reliability. As Valladares (2022, p. 1326) states, they "[...] guarantee that personal judgments and opinions [...] do not dominate over the rest and be controlled intersubjectively, to a significant degree, by trained collectives, who have credentials that [...] identify them as experts in a field".

Another essential aspect emerged from the analyzes made by the four papers, but not fully addressed in any of them, is uncertainty. Paper IV's authors, for example, state that the results obtained in the experiment do not support the claims that the water filter's magnetizer acts on water properties as it is amply advertised. However, they acknowledge that only a limited number of experimental measurements for each parameter were made and students should draw their conclusions based on the experimental setting adopted and the resulting outcomes. In the same venue, Paper I's author analyzes a disinformation spread on WhatsApp in which it is promised that a mixture of lemon, baking soda, and hot water would eliminate SARS-Cov-2. To debunk this disinformation, the author starts by declaring that the mixture serves as a buffer solution in the body, and there is no evidence that it acts on viral conditions. Here, stemming from the recognition that additional experiments could strengthen Paper IV's conclusions and the observation that there is no evidence supporting the claim analyzed in Paper I, it is crucial to acknowledge that these nuances of uncertainty –an inherent part of scientific practices– are often met with skepticism or outright denial by those unfamiliar with nature of science (Rosenberg *et al.*, 2022). People usually do not deal with uncertainty without anguish; the assurance offered by some health-related disinformation is appealing and comforting (Lu *et al.*, 2021). Therefore, when dealing with scientific disinformation, it is crucial to address uncertainty as a content to better comprehend science limitations, not ceasing to be a uniquely important tool for addressing socioscientific problems (Covitt and Anderson, 2022). It is plausible to address elements of uncertainty in both quantitative forms (e.g., error bars, ranges of predictions, statistical tests, etc.) and qualitative forms (e.g., alternative hypotheses, limitations of studies, questions for future research, etc.) (Covitt and Anderson, 2022). Yet, teaching about uncertainty in the mentioned context also means complexifying the usual science class exercise of labeling information as fact (scientifically correct) or fake (scientifically incorrect). Care should be taken not to reduce the problem of disinformation to a simple true-or-false dichotomy; instead, students must understand that truth can sometimes be inaccessible, and that knowledge may be uncertain, biased, or ambiguous (Hauge, 2022).

Incorporating subjects and practices related to the nature of science can foster a deeper understanding of how science is conducted and functions as a social institution. Teaching the nature of science in these terms emphasizes epistemological performance as a core competence across the curriculum (Valladares, 2021). Without this focus, science education risks falling short of its transformative potential.

3.2. Traditional and modern media functioning

One crucial dimension to better understand how scientific (dis)information circulates into society is to comprehend how scientific claims reach the lay public. This is a fundamental aspect of the nature of science, precisely regarding how science communication happens in and outside of scientific communities (Belova and Eilks, 2015; Cardoso and Gurgel, 2019; Siqueira and Arroio, 2022). Teaching and learning about science also include a broader epistemological understanding of scientific knowledge flow (Allchin, 2015). Taking this approach, it is highlighted following the role of traditional and modern media in providing access to and transforming scientific information.

Beginning with traditional media, Paper III's author highlights imprecisions about chemical concepts in newspapers, as exemplified by a U.S. newspaper's statement about ammonia's contribution to acid rain (potentially a case of scientific misinformation). This serves as an opportunity to delve into two key contents: mediation and the role of media as gatekeepers. First, this mediation process –or conveying knowledge to lay public– is not a neutral enterprise (Funk *et al.*, 2019). Therefore, students need to understand that media outlets have, in general, commercial or governmental purposes (Cardoso and Gurgel, 2019; Höttecke and Allchin, 2020; Kellner and Share, 2019). This implies that even reputable media may occasionally prioritize sensationalism over scientific accuracy to attract new audiences (Reid and Norris, 2015).

Through this mediation process, specialized journalists serve as curators of scientific information, meaning they select

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what readers deem worthy of access (Höttecke and Allchin, 2020). Consequently, scientific information is adapted or reconstructed to reach non-specialist audiences. This transposition (or reconstruction) effort inevitably "alters" scientific knowledge and, in some cases, can make it inaccurate, especially when professionals involved do not have sufficient expertise on the subject in question (Cardoso and Gurgel, 2019). Besides, mediation efforts and the quality of scientific information published are impacted by the quantity of information sources and the time allocated for editing and publication (typically short due to the pressure to "publish first") (Reid and Norris, 2015). Therefore, the role of journalists is crucial in communicating science to the public, yet this mediation may lead to undesirable outcomes. For instance, in 1983, the Brazilian magazine Veja reported on a hybrid fruit supposedly made of tomato and cow fibers. However, the original story was an April Fools' joke published by the British magazine New Scientist, which Veja mistakenly presented as accurate and only retracted days later (Stroppa, 2018). On the other hand, some sectors of journalism have positively contributed to science communication by, over the last 70 years, highlighting the importance of scientific knowledge on the climate crisis and indirectly mobilizing political action (Höttecke and Allchin, 2020).

The merchandizing context analyzed by paper IV's author also extends the debate over the mentioned mediation process of scientific knowledge. Advertising primarily serves commercial interests, often at the expense of information quality. They typically offer minimal, if any, evidence, and the evidence for claims is frequently abbreviated or falsified (Belova and Eilks, 2015; Belova *et al.*, 2015; Kellner and Share, 2019). The enduring historical and cultural trust between the public and science remains a consistent element in advertising. This trust explains why scientific language and references to scientists are often emphasized to convey persuasive marketing messages—such as in the case of the filtering device analyzed in Paper IV, which falsely claims to reduce water acidity, while offering little rigor regarding the accuracy of the underlying concepts and procedures. Being well-informed about scientific knowledge and practices may be enough to avoid falling for misleading claims in advertisements (Dodds *et al.*, 2008). However, learning both with and about advertising, focusing on its rhetoric and convincing strategies, can increase even more chemistry's contribution to the development of science and media literacy (Belova and Eilks, 2015).

When it comes to social media such as Facebook, WhatsApp, and Instagram (platforms where all the messages analyzed by papers I and II broadly circulated through), their impact in the mediation of scientific knowledge increases and complexifies exponentially. This statement is supported by the evidence that false news is 70% more likely to be retweeted than fact-checked information (Vosoughi *et al.*, 2018). This tendency to overlook systematic and well-founded knowledge is explained by social media architecture (Kellner and Share, 2019). Some social media platforms were made for people to react and interact, via commenting, liking, reposting, sharing, upvoting, and so on, prioritizing popularity and summarized communication over information accuracy (Bimber and Zúñiga, 2020). The consistent impact of social media architecture on the quality of scientific information justifies its incorporation as a content into chemistry curricula, particularly when addressing issues of disinformation.

Social media architecture provides other important contents to address regarding how scientific information reaches out to the public. From the contexts analyzed by papers I and II, it is worth highlighting three important aspects: filter bubble, economy of attention, and eco chambers. While the internet offers unparalleled access to diverse information, it can also have the opposite effect by reinforcing existing beliefs and marginalizing opposing viewpoints (Pivaro and Girotto Júnior, 2020). The filter bubble effect is a consequence of the logic of communication behind social media, which, based on user's past behaviors, tailor personalized contents to please and keep them connected (Schulz and Roessler, 2012). This business model is called the attention market, through which big tech companies compete for users' attention, processing and selling it to advertisers (Wu, 2019). One side effect of this logic is the formation of echo chambers, communities on social media formed by people with similar preferences, where false scientific ideas are more likely to be endorsed than questioned or challenged (Höttecke and Allchin, 2020).

3.3. Values and ideologies

Characterizing certain misleading science-related information as disinformation, instead of fake news, post-truth or pseudoscience has one fundamental reason: to shade light on purposes and motivations of those who create and disseminate disinformation (Swire-Thompson and Lazer, 2022). This is why it is crucial to recognize the relationship between information and power, especially when cases of scientific disinformation are not merely due to a lack of scientific literacy but are instead driven by anti-democratic agendas (Arroio, 2020; Feinstein and Waddington, 2020; Pivaro and Girotto Júnior, 2020). For instance, the well-documented efforts of corporations to undermine the scientific consensus on the harmful effects of smoking and the reality of climate change are particularly notable (Oreskes and Conway, 2010). These controversies were intentionally manufactured to sideline science-based public policies and prioritize economic and ideological agendas. In her book *Why Trust Science*, Oreskes (2019) argues that the primary strategy of science deniers is to create the impression that the relevant science is unsettled and that the pertinent scientific issues are still open to debate. Oreskes' solution involves two dimensions, both explored in this paper. First, in dialogue with the Nature of Science's framework, she advocates teaching how science works, emphasizing that there are often sound reasons to trust established scientific claims. The second approach is to expose the ideological and economic motivations behind the science denial, revealing that the objections are not rooted in science but are instead political and ideological. For science educators, Valladares (2021) recommends revealing the conflicts of interest, ideological biases, and economic motivations behind science denial, emphasizing it as a political strategy to undermine public trust and highlighting the need to understand the social, political, economic, historical, and epistemic dynamics of science.

Therefore, efforts to discredit scientific knowledge without the epistemological rigor, or to sway public trust towards the "wrong science" (knowledge outside the specialist consensus) (Allchin, 2021), must be examined in the context of underlying values and ideologies. This approach will offer a deeper understanding of the scientific disinformation at hand. For instance, the messages analyzed in papers I and II propose simplistic and miraculous remedies to fight SARS-CoV-2, such as adopting an alkaline diet and using vinegar for hand sanitization. Simultaneously, these claims cast doubt on the health protocols recommended by expert-based organizations like the World Health Organization (WHO). In fact, usually behind movements like anti-vax and anti-mask –such as in the case of corporations trying to deny anthropological climate change– lay down anti-science, anti-technocratic, and anti-state views, along with the

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protection of values based on self-determination (Das and Ahmed, 2021; Oreskes, 2019).

In this sense, there is evidence that as science-literacy and numeracy increased among US-Americans, concern with climate change decreased, and what influences positive awareness among them is more cultural affiliation than scientific reasoning capacity (Kahan *et al.*, 2012). In another study, African-US-Americans presented lower trust in the national healthcare system, as a possible result of a legacy of racial discrimination in medical research and in the national health system (Boulware *et al.*, 2003). Therefore, to understand and then change how people grapple with scientific knowledge, it is necessary to comprehend their social and cultural context, addressing values, culture, history and ideologies (Feinstein and Waddington, 2020; Kahan *et al.*, 2012; Silva and Arroio, 2023). However, caution must be taken when conveying those contents in classroom to avoid the false conclusion that certain values and ideologies are anti-science by nature. Or even that to better understand or make science is necessary to adopt a "neutral perspective", treating values as corrupting influences. First, during the 1960s, the political left in USA criticized Science for its military applications, while today, those on the political right critique it for highlighting flaws in contemporary capitalism (Oreskes, 2019). Second, science's past and present give plenty of examples of when values have been in favor of a more humanized science. Socialist values, for example, were crucial to some geneticists' critique of eugenic thinking (Oreskes, 2019); and indigenous' philosophies have been decisive to improve the quality of scientific conclusions regarding analyses of risk and uncertainty in environmental disasters (Covitt and Anderson, 2022). In sum, science as an institution has historically been shaped by values - such as technical and economic progress (Lacey and Mariconda, 2014). This is no different for science education which today formally prioritizes practices, goals, and subjects that reflect values like social participation, emancipation, and justice (Silva and Sasseron, 2021; Silva and Arroio, 2023; Valladares, 2021).

Therefore, discussions around those contents seek to contribute to rethinking the teaching of acid-base theories in light of new cultural phenomena. It no longer makes sense for teaching to continue to focus solely on the transmission of traditional contents, given that they are covered in primary and secondary education, and after the many hours spent training students, they are still vulnerable to the information disorders circulating on social media (Osborne and Pimentel, 2023). Scientific education needs to contribute to the development of these students' critical thinking and certainly the teaching of acid-base theories content can lead to this criticality when they are embedded in today's social context.

4. Conclusions

This paper is grounded in the thesis that scientific disinformation and misinformation related to acid-base theories are more effectively addressed through multidisciplinary approaches. Integrating content from various fields enriches chemistry education by reflecting the complexity of contemporary, real-world issue. This approach promotes a more critical understanding and supports meaningful transformation within the contemporary information ecosystem, helping to mitigate its adverse impacts on society.

In this work, analyses of publications on how acid-base theories are addressed in cases of informational disorders revealed that a disciplinary approach heavily focused on chemistry content is often used to debunk erroneous messages, even when the contexts involve socioscientific issues like the COVID-19 pandemic. On the other hand, this work defends and gives examples of how other contents (nature of science; traditional and modern media functioning; and values and ideologies) can be brought together with acid-base theories to a better understanding of disinformation and misinformation discussed in the analyzed papers.

It should be noted that this paper does not present an exhaustive discussion of content possibilities. Other topics are equally relevant and encouraged, such as media language and semiotics, audience interpretation and positionality, and critical approaches to science. The possibilities are vast since critical thinking is not a formula, but a journey (Kovach and Rosenstiel, 2011). It is important to emphasize that merely addressing a catalog of content is not sufficient to generate meaningful change in addressing the harms caused by disinformation. Likewise, although teaching analytical procedures—such as identifying sources, genres, and formats of manipulated or falsified news—can be useful, they are not enough to tackle the complexity of the issue. What is required is the alignment of content with adequate resources, pedagogical principles, methodologies, goals, and competencies capable of fostering an education that is truly transformative.

Finally, the concepts of acids and bases are deeply intertwined with our daily lives. As the 100th anniversary of their theories approaches, this milestone offers an opportunity to reconsider how these concepts are taught and learned at all educational levels in an era dominated by social media. It is a chance to cultivate critical thinking in the current generation, laying the foundation for the next century of acid-base theories.

Authors' contribution

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Conflict of interest

The authors declare that there is no conflict of interest.

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Exploring the interplay of innovation competence and chemistry mastery: insights from educational practices and factors of influence

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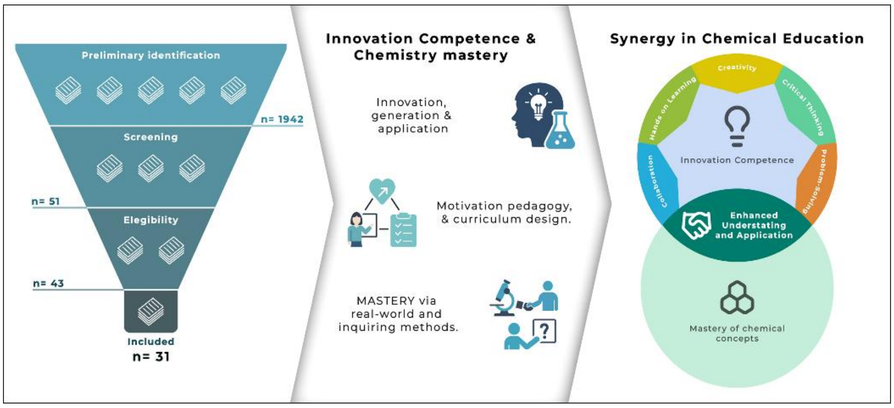
Abstract

Innovation competence is a critical skill today, enabling individuals to generate and apply innovative ideas. Its influence on students' mastery of chemical concepts and the factors shaping this relationship remain underexplored. This scoping review analyzes 31 studies to address three research questions: (1) How does innovation competence influence students' mastery of chemical concepts? (2) What factors shape this relationship? (3) What strategies can educators implement to foster innovation competence and chemical mastery? Findings reveal a positive correlation between innovation competence and chemical mastery, influenced by motivation, teaching strategies, and curriculum design. Practical strategies include problem-based learning, collaborative projects, and hands-on activities. While these insights provide valuable guidance, further research is needed to fully understand the interplay between innovation competence and chemical learning. This study offers actionable recommendations for enhancing teaching practices and advancing future research in chemistry education.



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Highlights

- Fluency, Innovation skills link strongly with chemical concept mastery.
- Motivation and teaching design shape students learning outcomes.
- Review of 31 studies shows chemistry–innovation connections.
- Innovative methods improve skills and concept understanding.
- Educational practices significantly impact chemistry learning.

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1. Introduction

Education is essential in fostering the development of a generation of innovators and problem solvers. The National Science, Technology, and Innovation (DSTIN) Policy 2021–2030 (MySTIE) and the Malaysian Science, Technology, Innovation, and Economic Framework 10–10 provide an overview that summarises the significance of science, technology, and innovation's (STI) role in making Malaysia a high-tech nation. By 2030 (DSTIN (2013-2030), the country needs to increase its Gross Domestic Product (GDP) to RM 3.4 trillion and reduce its dependence on foreign labor. Transforming Malaysia into a country that benefits from an ecosystem that fosters innovation is only possible if highly qualified STEM experts who can meet this demand are available. As a result, students must be prepared for professions in STEM fields with the innovative skills needed to compete globally (The United States Department of Education, 2016).

Innovation efficiency is needed to solve many global problems, especially in chemistry. The field of chemistry is also a focus area. It is linked to achieving some of the Sustainable Development Goals (SDG) of the United Nations to achieve a better and more sustainable future by 2030, such as in the field of nanotechnology, sustainable energy transition, smart cities, innovative industries, and other social and environmental issues (Droescher, 2018; Gomollón-Bel, 2020; Luo, 2018). The global world recognizes the efficiency of innovation, especially when it is considered that this factor can help prepare students to solve complex problems and positively affect individual personality quality (Atamanyuk *et al.*, 2021; OECD, 2019). Therefore, whether it is the view of scholars or educational policymakers, developing students' innovation competence is necessary to remain relevant in society (Krstikj *et al.*, 2022; Ojeda *et al.*, 2021). Cultivating innovation competence enables students to foster creativity, generate new ideas, solve problems, and identify new opportunities for problem-solving (Fredagsvik, 2023).

Chemistry students need to construct knowledge actively, work together in groups, and solve problems related to the real world (Anim-Eduful and Adu-Gyamfi, 2023). Through this method, students can be encouraged to connect the content of chemical concepts with real situations experienced by students. Innovative learning resources will be able to influence students' active learning (Clark *et al.*, 2018; Dunnigan *et al.*, 2020). In addition, carrying out a research project related to students' daily lives can enhance the mastery of analytical skills and a higher level of critical thinking skills (Jaleniauskiene and Kasperiuniene, 2022; Muna, 2021). Simultaneously, effective teaching practices can make learning chemistry more enjoyable, improve performance, and help students meet the required skills, especially in chemistry (Kalkbrenner and Horton-Parker, 2016).

One of the biggest obstacles to solving problems in a real-world context in chemistry is students' need to understand the basic concepts of chemistry (Mahaffy *et al.*, 2018). If students do not have a solid knowledge base, they may be unable to think creatively about how to apply that knowledge to solve problems (Ellah *et al.*, 2019). The study highlights chemistry students' challenges, revealing their difficulties in explaining natural phenomena based on acquired knowledge (Kanapathy *et al.*, 2019). Moreover, students encounter obstacles in problem-solving within the context of the natural world, struggling to generate diverse ideas—whether original concepts from their learned material or elaborations on existing ideas (Handayani *et al.*, 2021). High school chemistry students, despite exposure to relevant concepts, occasionally struggle with analyzing and interpreting chemical data (Lewis, 2020; Salleh *et al.*, 2023). Therefore, it highlights the need to develop innovation competence among chemistry students, which must be implemented immediately and optimally across disciplines, particularly at the secondary school level.

Recognizing the importance of meeting students' innovation competencies in equipping them to solve problems, especially the importance of chemistry in addressing real-life challenges and preparing students for the future, requires changes in the teaching and learning process. However, educational change requires dedication and transformation in the learning process. Hence, this study aims to review the synergistic relationship between innovation competence and chemical concept mastery and how their integration can enhance student learning in chemistry. Exploring this relationship aims to uncover how innovation competence and chemical concept mastery can mutually reinforce and amplify each other, resulting in more comprehensive and effective learning outcomes for students and answer the research questions:

- How does innovation competence influence the mastery of chemical concepts in students?
- What factors influence the relationship between innovation competence and mastering chemical concepts?
- What practical strategies can educators implement to foster innovation competence and mastery of chemical concepts in the classroom?

2. Method

The methodology for this study followed a scoping review framework, a structured approach well-suited for exploring emerging evidence where specific research questions or systematic review criteria are not yet clearly defined. Scoping reviews aim to examine the breadth and depth of available evidence, offering a comprehensive understanding of how research has been conducted in a field and the types of evidence available to inform practice.

This approach systematically identifies, categorizes, and maps existing studies to provide an overview of the current knowledge state while highlighting literature gaps. Framework by Arksey and O'Malley (2005) outlines four key objectives of scoping reviews: clarifying definitions and conceptual boundaries, summarizing research findings, identifying research gaps, and informing future investigations. For this review, a thorough search of relevant databases was conducted using predetermined inclusion criteria. Studies were selected, analyzed, and categorized into themes, which included definitions and measures of innovation competence, its relationship with chemical concepts, influencing factors, gaps in the literature, and implications for practice. By mapping the findings, the review delineated the existing evidence and pinpointed areas lacking rigorous investigation.

2.1. Preliminary identification

During the identification stage, research materials related to the developed research question on the relationship between innovation competence and mastery of chemical concepts were searched for. The identification process was initiated by keyword identification, where "classification of cervical cell" was one of the key phrases. Synonymous phrases were identified from dictionaries, thesauri, encyclopaedias, and previous research. With the help of these phrases, the search strings were constructed for the Web of Science and Scopus databases. Using these, an advanced search retrieved 1,942 from these databases.

Scopus: The following string was used:
TITLE-ABS-KEY (innovation AND competence AND education) AND (LIMIT-TO (SUBJAREA,"CHEM") OR LIMIT-TO (SUBJAREA,"SOCI")) AND (LIMIT-TO (DOCTYPE,"ar")) AND (LIMIT-TO (PUBSTAGE,"final")) AND (LIMIT-TO (LANGUAGE,"English"))
Access date: July 2023

Web of Science: The search was done by the following search parameters: Innovation competence (All Fields) AND Article or Review Article (Document Types) AND ((2020 OR 2021 OR 2022 OR 2023) Publication Years) AND Education Educational Research (Web of Science Categories)
Access date: July 2023

2.2. Screening

In the screening phase, the retrieved research items were screened for their content to align with the research questions. The content-related criteria were directed towards the selection of studies that addressed innovation competence in education. Initial screening excluded 1,891 publications, leaving 51 papers for further review based on inclusion and exclusion criteria. The first criterion emphasized literature type, prioritizing journal articles as primary sources of practical recommendations. Reviews, meta-analyses, books, book series, chapters, and conference proceedings were excluded. Additionally, publications were restricted to English and covered a 5-year timeline (2019–2023).

Inclusion criteria: English language, journal articles, final publication stage, and topics related to chemistry education and social sciences.

Exclusion: Non-English, conference papers, reviews, and subjects not from the domain of either chemistry education or social sciences.

2.3. Eligibility

Following the inclusion and exclusion criteria, the sample to be reviewed was filtered further to ensure clarity and transparency. At this stage, 51 articles were screened, and their titles and content were reviewed in detail for confirmation of relevance to the study objectives. This resulted in 12 publications being excluded because they did not fit within the empirical, data-driven scope of the study. This resulted in 31 articles being considered for in-depth evaluation. The strict process of eligibility determination underlines rigorous methodology and therefore assures the integrity and validity of the review findings. On that basis, such a systematic approach achieved appropriate alignment with the research objectives upheld to high-quality standards for selecting the articles.

2.4. Data analysis and abstraction

A detailed data analysis was performed of data from the 31 selected publications to identify the themes of related topics and subtopics, as described by **Fig. 1**. From this analysis, three themes emerged: **(1)** the relationship between innovation competence and mastery of chemical concepts; **(2)** factors influencing that relationship; and **(3)** implications for practice. Each theme was explored for more sub-themes or ideas emerging from the evidence in the context of the research. The data were discussed in collaboration with co-authors to ensure definitive interpretations and alignment with the research objectives. The results were reviewed to address any inconsistencies in the thematic analysis, ensuring the clarity, relevance, and appropriateness of the sub-themes. To avoid redundancy or confusion, the review focused exclusively on studies that directly aligned with the core research themes. This included only studies that explored the relationship between innovation competence and mastery of chemistry, as these were central to the research topic. By narrowing the scope in this way, the review provided a more focused and meaningful exploration of the factors influencing innovation competence and chemistry mastery within educational practices.

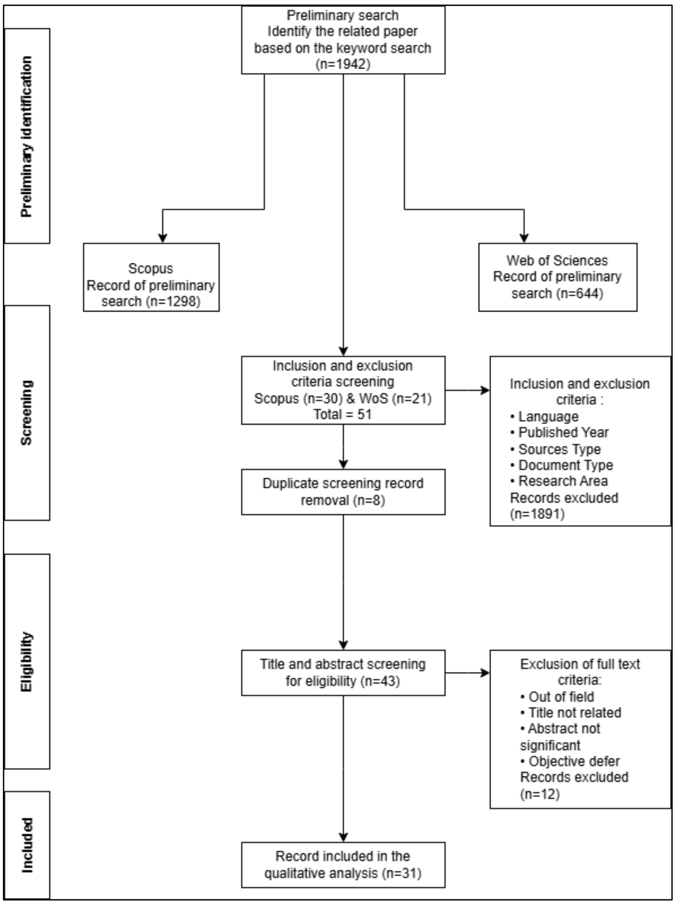

Figure 1. Flowchart of study selection adapted from PRISMA Statement.
Source: Guidance for Conducting Systematic Scoping Reviews (Peters *et al.*, 2015).

3. Result and discussion

3.1. How does innovation competence influence the mastery of chemical concepts in students?

Innovation competence contributes much to the students' mastering of chemical concepts, deepens their theoretical knowledge and practical skills, and develops their innovative thinking and active learning (Laliyo *et al.*, 2023; Rusmansyah *et al.*, 2019). It gives students the power to effectively present their ideas and solve problems that deepen their knowledge of chemical concepts (Margallo *et al.*, 2019). In these innovative activities of hands-on experiments, design challenges, and problem-based learning, the connection of theory with real situations is made (A. Solodikhina and M. Solodikhina, 2023); chemistry becomes more relevant and meaningful. These approaches not only enhance their mastery of chemical concepts but also encourage creativity in applying the same knowledge in new contexts.

Besides, innovation competence is a basis for acquiring essential critical skills: concentration planning, project management, social collaboration, and networking (Keinänen and Kairisto-Mertanen, 2019; Ong *et al.*, 2023). It has been revealed that a profound understanding of chemistry principles and their practical applications is an important prerequisite for developing innovative problem-solving skills. Active problem solvers build a holistic view to approach chemical phenomena, allowing them to explore, experiment, and apply chemical concepts innovatively (Margallo *et al.*, 2019). Such an interactive relationship of innovation competence to mastery in chemistry provides students with academic knowledge and practical skills.

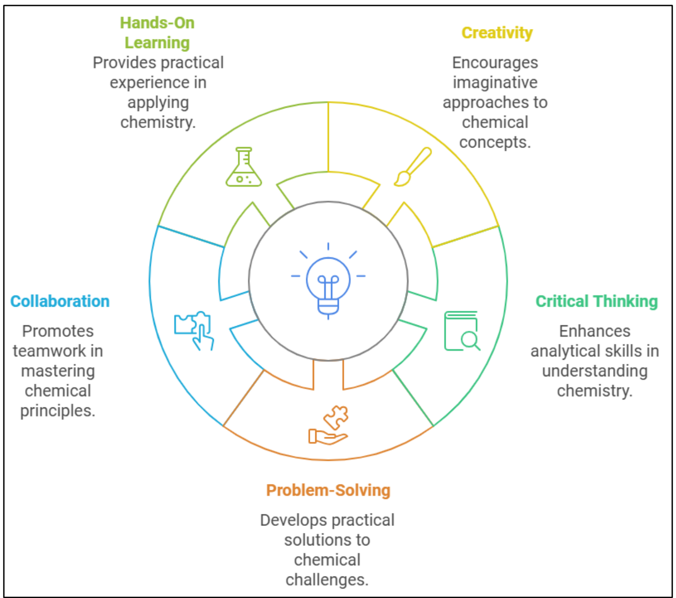

Figure 2. Innovation competence and its influence on students' mastery of chemical concepts.
Source: Elaborated by the authors.

Such research identified a strong positive association between innovation competence and chemistry concept mastery. Hence, an emphasis on skills in problem-solving, collaboration, critical thinking, and hands-on learning is underscored to facilitate a more profound understanding and application of chemistry (**Fig. 2**). Innovation competence will enable students to approach problems more creatively, solve complex problems, and work effectively in teams. These competencies are not only essential for solving real-world problems but also critical to improving both the theoretical and practical aspects of chemistry education.

Critical examination shows that while innovation competence supports creativity and problem-solving, it is not a panacea that assures deep conceptual understanding in chemistry. It may simply be that incorporating innovative capacity in learning automatically gives rise to improved understanding of chemistry concepts, disregarding challenges such as the differing degrees of learner engagement with innovative methodologies and the requirement for tailored interventions in keeping with individual learning styles. For instance, if students lack a solid foundation in chemistry, innovation-oriented activities will be challenging.

However, while collaboration and hands-on learning are essential for developing innovation competence, their effectiveness strongly depends on the quality of the collaborative processes and the nature of the learning activities. If not guided in an organized way, students might not be able to benefit fully from these strategies and might end up with a superficial understanding instead of deep conceptual mastery. For the latter, even though innovation competence pushes towards diversification of approaches, students will not apply their creative solutions if they do not have the critical thinking skills and scaffolding that connect abstract chemical principles to real-life scenarios.

3.2. What factors influence the relationship between innovation competence and mastering chemical concepts?

The relationship between innovation competence and understanding of chemical concepts is complex and influenced by various factors that interact across the educational, environmental, and individual levels (Hero *et al.*, 2021). Innovation competencies, such as creativity, problem-solving, critical thinking, and collaboration, would provide the necessary competencies to let students grasp chemistry concepts thoroughly and apply them in authentic real-life situations (Rampersad, 2020). However, the development of this competency does not solely depend on the learner; rather, it depends upon the educational methods used, the contextual variables surrounding the learner, and the inherent attributes that the students bring to the learning process. Understanding the way these determinants influence the chemical concept proficiency is vital for developing appropriate pedagogical strategies that foster innovation and prepare students with skills suitable for excelling in chemistry as a discipline (Ramírez-Montoya, 2021). In this context, a comprehensive exploration of these influencing factors is necessary to fully appreciate the dynamics in achieving innovation competence and chemical concept mastery.

The data provided in **Table 1** outlines that the relationship between innovation competence and mastering chemical concepts is influenced by several factors, which can be categorized into educational strategies, environmental conditions, and individual attributes. Furthermore, individual factors such as personality traits, flexibility, motivation, and personal characteristics play an essential role in students' competence and skills in mastering chemical concepts (Keinänen and Kairisto-Mertanen, 2019). Empirical evidence supporting the effect of these factors emphasizes their importance in shaping students' ability to build innovation competencies in their learning (Krstikj *et al.*, 2022).

By understanding the factors listed in the **Table 1**, educators and academic institutions can create a learning environment to foster innovation competence and an integrated holistic understanding of chemical principles. Each identified factor innovative models, blended learning, active learning, ecological environment, organizational support, learning strategies, personal characteristics, and motivation is synergistic in augmenting students' capabilities in thinking critically, solving problems creatively, and collaborating effectively. Taken together, these components create a dynamic learning environment that can significantly boost creativity and understanding of chemical principles, provided they are appropriately absorbed and adapted to the needs of the student (Charosky *et al.*, 2022; Ovbiagbonhia, 2021). These skills will be very important for the student to learn, not just the abstract concepts of chemistry, but also to apply these creatively to arrive at a deeper and more practical understanding of the subject (States *et al.*, 2023).

Moreover, by integrating these factors into chemistry education, educators can foster students' innovation competencies, empowering them to drive meaningful progress and contributions in chemistry while embracing lifelong learning and professional development opportunities.

Table 1. Summary of Findings: Influence Factors between Innovation Competence and The Mastery of The Concept.

Category	Factor Influenced	Impact on Innovation Competence and Chemical Concepts
Educational Strategies	Active Learning	Enhances fundamental understanding, collaboration, and problem-solving skills (Liliasari *et al.*, 2021; Zubair *et al.*, 2023)
	Innovative Models	Improves creative and critical thinking, practical application of knowledge (Krab-Hüsken *et al.*, 2023)
	Blended Learning	Makes abstract concepts tangible, improves subject-specific knowledge (Krab-Hüsken *et al.*, 2023; Obada *et al.*, 2023)
Environmental Conditions	Ecological Environment	Enhances innovation intention and effectiveness (Jenniffer *et al.*, 2022)
	Organizational Support	Provides resources and expertise for innovation (Sinaga *et al.*, 2019)
Individual Attributes	Learning Strategies	Enhances idea generation and promotion (Kirchhoff *et al.*, 2023)
	Personal Characteristics	Influences idea generation, networking, and decision-making (Brändle *et al.*, 2023; Kirchhoff *et al.*, 2023)
	Motivation and Attitudes	Affects competence development, requires diverse teaching methods (Anwar *et al.*, 2022; Brändle *et al.*, 2023)

Source: Elaborated by the authors using data from Scopus AI.

3.3. What practical strategies can educators implement to foster innovation competence and mastery of chemical concepts in the classroom?

To effectively foster innovation, competence, and mastery of chemical concepts, educators must implement practical strategies that engage students in both critical thinking and hands-on learning. Innovation competence includes skills relevant to mastering both the theoretical and practical aspects of chemistry: the ability to think creatively, solve complex problems and work collaboratively. Combining active learning, technology integration, real-world applications, and collaborative exercises in the learning environment fosters an environment where students not only gain a deeper understanding of chemistry concepts but also develop the skills necessary for innovation. These types of strategies help learners connect abstract chemical concepts to real-world problems, promoting deeper learning and development of critical innovation competencies.

Table 2 shows studies emphasizing the importance of implementing various practical strategies to produce innovation and creativity in different contexts. Techniques and methods are crucial factors in moulding creativity and fostering innovation (Hernández-Torrano and Ibrayeva, 2020), underscoring the significance of adaptability in thinking and comprehension of thinking processes. In education, universities are urged to prioritize teaching and evaluating innovation efficiency. They should deliberately design the learning environment to foster innovative competence among students (Ovbiagbonhia *et al.*, 2019). There is a call for developing and implementing an innovation competency framework in the national curriculum that aims to integrate education to build innovation competency systematically. Design learning approaches stimulate practical higher-order thinking skills, providing students with opportunities to develop the innovative mindset and skills needed to succeed in various domains (Cai and Tang, 2022). The practical implications of this study offer educators valuable rewards and collaborative educational practices that empower students to face future challenges and opportunities.

Table 2. Summary of findings: practical strategies for enhancing innovation competence and mastery of chemical concepts.

Practical Strategies	Impact on Understanding Chemical Principles
Design Thinking	Empowers innovation competencies and problem-solving skills (Nasir *et al.*, 2023; Obolewicz *et al.*, 2023)
Hands-on Pedagogy	Increases curiosity, engagement, peer learning, and collaboration (Spaan *et al.*, 2023)
Gamification and Experiential Learning	Enhances retention of key concepts and deep understanding of chemical processes (Czok *et al.*, 2023; Peeters *et al.*, 2023)
Innovative Learning Materials	Facilitates independent learning and better performance (Nagpal *et al.*, 2023)
Project-Based Learning (PBL)	Improves conceptual understanding and crucial learning skills (Chiu, 2021; He *et al.*, 2023)
STEM Integration	Enhances critical thinking and student engagement (Brunnert and Tausch, 2023)
Active Learning Strategies	Promotes higher-order cognitive skills and practical application of knowledge (Shidiq *et al.*, 2022)
Student-Centred Instruction	Improves understanding of concepts and increases interest in subject (Lenihan *et al.*, 2020)

Source: Elaborated by the authors using data from Scopus AI.

Each strategy is designed to encourage active and creative participation by students, with the goal of not merely increasing their understanding of the chemical concepts but inspiring them to apply these ideas in practical and imaginative ways. The **Table 2** provides a comprehensive set of strategies for educators looking to integrate these useful strategies to enhance student learning outcomes in chemistry.

4. Conclusions

In conclusion, the comprehensive literature review of 31 articles shows a conclusive and consistent association between innovation competence and students' understanding of chemical concepts. The results underline the critical role of developing innovation competencies, especially creativity, problem-solving, and collaboration, in the domain of chemistry education. There is a strong positive relationship in that when students have these competencies, their ability to learn and apply complex chemical concepts is significantly enhanced. **Figure 3** illustrates the interplay between innovation competence and the mastery of chemistry concepts among students. The insights presented here show that fostering innovation competence can lead to better academic performance and a deeper understanding of complex chemical principles.

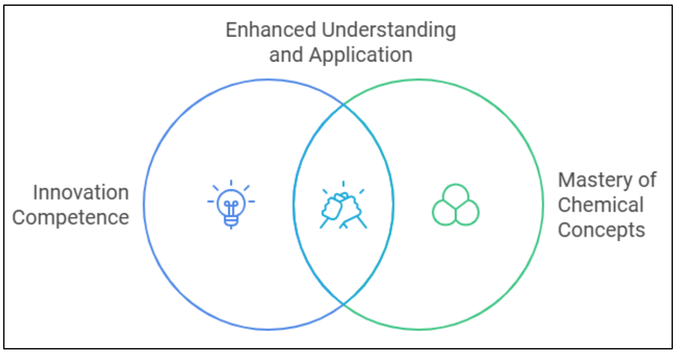

Figure 3. Interplay between innovation competence and the mastery of chemical concepts.
Source: Elaborated by the authors.

The main factors influencing this relationship include active learning, innovative models, blended learning environments, and organizational support, which together create an ecosystem that supports conceptual understanding and innovation skills development. Instructional methods such as Design Thinking, Hands-on Pedagogy, and Project-Based Learning (PBL) are particularly effective in driving this integration because they engage students in hands-on problem-solving and real-world application of chemistry concepts. The review suggests that future research should examine the enduring effects of these instructional strategies, especially within varied educational contexts, and their implications for students' creativity and critical thinking abilities over time. Additionally, it is advisable to conduct further research into the impact of personalized learning methods and the significance of technology in fostering innovative capabilities.

However, the review also highlights several limitations, such as the small scope of some of the studies regarding sample size and geographical context. In addition, the lack of standardized metrics for assessing innovation competence in chemistry education makes cross-study comparisons difficult. This should be addressed in future research by using much larger samples that are diverse in terms of backgrounds and by developing strong measures of innovation competencies applicable to all. This will yield more generalizable insights and help to hone in on the strategies necessary for creating more profound and meaningful learning experiences within chemistry education.

Authors' contribution

Conceptualization: Norliyana binti Md. Aris; Nor Hasniza binti Ibrahim; **Data curation:** Norliyana binti Md. Aris; **Formal Analysis:** Norliyana binti Md. Aris; Noor Dayana binti Abd Halim; **Funding acquisition:** Not applicable; **Investigation:** Norliyana binti Md. Aris; Noor Dayana binti Abd Halim; **Methodology:** Norliyana binti Md. Aris; Nor Hasniza binti Ibrahim; **Project administration:** Norliyana binti Md. Aris; **Resources:** Not applicable; **Software:** Not applicable; **Supervision:** Nor Hasniza binti Ibrahim; Johari Surif; **Validation:** Noor Dayana binti Abd Halim; Johari Surif; **Visualization:** Norliyana binti Md. Aris; **Writing – original draft:** Norliyana binti Md. Aris; **Writing – review & editing:** Nor Hasniza binti Ibrahim; Noor Dayana binti Abd Halim; Johari Surif.

Data availability statement

Data from Scopus and Scopus AI were analyzed by the authors and are included in the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Phytochemical, antioxidant, and enzyme inhibition potential exploration of *Nyctanthes arbor-tristis via in vitro* and *in silico* methods

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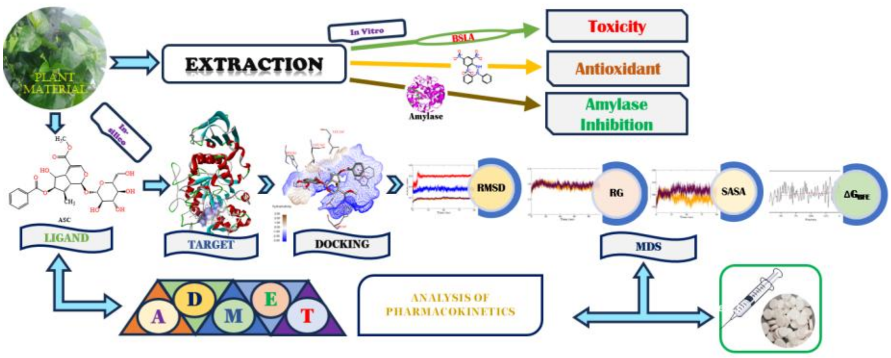
Abstract

Secondary metabolites in medicinal plants have been found to possess a broad spectrum of therapeutic properties. This study investigates the sequential extraction, quantitative phytochemicals, and bioactivity evaluations of Nyctanthes arbor-tristis leaf growing in Nepal. Methanolic extract contains the highest phenolics and resulted in the lowest IC50 values of 56±3 µg/mL and 157±3 µg/mL, in antioxidant and α-amylase inhibition assays, respectively. Hexane extract was found to contain abundant flavonoids and to be the most lethal to brine shrimp napuili with LC50 of 87±5 µg/mL. Phytochemicals arborside-C (ASC) and arborside-D (ASD) were found to be the most potent ligands to bind with α-amylase (PDB ID: 4GQR), resulting from docking and molecular dynamics simulation outcomes. The free energy changes calculated by the MMPBSA method and ADMET profiling of hit candidates supported by the spontaneity of complex formation reactions and their pharmacokinetic efficacy, respectively. This study proposes two compounds as hit candidates for the α-amylase target. Biological characterization using an in vivo approach is further recommended to assess their precise pharmacological validation.



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1. *Nyctanthes arbor-tristis;*
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4. arborside-C;
5. arborside-D.

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Rogéria Rocha **Gonçalves**

Highlights

- Ultrasonic sequential extraction of phytochemicals from *N. arbor-tristis* leaf.
- Quantitative phytochemical study (TPC and TFC).
- Assessment of in vitro antioxidant, toxicity, and α-amylase inhibition bioactivities.
- Molecular docking and molecular dynamics simulations (MDS) of phytocompounds.
- Identification of hit compounds against pancreatic amylase through in silico methods.

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1. Introduction

Nepal is home to a wide array of plants, with an estimated 9,000 different species of flowering plants (Khakurel *et al.*, 2022; Kunwar *et al.*, 2016). Plants have medicinal values due to secondary metabolites and are a good source of therapeutics (Kumar *et al.*, 2022). Biosynthesis, extraction, identification, structural elucidation, quantification, and physical and chemical properties of phytochemicals are the key to the drug discovery process (Butler, 2004; Choo and Chai, 2023).

Nyctanthes arbor-tristis L., a plant of the Oleaceae family, commonly called Night jasmine, is a typical shrub with bright, highly scented blooms that bloom at night and fall off before daybreak (Dewi *et al.*, 2022). The plant is 10 to 30 m in height, and its leaves are 2–6 cm broad, 6–12 cm long, simple, petiolate, exstipulate, and reticulate venation (Solanki *et al.*, 2021). *N. arbor-tristis* is distributed worldwide and found in tropical and subtropical regions, ranging in height up to 1500 m geographically in the Himalayan region of India, Nepal, and Pakistan (Jain and Pandey, 2016).

N. arbor-tristis leaves have a variety of chemical components, including alkaloids, glycosides, flavonoids, terpenoids, and tannins (Bb *et al.*, 2015; Meshram *et al.*, 2012). The therapeutic benefit is related to the presence of possible phytochemicals such as nyctantic acid, β-sitosterol, oleanolic acid, friedelin, arborside-A, arborside-B, arborside-C, arborside-D, arbortristoside-A, arbortristoside-C, benzoic esters of loganin, 6-β-hydroxyloganin, mannitol, astragalin, ascorbic acid, and methyl salicylate found in the leaves (Agrawal and Pal, 2013; Sah and Verma, 2012; Meshram *et al.*, 2012). The pharmacological studies showed their potential as antibacterial, anti-inflammatory, analgesic, antidiabetic, cough-suppressant, antioxidant, antimalarial, anti-arthritic, antispasmodic, antipyretic, immunostimulant, anthelminthic, antileishmanial, hepatoprotective, anti-allergic, antiviral, and CNS depressive (Sah and Verma, 2012; Laware and Shirole, 2023; Rawat *et al.*, 2021). Each component of this plant has been used to treat various ailments in Ayurveda, including arthritis, digestive issues, tonics, laxatives, diuretics, asthma, cough, discomfort, hemorrhoids, and irregular menstrual periods (Dewi *et al.*, 2022; Kushwah *et al.*, 2023).

Diabetes Mellitus (DM), type I and II are chronic hyperglycemia disorders. Type I diabetes is caused mainly by β-cell death of the pancreas that results in a reduction in insulin secretion, in contrast, type II diabetes (T2D) is characterized by insulin resistance in the cells (Mohamed *et al.*, 2023). The ingestion and absorption of dietary carbohydrates substantially increase postprandial blood glucose levels (Sugandh *et al.*, 2023). The enzyme α-amylase breaks down starch into glucose fragments by breaking down the glycosidic linkages (Proença *et al.*, 2019). Human pancreatic α-amylase enzyme (HPAE) inhibition is a conventional method utilized to treat T2D (Ogunyemi *et al.*, 2022). An *in silico* methodology has effectively decreased the expenditure associated with experimental procedures and the temporal requirements for determining complicated structures (Stănciuc *et al.*, 2020). It aims to ascertain the potential method of binding orientation, binding affinity, and stability of the molecules to enzymes (Vasanthkumar *et al.*, 2021). Molecular docking calculations and molecular dynamics simulations are used to test millions of possible binding orientations at receptor binding sites based on protein structures to propose the pharmacological significance of various chemicals in plant metabolites (Hollingsworth and Dror, 2018; Zhao *et al.*, 2021). The structure-based drug design (SBDD) utilizes docking and simulation programs for virtual estimation of drug-likeness, and further ADMET prediction anchors to drug-like molecules through its pharmacokinetic evaluation that binds with a particular receptor protein to manipulate its function (Lolok *et al.*, 2022).

In this study, an assessment of the *in vitro* antioxidant, α-amylase inhibition, brine shrimp lethality assay (BSLA), and estimation of TPC and TFC of the different extract fractions of *N. arbor-tristis* leaf were carried out. Molecular docking virtual screening, molecular dynamics simulations, and ADMET predictions of the compounds found in the plant leaf were further used to understand their mechanism and pharmaceutical aptitude towards HPAE. The result of this study can be used to justify and validate the potential of the *N. arbor-tristis* in antioxidation, cytotoxicity, and, importantly, α-amylase inhibition.

2. Experimental

2.1. Chemicals

Solvents hexane, chloroform, ethyl acetate, acetone, methanol (Qualigens Fine Chemicals), sulfuric acid, hydrochloric acid, aluminum chloride, sodium carbonate, dimethyl sulphoxide (DMSO), and sodium dihydrogen phosphate (Thermo-Fisher Scientific India) were used. Gallic acid (Hi-media Laboratories), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin (Wako Pure Chemicals, Osaka, Japan), Folin–Ciocalteu's phenol reagent (FCR), ascorbic acid, acarbose, and α-amylase (Hi-media Laboratories) were used, which were imported from India.

2.2. Plant collection and identification

The *N. arbor-tristis* leaves were collected in Sindhupalchok district, Nepal (altitude: 1350 m, latitude: 27°46'14" N, longitude: 85°48'59" E). The plant (voucher code 01KATH160201) was identified and verified at the National Herbarium & Plant Laboratories (KATH) in Lalitpur, Nepal.

2.3. Preparation of plant extracts

The collected and dried 1 kg leaves of *N. arbor-tristis* were powdered using an electric grinder. Through an ultrasonic extraction process, different leaf extracts, hexane extract (**HE**), chloroform extract (**CE**), ethyl acetate extract (**EAE**), acetone extract (**AE**), methanol extract (**ME**), and distilled water extract (**DWE**), were prepared in six different solvents, hexane, chloroform, ethyl acetate, acetone, methanol, and distilled water, respectively through a sequential extraction (solid-liquid fractionation) in increasing polarity order of the solvents.

2.4. Preliminary phytochemical profiling

A phytochemical study of *N. arbor-tristis* leaf was conducted to profile the various natural constituents in the extracts using a standard protocol (Banu and Cathrine, 2015).

2.5. Phenolic content (TPC) determination

Folin-Ciocalteu colorimetric analysis based on an oxidation-reduction reaction was used to TPC with minor modifications (Gautam *et al.*, 2022). From the serially diluted concentration of standard gallic acid stock (500 to 25 μg/mL), 20 μL of each was dispensed in a 96-well plate containing 100 μL of Folin-ciocalteu reagent and incubated for 5 min at room temperature in the dark. 80 μL of 7% Na_2CO_3 was added to the reaction mixture and further incubated for another 2 h at 23 °C. The resulting blue-colored mixture was subjected to measuring absorbance using a spectrophotometer at 765 nm in triplicate, and a calibration curve was plotted. The exact process was repeated for all plant extract fractions, and the TPC of each extract was calculated as gallic acid equivalent per gram (GAE/g) using a calibration curve.

2.6. Flavonoid content (TFC) determination

The aluminum chloride colorimetric assay was used to measure the TFC of the extracts described in previous work (Chandra *et al.*, 2014). Briefly, the stock solution of standard quercetin in methanol was serially diluted (250 µg/mL to 25 µg/mL) and added to the microplate well. 2% aluminum chloride in methanol (100µL) was added to it and incubated for 10 min in the dark. The absorbance of the reaction mixture was measured at 425nm through a spectrophotometer. The calibration curve was plotted, the exact process was repeated for all plant extract fractions, and TFC was calculated as quercetin equivalent per gram (QE/g).

2.7. Antioxidant activity assay

Using 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) (Blois, 1958), an antioxidant activity assay was done on the protocol described in previous literature, with some modifications (Sabudak *et al.*, 2013). The stock solution of standard ascorbic acid was resolved into concentrations of 30 µg/mL to 2.5 µg/mL through serial dilution. 2 mL of each concentration of ascorbic acid was mixed with 2 mL 0.2 mmol/L DPPH solution in triplicate and kept in the dark for 30 min. The absorbance was measured at 517 nm against methanol and DPPH as a blank. The exact process was repeated for each extract fraction (triplicate) at different concentrations, and absorbance was measured. Using a graph plot between the percentage scavenging activity of extracts vs concentrations in GraphPad Prism, the half-maximum inhibitory concentration (IC_{50}) of each extract was calculated. **Equation 1** was applied to evaluate the %DPPH radical scavenging.

$$\%\ \text{DPPH Scavenging} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{(A_{\text{blank}})} \times 100\% \qquad (1)$$

2.8. α-Amylase inhibition assay

The α-amylase inhibition activity was performed using the 3,5-dinitrosalicylic acid (DNSA) method, with some modifications (Mustafa *et al.*, 2021). 10% dimethyl sulfoxide (DMSO) was used to dilute the *N. arbor-tristis* leaf extracts and generate various concentrations. The diluted solutions in different test tubes were mixed with DMSO, buffer, and NaCl at a pH of 6.9. This mixture was added with α-amylase (1,4-α-D-glucano-glucanohydrolase) solution (200 µL) and incubated for 10 min at a temperature of 30 °C. The starch solution (0.1%) was added to each tube in a volume of 200 µL, and the tubes were incubated for 3 min. The process was stopped by adding DNSA reagent (200 µL) and warmed in a water bath for 10 min at 85-90 °C. After reaching room temperature, the reaction mixture was diluted by adding distilled water (5 mL), and the absorbance of the reaction mixture was measured at 540 nm. The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µL of buffer. The standard acarbose solution was taken as a positive control. The percentage of amylase inhibition was estimated using **Eq. 2**.

$$\%\ \text{inhibition} = \frac{Abs.blank - Abs.\ sample}{Abs.blank} \times 100\% \qquad (2)$$

By plotting the extract concentrations against the percentage of α-amylase inhibition in the dose-inhibition curve using GraphPad Prism, the IC_{50} value was estimated for each extract.

2.9. Brine shrimp toxicity assay

The brine shrimp toxicity assay is a valuable introductory screening tool to determine the potential toxicity of various compounds (Niksic *et al.*, 2021). It involves assessing their potential to induce mortality in laboratory-cultured brine shrimp (*Artemia salina*) nauplii, and the protocol is based on previous work (Majumder *et al.*, 2019). Artificial seawater was prepared, and brine shrimp eggs were hatched for 48 h. A stock solution of each extract was successfully diluted (1000 µg/mL to 62.5 µg/mL) using the serial dilution method. Varying amounts of plant extract were applied to ten nauplii and left for 24 h. DMSO was used as a blank, and potassium dichromate as a positive control. The mortality endpoint was observed for each extract fraction after application to the prepared solution in a triplicate format. The lethality percentage for each concentration was determined by counting the number of dead and live nauplii. **Equation 3** was used to calculate the percentage mortality of the nauplii.

$$\%\ \text{Mortality} = \frac{\text{No. of dead shrimps}}{\text{Total No. of shrimps}} \times 100\% \qquad (3)$$

The LC_{50} represents the concentration at which the tested extract kills 50% of the brine shrimp nauplii. It was calculated using GraphPad Prism.

2.10. Computational tools

2.10.1. Ligand selection

The previously isolated compounds (iridoids, flavonoids, and phenolic compounds) from different extracts of the leaves of *N. arbor-tristis* were taken as candidate ligands as HPAE inhibitors (**Table 1**). Molecular structures of some of the selected ligands are presented in **Fig. 1**. The **Supplementary Information** (**Table 1S** and **Fig. 1S**) lists other detailed information and structures of all selected ligands.

Table 1. Some of the top candidate ligands selected from *N. arbor-tristis* leaf.

Ligands	Ligand ID	Molecular weight (g/mol)	PubChem CID	Reference
Arborside-A	ASA	614.6	182902	Dewi *et al.* (2022)
Arborside-B	ASB	494.5	182903	Dewi *et al.* (2022)
Arborside-C	ASC	510.5	182904	Agrawal and Pal (2013)
Arborside-D	ASD	556.5	101685135	Agrawal and Pal (2013)
Arbortristoside-A	ATSA	566.5	6442162	Vishwakarma *et al.* (2022); Rathore *et al.* (1989)
Arbortristoside-C	ATSC	552.5	23955893	Dewi *et al.* (2022)
Astragalin	AG	448.4	5282102	Sah and Verma (2012)

Source: Elaborated by the authors.

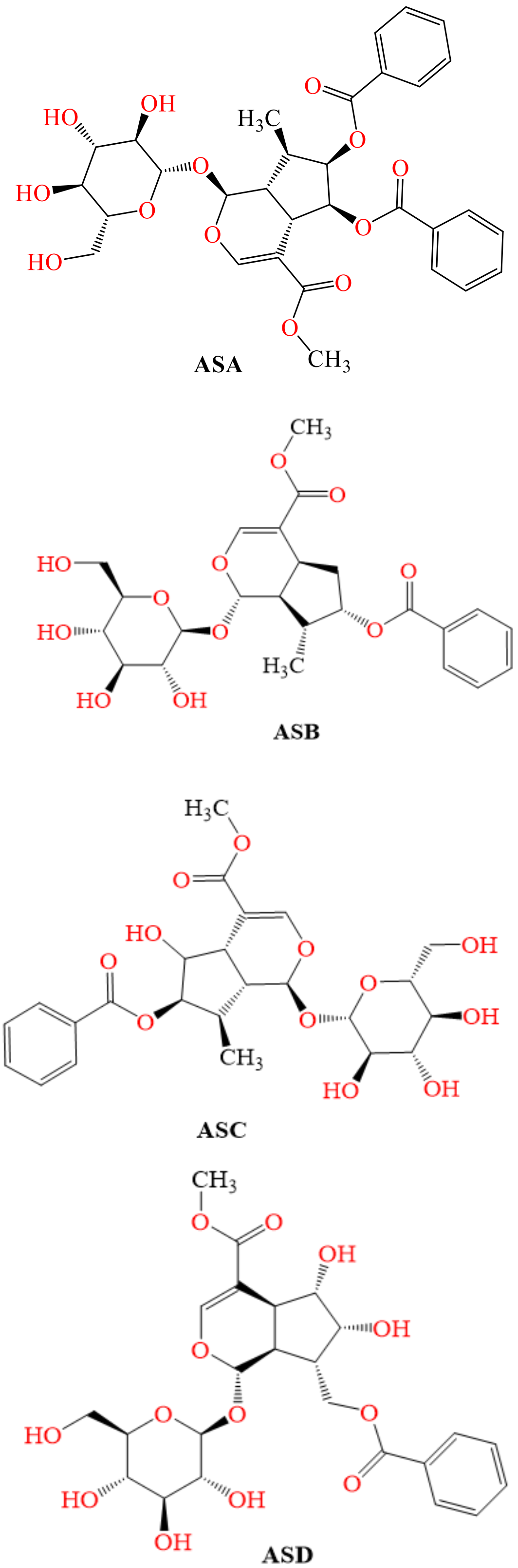

Figure 1. Molecular structures of some of the top selected ligands, **ASA** (Arborside-A), **ASB** (Arborside-B), **ASC** (Arborside-C), and **ASD** (Arborside-D).
Source: Elaborated by the authors.

2.10.2. Ligand preparation

The 3D structures and coordinates of ligands were retrieved from the PubChem database in sdf format with the respective PubChem CID mentioned in **Table 1**. The bond order and molecular formula of compounds were verified and converted into pdb format using the PyMOL software (version 2.5.5) (Yuan *et al.*, 2017). Using the conjugate gradient algorithm, the Universal Force Field (UFF) for 5000 cycles at 10^{-8} units of energy convergence was chosen after adding the hydrogen atoms in the Avogadro program (version 1.2.0) for molecular structure optimization (Hanwell *et al.*, 2012). AutoDock Tools converted pdb into pdbqt, which is required for molecular docking (Trott and Olson, 2009). Root mean square deviation (RMSD) between the docked ligand (myricetin) and native ligand (myricetin) in the crystal structure of the protein (PDB ID: 4GQR) was calculated to be less than 2 Å for docking protocol validation (Shrestha *et al.*, 2024) (presented in **Fig. 2S** in **Supplementary Information**).

2.10.3. Target selection and preparation

HPAE (PDB ID: 4GQR) with a resolution of 1.20 Å, with an X-ray crystallographic structure, was restored from the RCSB database (**https://www.rcsb.org/**) (Berman *et al.*, 2000). The protein structure visualization and processing were done using the PyMOL program. The active sites (catalytic triad, ASP197, GLU233, and ASP300) of the enzyme were determined using a co-crystallized receptor-ligand complex structure (Liu *et al.*, 2017a). The protein was cleaned in PyMOL software by removing water molecules, ions, and non-standard residues, and the apo structure was stored as a pdb file. The protein was processed by adding polar hydrogen atoms and Kollman charges in AutoDock Tools and converted into pdbqt format, which was required for molecular docking. The grid box was set to cover all the active site residues. The grid box center (16.731, 17.235, and 42.467) and the grid box size (x = 44, y = 46, z = 44 in Å with spacing of 0.375 Å) in the receptor protein were selected.

2.10.4. Molecular docking calculations

The binding mechanism (pose, orientation, and location) between the ligand and the receptor was investigated using molecular docking studies. The AutoDock Vina software (version 1.5.7) was used to conduct rigid molecular docking calculations (Trott and Olson, 2009). The chosen molecules were docked and examined based on the possible protein-ligand interactions and the lowest binding affinity (docking score). During molecular docking, the ligand remained flexible in the active site pocket of the protein despite the protein's rigidity. Control parameters such as the number of modes, energy range, and exhaustiveness were 20, 4, and 64, respectively, for all docking computations. The stable protein-ligand complex with the highest binding affinity was ultimately determined using a scoring function. Biovia Discovery Studio Visualizer (version 21.1.0.20298) was used for the visualization of protein-ligand interactions (Shaweta *et al.*, 2021). For the stability assessment in terms of geometrical and thermodynamic parameters, a molecular dynamics simulation of the complex with the pose with the best binding energies was selected.

2.10.5. Molecular dynamics simulation (MDS)

The MDS of the ligand-protein adducts were performed using the GROMACS software (version 2021.2) (Abraham *et al.*, 2015). The Charmm27 force field from the swissparam server (**https://www.swissparam.ch/**) (assessed on January 10, 2024) was used for both the ligand and the receptor (Zoete *et al.*, 2011). Utilizing the TIP3P water model, a triclinic box system was solvated. 12 Å spacing was chosen to minimize erroneous interactions between the periodic images. The system was neutralized, and an isotonic solution of NaCl was employed. At a physiological temperature (310 K), the system was equilibrated in four stages, each lasting 200 ps. For NVT equilibrium, the initial two stages were completed, and for NPT equilibrium, the final two phases were chained. The final production run was conducted for 100 ns without any constraints, and several parameters, including RMSF, SASA, RMSD, and R_g, were retrieved from the MDS trajectory using the built-in modules of the GROMACS program.

2.10.6. Binding free energy changes (ΔGBFE) estimation

The MMPBSA method was used to calculate the change in the binding free energy (ΔG_{BFE}) of the adduct (Onufriev and Case, 2019). The viability and spontaneity of the forward reaction were evaluated based on the assessment of free energy changes. The binding free energies of the complex, protein, and ligand were determined using the gmx-MMPBSA module on an equilibrated trajectory segment of 200 frames for 20 ns. **Equations 4** and **5** were used to calculate the binding free energy change during complex formation (Wang *et al.*, 2019).

$$\Delta G_{BFE} = G_{complex} - G_{receptor} - G_{ligand} \quad (4)$$

$$\Delta G_{BFE} = \Delta H - T\Delta S = \Delta E_{MM} + \Delta G_{SOLV} - T\Delta S \quad (5)$$

where,
$\Delta E_{MM} = \Delta E_{IN} + \Delta E_{vdw} + \Delta E_{ELE}$
$\Delta G_{SOLV} = \Delta G_{PB} + \Delta G_{SA}$
ΔG_{BFE} = Binding Free Energy changes
ΔE_{MM} = Energy change in gas phase molecular mechanics
ΔE_{IN} = Internal energy of the system
ΔE_{vdw} = van der Waals energy
ΔG_{SOLV} = Electrostatic solvation energy
ΔG_{PB} = Polar contributions in solute-solvent system
ΔE_{ELE} = Electrostatic energy
ΔS = Entropy changes of the system
ΔG_{SA} = Nonpolar contributions in the system

The entropy term (**TΔS**) was not considered in the binding free energy calculation because of significant technical costs and errors raised during computational calculations (Wang *et al.*, 2019).

2.11. ADMET profiling

Swiss ADME (**http://www.swissadme.ch/**), ProTox-II (**https://tox-new.charite.de/protox_II/**), and pkCSM (**https://biosig.lab.uq.edu.au/pkcsm/**) servers (accessed on January 19, 2024) were used for calculating absorption, distribution, metabolism, excretion, and toxicity parameters of hit compounds and reference drugs (Acarbose and Miglitol) (Banerjee *et al.*, 2018; Pires *et al.*, 2015).

2.12. Statistical evaluation

All the *in vitro* experimental results were taken in triplicate (n = 3). TPC, TFC, and binding free energy results were presented as mean ± SD (standard deviation), and quantitative biological activity tests (DPPH assay, amylase inhibition, and BSLA) were calculated in terms of mean ± SEM (standard error of mean) for a more reliable IC_{50} calculation. GraphPad Prism (version 9.4.1) was used to calculate IC_{50} values of bioactivities. TPC and TFC were calculated using Microsoft Excel 2021.

2.13. Computational resources

Molecular docking calculations, data interpretation, and visualization were done using Windows 11 (8 GB RAM, 8-core CPU processor). Molecular dynamics simulation and binding free energy calculations were performed in Ubuntu 20.04.06, an LTS operating system, a 24-core processor machine with a 24 GB GPU accelerator.

3. Results and discussion

3.1. Qualitative estimation of phytochemicals

Qualitative analysis of the phytochemicals gives a preliminary idea of constituents present in the extracts and helps to quantify and further characterize (Olayinka *et al.*, 2010). The FT-IR analysis of the extract is presented in the **Supplementary Information** in **Fig. 3S**. Different extracts showed distinct results in screening following polarity and phytoconstituents present in the leaf of *N. arbor-tristis*. Alkaloids, flavonoids, terpenoids, glycosides, phenolic compounds, steroids, carbohydrates, and quinones were identified (**Table 2**) as the primary ingredients.

Table 2. Phytochemical screening of the various extracts.

Class of phytochemicals	HE	CE	EAE	AE	ME	DWE
Alkaloids	–	+	+	+	+	+
Phenolic Compounds	–	+	+	+	+	+
Flavonoids	–	+	+	+	+	+
Terpenoids	+	+	+	+	+	–
Cardiac Glycosides	–	–	+	+	+	+
Carbohydrates	–	+	+	+	+	+
Proteins	–	–	+	+	+	+
Triterpenoids	+	+	+	+	–	–
Tannins	–	+	+	+	+	+
Resins	–	–	–	+	+	+
Steroids	–	+	+	+	+	–
Quinones	+	+	+	+	+	–
Saponins	–	–	–	–	–	–

Note: + refers presence; – refers absence. **HE** (Hexane extract); **CE** (Chloroform extract); **EAE** (Ethyl acetate extract); **AE** (Acetone extract); **ME** (Methanol extract); **DWE** (Distilled water extract).
Source: Elaborated by the authors.

3.2. Quantitative estimation of phytochemicals

The yield percentage was found to be the highest for the extract **CE** (7.3%) among all extract fractions. Phenolic compounds and flavonoids are natural products that have the potential for pharmacological activity, like antioxidant, antidiabetic, anti-inflammatory, and Anticarcinogen (Zain and Omar, 2018). TPC of different fractions was determined using Folin-Ciocalteu reagent with slight modification with the help of a standard gallic acid calibration curve (Y = 0.0039X + 0.0568, and R^2 = 0.9957), likewise, TFC was calculated through spectrophotometry of the colored solution of aluminum chloride reagent with extract with the help of a standard quercetin calibration curve (Y = 0.0068X + 0.00704, and R^2 = 0.9997). The standard calibration curves are included in the **Supplementary Information** (**Fig. 4S**). The % yield, TPC, and TFC of all fractions are listed in **Table 3**. Extracts of **AE** and **ME** fractions of *N. arbor-tristis* were found to have a high content of the phenolic compound of 137±4 mg GAE/g and 139±4 mg GAE/g, respectively. TFC was found high in extract **CE** (369 ± 4 mg GAE/g) and **HE** (286 ± 10 mg GAE/g) fractions.

Table 3. The yield, TPC, and TFC of different extract fractions.

Extracts	Chemical contents		
	Yield%	TPC (mg GAE/g)	TFC (mg QE/g)
HE	0.45	18 ± 4	286 ± 10
CE	7.3	46 ± 4	369 ± 4
EAE	1.55	78 ± 5	92 ± 4
AE	2.3	137 ± 4	62 ± 3
ME	3.15	139 ± 4	68 ± 4
DWE	2.97	56 ± 4	17 ± 2

Note: TPC and TFC = triplicate average ± SD. **HE** (Hexane extract); **CE** (Chloroform extract); **EAE** (Ethyl acetate extract); **AE** (Acetone extract); **ME** (Methanol extract); **DWE** (Distilled water extract).
Source: Elaborated by the authors.

3.3. DPPH scavenging assay

The DPPH radical scavenging assay gives the *in vitro* quantitative figure of the antioxidant potential of metabolites found in phytochemicals (Sethi *et al.*, 2020). *N. arbor-tristis* extract fractions displayed active antioxidant potentials towards DPPH free radicals. Extracts **ME** and **AE** were found to be the most potent antioxidants with IC_{50} values of 56 ± 3 g/mL and 79 ± 3 µg/mL, respectively, which were found to be marginally higher IC_{50} values than standard ascorbic acid (**Table 4**). Other extracts (IC_{50} > 100 µg/mL) showed mild antioxidizing phenomena. The order of the DPPH scavenging capacity of the extracts can be illustrated as **ME>AE>DWE>EAE>HE>CE**.

Table 4. Comparative antioxidant, amylase inhibition, and toxicity results containing values of various extracts with respective positive control.

Extracts and positive controls	Evaluated bioactivity		
	DPPH scavenging (IC_{50} in µg/mL)	α-Amylase inhibition (IC_{50} in µg/mL)	Brine shrimp lethality assay (LC_{50} in µg/mL)
HE	120 ± 5	547 ± 9	97 ± 2
CE	410 ± 6	386 ± 15	295 ± 7
EAE	126 ± 2	1656 ± 8	161 ± 5
AE	79 ± 3	919 ± 9	240 ± 5
ME	56 ± 3	157 ± 3	175 ± 3
DWE	104 ± 6	1799 ± 7	998 ± 10
***Ascorbic acid**	17 ± 3	–	–
***Acarbose**	–	52 ± 1	–
***$K_2Cr_2O_7$**	–	–	152 ± 2

Source: Elaborated by the authors.

3.4. Alpha-amylase inhibition assay

The α-amylase inhibition activity of different extracts is listed in **Table 4**. Among the fractions, **ME** of IC_{50} 157 ± 3 **µg/mL** was found to be a significant α-amylase inhibitor compared to other fractions. Extracts **HE** and **CE** exhibited moderate inhibition, whereas **EAE**, **AE**, and **DWE** showed weak inhibitory activity to the amylase enzyme. The extract **ME** showed a good amylase inhibition activity compared with standard acarbose (52 ± 1 µg/mL), signifying the antidiabetic potential of *N. arbor-tristis*. The amylase enzyme inhibition potential of the extracts was found to be in the following order: **ME > CE > HE > AE > EAE > DWE**.

3.5. Brine shrimp toxicity evaluation

Brine shrimp cytotoxicity assay portrayed a moderate toxicity in all extracts. LC_{50} of **HE** (97 ± 2 µg/mL) was found to be the least toxic to brine shrimp larvae. LC_{50} values of extracts **CE**, **EAE**, **AE**, and **ME** were found to be moderate, and comparable to each other and the positive control potassium dichromate (LC_{50} < 300 µg/mL). The comparative illustration of all the extracts with lethal concentration is mentioned in **Table 4**.

The trending fitting curve of the observed data of different bioactivity is presented in **Figures 5S**, **6S**, and **7S** in **Supplementary Information**.

3.6. Computational virtual screening

3.6.1. Binding affinities from molecular docking calculation

The most conventional method to inhibit HPAE is to bind it with a suitable ligand/drug at its orthosteric site (Cele *et al.*, 2022). Molecular docking is an easy, preliminary, virtual, and rapid computational method to compute and analyze the compatibility of any molecule (guest) and its possible therapeutic activity with the active macromolecular protein (host) through Host-Guest interaction (Das *et al.*, 2024). Further viability and stability of the docked complex were assessed using MDS. The compounds found in the leaves of *N. arbor-tristis* were examined to determine their HPAE binding capacity through computation. Most of the candidate ligands scored better in molecular docking than the native ligand myricetin (–33.1 kJ/mol) with the amylase receptor (PDB ID: 4GQR) (Bitew *et al.*, 2021), and the calculations are shown in **Table 5**. Conventional hydrogen bonds, Pi-alkyl, Pi-Pi stacked, other hydrophobic interactions, and van der Waals interactions were the noticeable non-covalent interactions in the protein-ligand complexes. Among all ligands, **ASC** and **ASD** scored the same affinity of –33.5 kJ/mol, and it was found that these ligands exhibited a stable trajectory in MDS, which might be a consequence of the strong interactions in the adduct with a larger hydrogen bond count and proper orientation of the ligand with the receptor. By conventional hydrogen bond, ligand ASC interacted with amino acid residues, HIS305, GLU233, ASP197, and ASP300 (<3 Å). On the other hand, ligand **ASD** interacted with TRP59, TYR151, THR163, and HIS 201 through hydrogen bonding (<2.6 Å**)** along with other possible interactions. Such strong interactions (between ligands and active site triad) might provide stability to the complexes, which were further supported by the MDS results of both ligands, which are discussed later. Although **ASB** showed the highest docking score (–34.7 kJ/mol), it was found to be unstable in the amylase binding pocket (in MDS). The docking score and its validity through MDS signified the stability of the adduct at physiological temperature, which could result in the inhibition of the target enzyme (Omar *et al.*, 2022). Most of the ligands were found to interact with the catalytic triad of amylase (**APS197**, **GLU233**, and **ASP300**), along with ASP356, HIS305, ILE235, HIS201, TRP59, ALA106, and ALA198 (Chothani *et al.*, 2024; Renganathan *et al.*, 2021; Zahra *et al.*, 2024).

Table 5. Interactions between the compounds (top ligands, drug, native ligand) and the amino acid residues in ligand-protein complexes from molecular docking calculations.

Candidate Ligands	Docking Score (kJ/mol)	Interactions	Active site residues (Distance Å)
ASB	**–34.7**	Conventional Hydrogen Bond	GLU233 (2.47, 2.66), ASP300 (2.12)
		Pi-Alkyl	TRP59 (4.38, 5.10), HIS305 (4.60)
		Carbon Hydrogen bond	HIS305 (3.77)
		Alkyl	LEU165 (4.74)
		van der Waals	TRP58, ASP197, TYR62, HIS201, GLN63, VAL107, LEU162, THR163, ARG195, ILE235,
ASC	**–33.5**	Conventional Hydrogen Bond	ASP197 (2.45), GLU233 (2.07), HIS305 (2.79), ASP300 (2.73)
		Alkyl	LEU162 (3.90), LEU165 (4.94)
		Pi-Alkyl	TRP59 (4.20), HIS305 (2.79)
		Pi-Pi Stacked	TRP59 (4.06)
		van der Waals	TRY62, TYR151, THR163, ARG195, GLN63, ALA198, TRP58, LYS200, ILE235
ASD	**–33.5**	Conventional Hydrogen Bond	TRP59 (2.14), TYR151 (2.57), THR163 (2.21), HIS201 (2.13, 2.58)
		Pi-Alkyl	HIS299 (4.38)
		Carbon Hydrogen bond	GLU233 (3.34), ASP300 (3.38)
		Pi-Pi Stacked	TYR151(3.93)
		van der Waals	TRP58, GLN63, LEU162, ARG195, ASP197, ALA198, GLU60, LYS200, TYR62, ILE235
ASTA	**–33.5**	Conventional Hydrogen Bond	GLU233 (1.84), ASP300 (2.23)
		Pi-Alkyl	HIS201 (5.16)
		Carbon Hydrogen bond	TRP59 (3.80), HIS299 (3.60)
		Pi-Pi Stacked	TRP59 (4.98)
		Pi-sigma	TRP59 (3.86)
		Alkyl	LEU162 (5.44), LEU165 (4.42), GLU233 (5.80)
		van der Waals	TRP58, TYR62, GLN63, TYR151, THR163, ARG195, ASP197, ALA198, HIS305, GLY306
AG	**–33.1**	Conventional H-Bond	GLN63 (2.82), ASP197 (4.43)
		Pi-Pi Stacked	TYR62 (5.08), TRP59 (4.03, 4.66)
		van der Waals	TRP58, GLN63, HIS101, LEU162, LEU165, ALA198, GLU233, ILE235, GLY306
		Pi-Alkyl	ALA307 (5.05)
		Carbon Hydrogen bond	TRP59 (3.24)
		Pi-Pi Stacked	TRP59 (5.16)
		Pi-sigma	ILE235 (3.93)
		Pi-Donor Hydrogen bond	HIS299 (3.28)
		van der Waals	TRP58, TYR62, GLN63, TYR151, LEU162, ARG195, ASP197, ALA198, LYS200, HIS305, GLY306, GLY308
#Myricetin	**–33.1**	Conventional H-bond	GLN63 (2.13), ARG195 (2.33), GLU233 (2.31)
		Pi-Pi staked	TRP59 (4.36, 5.39), TYR62 (4.83)
		Van der Waals	HIS101, LEU162, LEU162, ASP197, ALA198, HIS299, ASP300
***Acarbose Yi *et al.* (2022)**	**–32.2**	Conventional H-bond	GLN63 (2.33, 2.36, 2.62), ARG195 (3.04), GLU233 (1.98, 2.33), ASP300 (2.35)
		Pi-donor hydrogen bond	TRP59 (3.78, 3.88)
		van der Waals	VAL49, ILE51, TRP58, TYR62, LEU162, THR163, ASP197, ALA198, HIS299, PHE256, GLY306, GLY306, ARG303, HIS305, TRP357

Note: #Native ligand; *Antidiabetic reference drug. **ASB** (Arborside-B); **ASC** (Arborside-C); **ASD** (Arborside-D); **ATSA** (Arbortristoside-A); **ATSC** (Arbortristoside-C); **Bold residues** (catalytic triad residues in the orthosteric side of Human pancreatic α-amylase).
Source: Elaborated by the authors.

The observations indicated that the conventional hydrogen bond between electronegative acceptor and hydrogen, Pi-Pi stacked link between two aromatic rings, Pi-alkyl interaction between the alkyl group and aromatic ring or unsaturation, van der Waals' interaction, and other noncovalent interactions were found to be present between ligand and protein complexes. Ligand **ASC** formed H-bonds with active site residues GLU233 (H-acceptor), ASP197 (H-acceptor), and ASP300 (H-acceptor) by accepting the hydrogen from the H-donor (–OH) site of the ligand, and residues HIS201 (H-donor) and HIS305 (H-donor) donated the hydrogen to the acceptor oxygen site of the ligand. LEU162, LEU165, TYR59, and HIS305 interacted with ASC to bind by hydrophobic interactions containing Pi-Pi stacking, Pi-alkyl, and alkyl-alkyl interactions. Similarly, in ligand ASD, residues TRP59 and THR163 acted as hydrogen acceptors. HIS201 and TYR153 played a role as hydrogen donors in forming hydrogen bonds, and residues HIS299 and TYR153 showed hydrophobic interaction with the ligand. Docking scores and interactions of the ligands displayed the potential binding capability of the ligands towards HPAE, which could eventually be the subsequent inhibitory action of compounds in physiological reactions. The comparison (docking score and interactions) of selected compounds with native ligand (myricetin) and the drug acarbose (–32.2 kJ/mol) further supported the effective interactions and stability of the complexes formed with amylase enzyme. Figurative (3D interaction with the hydrophobicity of protein and solvent accessibility surface (SAS) in 2D) illustrations of molecular docking calculations of major active compounds are presented in **Fig. 2**. Other calculated data and figures are included in the supplementary information (**Tables 1S**, **2S**, and **Fig. 8S**).

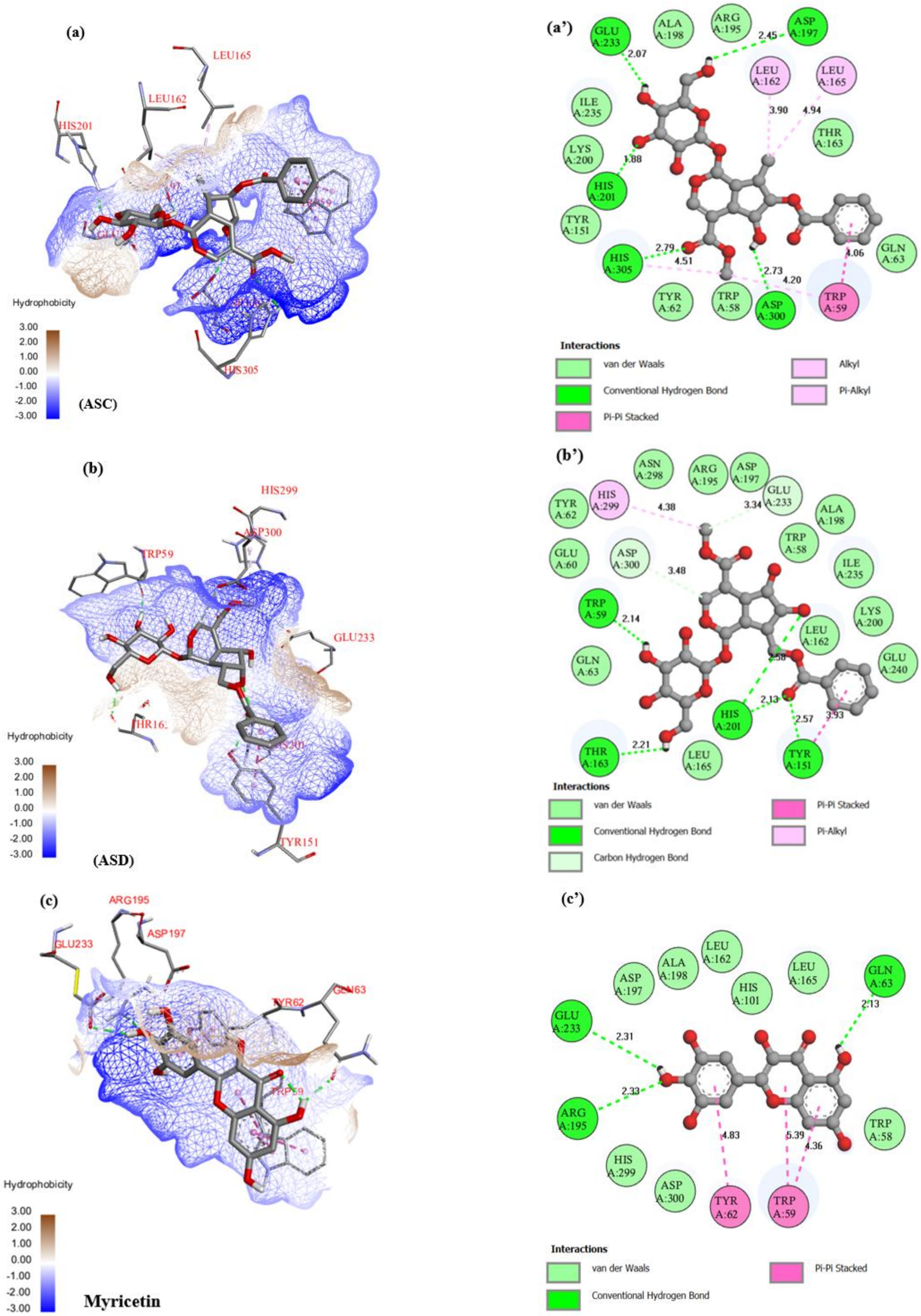

Figure 2. Interactive presentations of the protein-ligand complex of {(ASC **(a, a')**, ASD **(b, b')** and Myricetin **(c, c')**} 3D with the hydrophobicity of protein and 2D with solvent accessibility surface from molecular docking including bond length **(Å)**, the color of atoms in the ligand 2D structure, red, black, and grey are for **O**, **H**, and **C** atoms, respectively.
Source: Elaborated by the authors.

3.6.2. Molecular dynamics simulations (MDS)

3.6.2.1. Root mean square deviation (RMSD)

MDS trajectory was used to extract the RMSD of ligand and protein for a duration of 100 ns, and it was considered to compute the dynamics and stability of the simulated complex (Adcock and McCammon, 2006). The stability of the simulated complex is assessed by the ligand and protein backbone RMSD and the smoothness of curves (Aier *et al.*, 2016). Among the selected compounds, **ASC** and **ASD** displayed better simulation results as they showed a stable trajectory (**Fig. 3a**) with a value below 6 Å. The protein backbone curves were lower than those of the ligand, indicating that the receptor's geometry remained stable throughout the MDS. The ligand binding to the receptor did not change the 3D structure of the adduct throughout 100 ns of the simulation time. The compound **ASC** depicted a smooth trajectory with an RMSD lower than 6 Å, but the RMSD for **ASD** was stable below 4 Å. The stability of the trajectory of the protein backbone in complexes (RMSD 1.8 nm) and apo structure (RMSD 1.5 nm) was found to support posture conservation, and the stability of the ligands during simulation at the binding site of the protein suggests their good inhibition potential towards the HPAE. A comparative and figurative presentation of the RMSD of ligand and protein backbone relative to the protein backbone in the protein-ligand complexes is given in **Fig. 3**.

3.6.2.2. Root mean square fluctuation (RMSF)

RMSF measures the fluctuation of alpha carbon atoms and conformational changes of the protein backbone during the MDS (Martínez, 2015). **Fig. 3c** presents the RMSF of alpha carbon atoms of the protein backbone in the **ASC** and **ASD** complexes. A larger RMSF may account for the RMS deviation (Martínez, 2015). Small RMSF peaks and fluctuations (helix and sheet structure of protein) on most of the catalytic sites and binding catalytic triad (ASP197, GLU233, and ASP300) signified the binding of the ligands **ASC** and **ASD** to the protein, which indicated the effective interaction of ligands at the active site and provided rigidity to the fluctuation of the protein backbone. The unusual rise of the curve at residue numbers up to 0.6 nm around 56, 310, 350, and 460 might be caused due to the presence of fluctuating loops at a larger distance.

3.6.2.3. Radius of gyration (Rg)

The radius of gyration (R_g) focuses on the conformational change in the simulated protein-ligand complex from MD simulation, as shown in **Fig. 3d**. R_g provides the average separation between all dispersed elements from the molecule's central axis (Liu *et al.*, 2017b). The correlation between RMSD, RMSF, and R_g provides insight into the relationship between complex compressibility, delocalization of the ligand, and residual fluctuations. Low RMSD, the rigidity of protein residues at binding sites, and the unchanging R_g support the stability of the complex formed. The R_g was nearly constant at about 2.34 nm for both complexes and the apo structure (**Fig. 9S**), roughly equal to the Rg before complexes formed (2.35 nm). The minimal variation in R_g suggested no appreciable deformation of the receptor's geometry and compactness upon the binding of ligands (Ahmed *et al.*, 2022). Therefore, the adducts of **ASC** and **ASD** with HPAE remained stable in terms of compactness during the MDS.

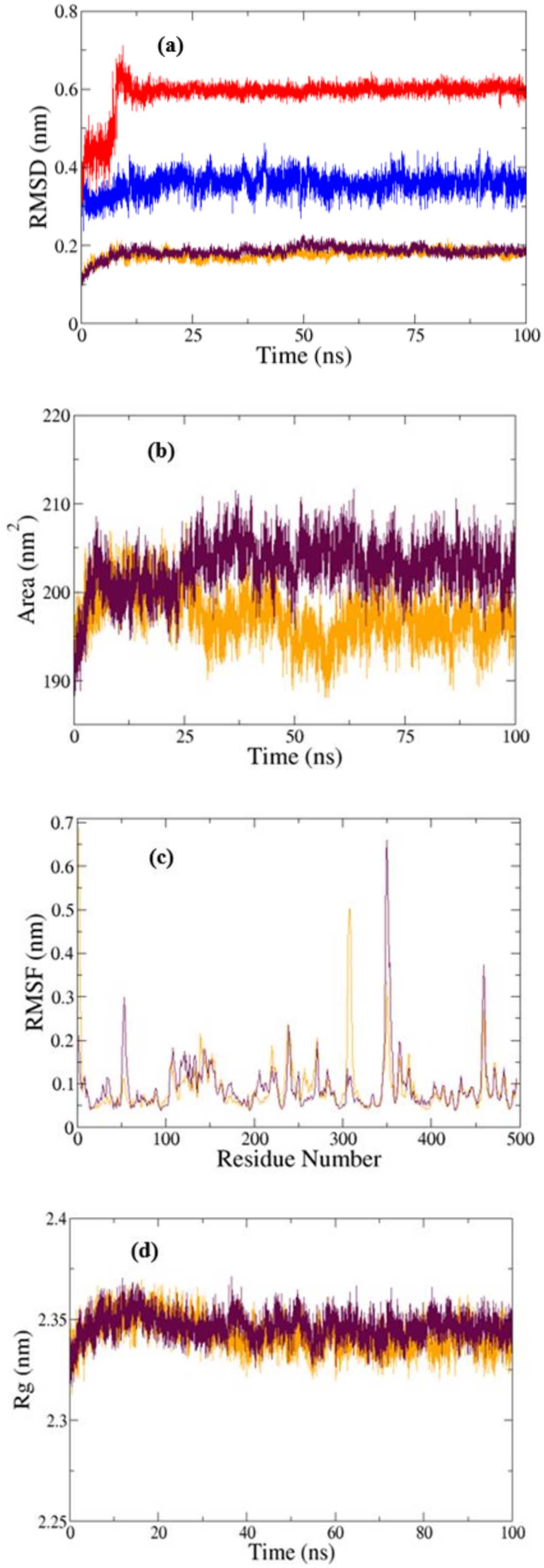

Figure 3. (a) Comparative MDS trajectory with RMSD of **ASC** (red) with protein backbone (maroon) and for **ASD** (blue) with protein backbone (orange), **(b)** SASA of protein in complex with **ASC** (maroon) and **ASD** (orange), **(c)** RMSF plot of protein backbones (maroon-colored curve for **ASC** and orange-colored curve for **ASD** complex) and **(d)** Radius of gyration of protein complexes with ligand **ASC** (maroon) and for **ASD** (orange).
Source: Elaborated by the authors.

3.6.2.4. Solvent accessible surface area (SASA)

The SASA measures the accessible surface of the protein to the solvents quantitatively and the change in compactness of the protein surface on adduct formation with a ligand. The trajectory observed in the SASA plot for each protein complex was regular up to 100 ns with minor fluctuations, as shown in **Fig. 3b**. The value of SASA, the steady areas about 197 and 204 nm^2, was observed for protein-ligand complexes with compounds **ASD** and **ASC**, respectively, suggesting some exposure in protein morphology after adduct formation. The comparison of the SASA of the apo structure (about 200 nm^2) with the complexes of compounds, **ASD** and **ASD**, signified no appreciable change in the SASA, and the value was found even lower for **ASD** complex after adduct formation which was strongly supporting result to interpret the negligible change in the surface area of the enzyme on complex formation and geometrical change in enzyme. The result implied that the change in accessibility of the solvent to the hydrophobic surface of the receptor after binding with the ligand was minimal (Zhang and Lazim, 2017). The apo protein structure MD simulation results are included in the supplementary information (**Fig. 9S**).

3.6.2.5. Hydrogen bond count

The hydrogen bond is a non-covalent interaction that has a significant role in providing stability to the protein-ligand complex in various biological processes (Chikalov *et al.*, 2011). A higher hydrogen bond count would enhance the stability of the complex. **Figure 4** illustrates the variation in hydrogen bond count on the course of MDS between the ligand and protein for 100 ns. Both the ligands (**ASC** and **ASD**) interacted with a high number of hydrogen bonds; **ASC** and **ASD** possess up to 4 or 5 hydrogen bonds most of the time and reach up to 8 hydrogen bonds in some transitions (**Fig. 4**). The result might suggest slightly more stability of the **ASD** complex due to the interaction with protein than the **ASC complex** in different time frames based on hydrogen bond counts.

3.6.2.6. Thermodynamic stability calculations

The binding free energy change (**ΔG_{BFE}**) from the MMPBSA calculation (**Eqs. 4** and **5**) showed the spontaneity of the complex formation reaction. The total change in binding free energy (**ΔG_{BFE}**) of the protein-ligand complexes was –138 ± 15 kJ/mol and –91 ± 15 kJ/mol for **ASC** and **ASD** complexes, respectively, as shown in **Table 6**. The equilibrated part of the trajectory of the last 10 ns was taken to calculate the **ΔG_{BFE}** in complexes.

The van der Waals interaction, polar solvation energy, electrostatic energy, and energy contributions of phases were calculated as energy components using the MMPBSA module. **ΔG_{BFE}**<0 suggested the spontaneous nature of the complex formation reaction, which could eventually support the active amylase inhibition potential of the ligands (Olsson *et al.*, 2008). The **ΔG_{BFE}** for the **ASC complex** was found to be quite lower than the ΔGBFE of the ASD complex, which signified the more stable complex formation of the former than the latter. So, the binding affinities, relatively lower RMSD, minimal RMSF, relatively higher hydrogen bond count, and negative **ΔG_{BFE}** (< –91 kJ/mol) of the protein-ligand complexes could strongly support the HPAE inhibition capability of ligands **ASC** and **ASD**. A figurative presentation of the moving average value of the MMPBSA calculation of free energy change is included in supplementary information (**Fig. 10S**).

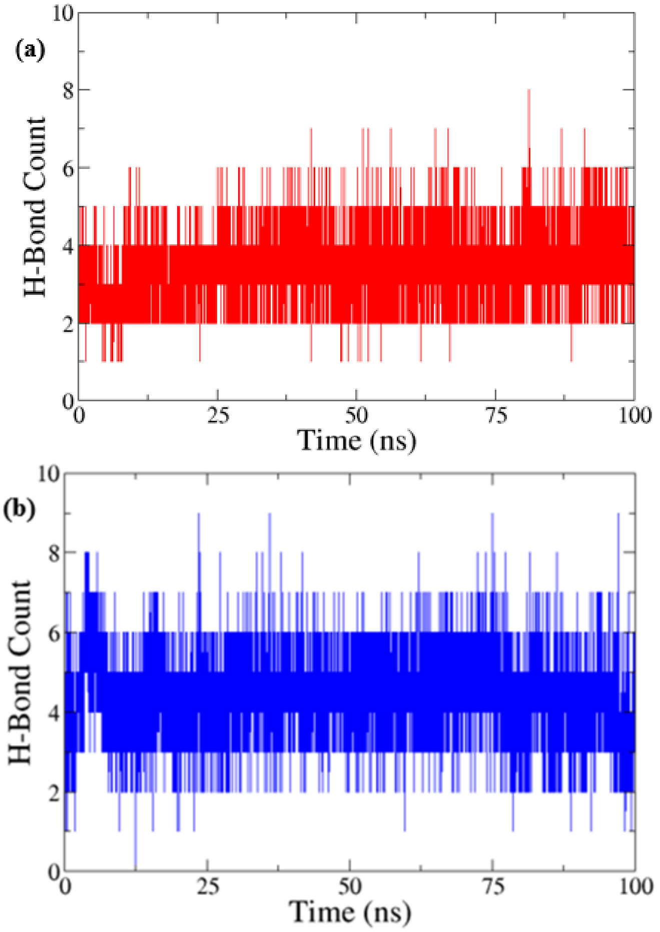

Figure 4. H-bond counts in **(a)** red for the **ASC**-protein complex and **(b)** blue for the **ASD**-protein complex.
Source: Elaborated by the authors.

Table 6. Thermodynamic parameters and their contributions to the total free energy change of complexes.

Binding free energy components	Energy change (kJ/mol) of the receptor with ASC	ASD
ΔV_{DWAALS}	−172 ± 14	−161 ± 14
ΔE_{PB}	252 ± 20	258 ± 23
ΔE_{EL}	−199 ± 22	−170 ± 19
ΔEM_{POLAR}	−18.7 ± 0.6	−18.5 ± 0.6
ΔG_{GAS}	−371 ± 25	−331 ± 15
ΔG_{SOLV}	234 ± 19	241 ± 11
ΔG_{BFE}	−137 ± 15	−91 ± 15

Note: **ASC** (arborside C); **ASD** (arborside D); value = Energy change ± SD; **ΔG_{BFE}** = Binding free energy; **ΔE_{GAS}** = Energy change in gas phase molecular mechanics; **ΔE_{ELE}** = Electrostatic energy; **ΔV_{DWAALS}** = van der Waals energy; **ΔG_{SOLV}** = Electrostatic solvation energy summation; **ΔG_{PB}** = Polar contributions in solute-solvent system.
Source: Elaborated by the authors.

3.7. Pharmacokinetics and pharmacodynamics of hit candidates

ADMET prediction helps to recognize the significance of ligands towards pharmaceutical efficacy, therapeutic aptitude, pharmacokinetics, and pharmacodynamics (Pires *et al.*, 2015). **Table 7** summarizes the possible results of the ADMET predictions of compounds and drugs. The ligands (**ASC** and **ASD**) were found in toxicity class 4, indicating a slightly toxic nature (Banerjee *et al.*, 2018). The lethal dose of 50% (LD_{50}) for **ASC** and **ASD** was predicted at 2000 mg/kg, which was lower than that of acarbose, which predicted the toxic nature of the ligand compared to the drug acarbose and less harmful than the drug miglitol (1190 mg/kg). Consensus LogP (<0.6) showed the lipophilicity of both compounds as reference drugs and was comparatively smaller than that of the drugs. None of them followed the Ro5 for drug-likeness except miglitol. Ligand **ASC** was found to be immunotoxic like acarbose, which might indicate the cause of interruption to the immune system (Zerdan *et al.*, 2021). All ligands were inactive regarding hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and phosphoprotein p53. The LD_{50} for **ASC** (2.544 mol/kg) and **ASD** (2.819 mol/kg) were found to be comparable to that of acarbose on oral rat acute toxicity. The logBB value of compounds (< –1) signified poor permeability to the blood-brain barrier (BBB), and the LogSP value (< –4) indicated the impermeability of compounds to the central nervous system (Banerjee *et al.*, 2018; Carpenter *et al.*, 2014). Metabolic properties of both ligands were found, as shown by the drugs. The gastrointestinal absorption factor for the compounds was predicted to be as low as that for drugs, which might depend on the molecular structure and solubility of the candidate. The total clearance measures mainly the hepatic, biliary, and renal clearance, which determines the steady-state dose concentration in the body, and was found to be higher for the compounds than for acarbose and miglitol, which signified that the body could easily release the compound residues through excretion than drugs.

Table 7. ADMET profile of best candidate ligands compared with standard drugs.

ADMET Parameters	Compounds (units)			
	ASC	ASD	*Acarbose	*Miglitol
Toxicity class	4	4	6	4
LD_{50} (mg/kg)	2000	2000	24000	1190
Lipinski rule (RO5)	No	No	No	yes
Consensus LogP	−0.31	−1.12	−6.22	−3.26
Immunotoxicity	Active	Inactive	Active	Active
Hepatotoxicity	Inactive	Inactive	Inactive	Active
Carcinogenicity	Inactive	Inactive	Inactive	Inactive
Mutagenicity	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive
Phosphoprotein p53	Inactive	Inactive	Inactive	Inactive
CYP2D6 substrate	No	No	No	No
CYP3A4 substrate	No	No	No	No
CYP2C9 inhibitor	No	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
Oral rat acute toxicity (mol/kg)	2.544	2.819	2.447	2.257
BBB permeability (logBB)	−1.456	−1.496	−1.717	−1.501
CNS permeability (logPS)	−4.27	−4.517	−6.438	−4.842
GI absorption	Low	Low	Low	Low
Total clearance log (mL/min/kg)	1.009	1.031	0.428	0.815

Note: **ASC** (Arborside C), **ASD** (Arborside D), and *Reference drugs.
Source: Elaborated by the authors.

The comparison of top candidates with the standard drugs acarbose and miglitol (Basnet *et al.*, 2023) implied the positive therapeutic behavior of compounds. Hence, the ADMET analysis of the compounds **ASC** and **ASD** revealed comparative therapeutic significance to the standard drugs.

Plant *N. arbor-tristis* has been reported on various biological activities like antioxidant, antidiabetic, and cytotoxicity in different methodologies to expose its medicinal significance. However, six different solvent extracts of leaves of *N. arbor-tristis* through sequential extraction in ascending polarity and their ethnomedicinal studies through *in vitro* and computational antidiabetic analysis using HPAE in detail have not been carried out yet.

This study showed the presence of different phytochemicals containing alkaloids, flavonoids, phenolic compounds, glycosides, and reducing sugars in the leaves of the selected plant. Quantitative phytochemical analysis revealed the presence of high phenolic contents in the acetone extract (**AE**) and methanolic extract (**ME**) of 137 ± 4 mg GAE/g and 139 ± 4 mg GAE/g, respectively (**Table 3**). Chloroform extract (**CE**) and hexane extract (**HE**) were found to have high TFC compared to other extract fractions.

The result portrayed the significant antioxidant nature of the leaf extract of *N. arbor-tristis,* as extract **ME** showed the best antioxidant activity with an IC_{50} of 56 ± 3 µg/mL among all leaf extracts, which was comparable to the antioxidant activity of the control ascorbic acid. Other extracts showed moderate antioxidizing potential toward DPPH free radicals. Akki *et al.* (2009) studied the DPPH scavenging assay of plant leaves in different solvents (pet ether, butanol, ethyl acetate, and butylated hydroxytoluene), and butanol extract showed the best scavenging against DPPH radical. Formerly, the medicinal properties of this plant have been explored in the flowers and seeds of the plant and found to have significant antioxidant properties of different extracts (Mishra *et al.*, 2016; Mishra *et al.*, 2022). The therapeutic potential of natural products is due to the presence of phytoconstituents containing iridoids, flavonoids, alkaloids, and others (Chauhan and Banerjee, 2024; Naseem *et al.*, 2024). The quantitative phytochemical assessment showed the presence of high phenolic content in extracts **AE** and **ME**, and similarly high flavonoid content in all extracts except **DWE**. A better antioxidant nature of **ME** might be due to the presence of functional components than in other extracts. These could act upon harmful reactive oxygen, reactive nitrogen, and free radical species in the human body to minimize alternation in cellular functioning, metabolism, and could prevent the formation of diseases (Yang *et al.*, 2016). A high content of metabolites containing flavonoids, phenolics, and iridoids in extracts is the backbone of their pharmaceutical significance, which has supported the observations of this study (Phuyal *et al.*, 2020).

In this study, extract **HE** was found to be most lethal to *Artemia salina* nauplii, and the lethal concentration of 97 ± 2 µg/mL in BSLA. The toxicity of the extracts, **ME** and **CE**, was found to be comparable to that of **HE**, and other extracts were found to be less toxic than the control potassium dichromate (**Table 4**). The results showed that **HE**, **ME**, and **EAE** were found (LC_{50} < 200 µg/mL) moderately toxic, other extracts (LC_{50} < 500 µg/mL) were found weakly toxic, and **DWE** (LC_{50} > 500 µg/mL) was found nontoxic (Niksic *et al.*, 2021). The BSLA assay helps to evaluate the potential of herbal plants towards anticancer activity through cytotoxicity screening (Meyer *et al.*, 1982).

The α-amylase inhibition assay helped estimate the potential antidiabetic activity of the different leaf extract fractions. A computational approach using human pancreatic amylase enzyme was carried out to analyze the possible effective interactions, the molecular mechanism of complex formation, and to support the *in vitro* antidiabetic activity. Methanolic extract fraction (**ME**) was the most potent amylase inhibitor with IC_{50}, 157 ± 3 µg/mL compared to other fractions and standard acarbose (**Table 4**). Extract **HE** and **CE** showed mild inhibition activity, and the rest of the fractions showed weak inhibition activity against *α*-amylase. A computational examination of compounds found in leaves of *N. arbor-tristis* (**Table 1**) was carried out. The *in vitro* experimental outcome and computational results helped to better understand the molecular mechanism and possible effective interaction of the ligand with the target protein. To predict the possible compound responsible for amylase inhibition, previously isolated and reported compounds of *N. arbor-tristis* characterized by GC-MS, LC-MS, ^{1}H NMR, and ^{13}C NMR were selected. Such structures were optimized, energy minimized, and screened through molecular docking and MDS. Among all the selected compounds, **ASC** and **ASD** were found to be bound most effectively to the amylase enzyme, which could be proposed as potential HPAE inhibitors, as interpreted by molecular docking calculation with a high binding affinity (–33.5 kJ/mol for both), reasonable geometric configuration, and thermodynamic parameters. Negative binding free energy (ΔG_{BFE} < 0), comparative pharmacokinetic and pharmacodynamic properties of the compounds compared with the drugs Acarbose and Miglitol (**Table 7**), shown by ADMET profiling, provided strong evidence of spontaneity of the complexes along with binding efficacy to HPAE and drug likeness through computer-based assessments. The amylase inhibition potential of the extracts and stability of the enzyme-ligand complexes on computational screening portrayed the extracts' HPAE inhibition capability and the plant's antidiabetic potential.

Overall, the results of this study illustrated and supported the ethnomedicinal importance of the leaf extract of *N. arbor-tristis*. The high content of potential bioactive metabolites, *in vitro* experiments, and computational virtual screening outputs supported the good antioxidant and HPAE inhibition potential of the plant.

4. Conclusions

The study showed that the methanolic extract (**ME**) was found to be the most significant extract in all *in vitro* antioxidants, *α*-amylase inhibition, and brine shrimp lethality assays; however, other fractions showed moderate responses to the bioactivity evaluations. Among the selected ligands, arborside-C (**ASC**) and arborside-D (**ASD**) showed significant affinity to the human pancreatic α-amylase enzyme, as determined through molecular docking, molecular dynamics simulation, and ADMET profiling. Polar regions of the ligands (mainly the hydroxyl group) were found to be effective binding sites to the target protein amylase in molecular docking analysis. The integration of *in vitro* and *in silico* analysis in this study demonstrated the potential amylase inhibitory capability of plant phytoconstituents. As *in silico* analysis gives the possible therapeutic estimation through virtual screening at a molecular level, the phytochemicals isolated from this plant (hit candidates arborside-C, and arborside-D) were proposed to be used for *in vivo* bio-characterization, validation, and optimization to estimate their medicinal significance to treat hyperglycemia through the amylase enzyme inhibition mechanism.

Authors' contribution

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Data availability statement

The data will be available upon request from the main and corresponding authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Supplementary Information

FT-IR Analysis of the extract

FTIR spectroscopic analysis provides significant insights into the chemical composition and structural characteristics of the analyzed compounds. FT-IR (PerkinElmer Spectrum IR; Version 10.6.2) analysis of all the extracts was conducted at the Amrit Campus in Kathmandu. The spectroscopic analysis of FTIR enables the identification of functional groups found in the extracts (Grasel *et al.*, 2016). The comparative spectral peaks of extracts are presented (**Fig. 3S**). Alcohols, alkyl groups, and carbonyl groups were found most abundant in the extract fractions.

Ligand structures

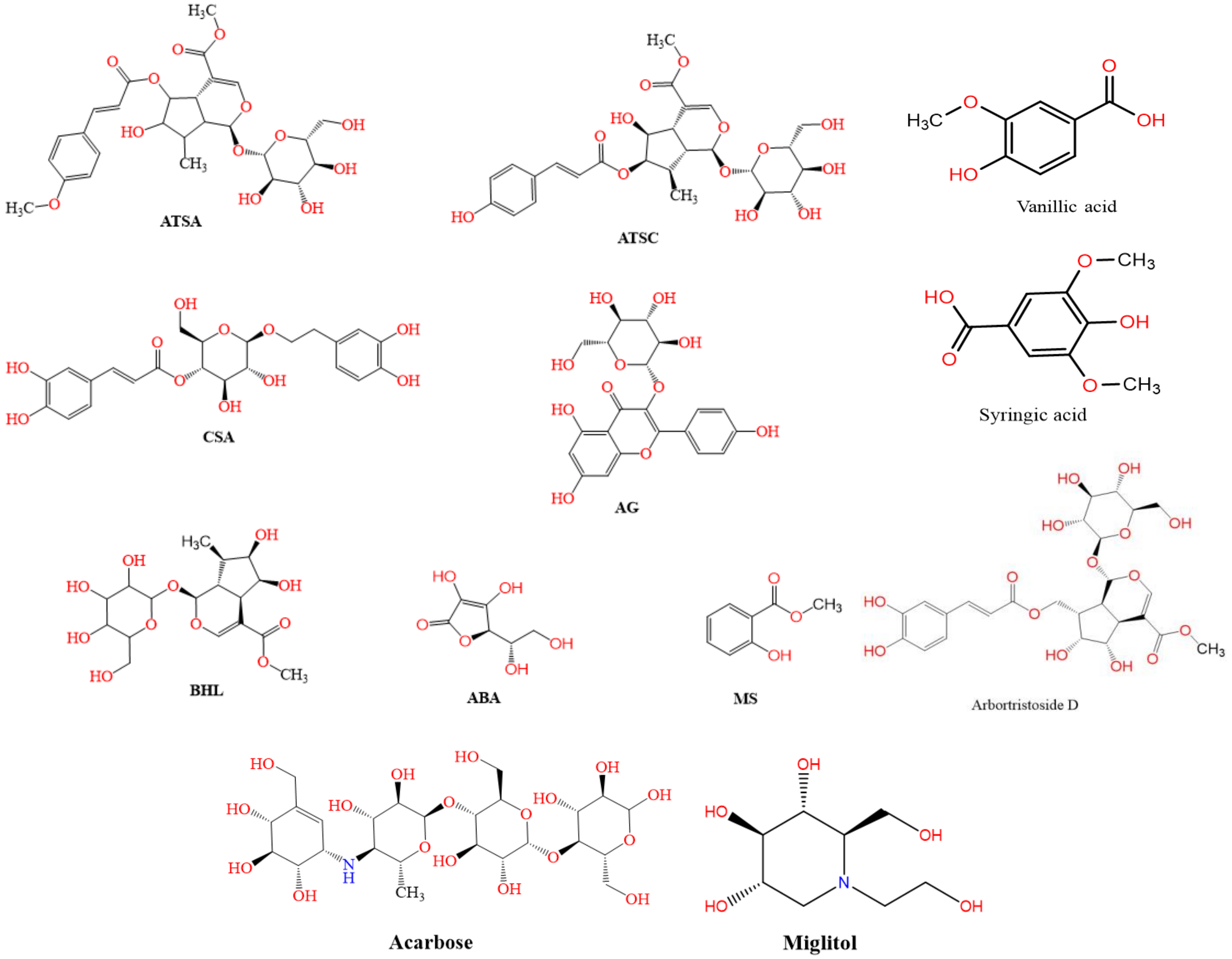

Figure 1S. Molecular structures of selected ligands for molecular docking and standard drugs (acarbose and miglitol) drawn in ChemDraw 16.
Source: Elaborated by the authors.

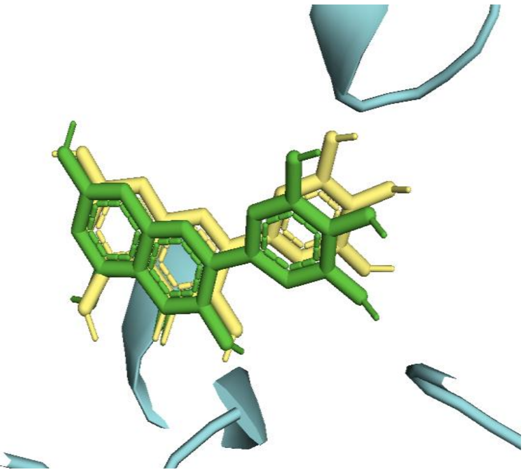

Figure 2S. (RMSD <2 Å), Superimposition of the native ligand myricetin (green) with docked ligand (yellow) myricetin, visualized in PyMOL software.
Source: Elaborated by the authors.

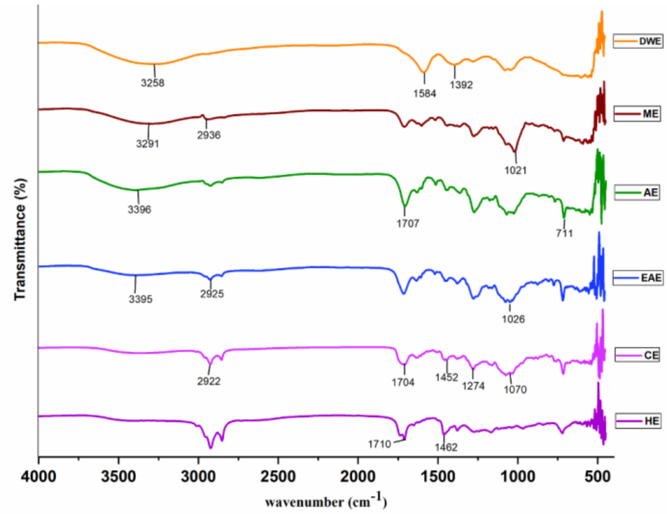

Figure 3S. FTIR analysis of extract fractions.
Source: Elaborated by the authors.

Standard calibration curves for TPC and TFC

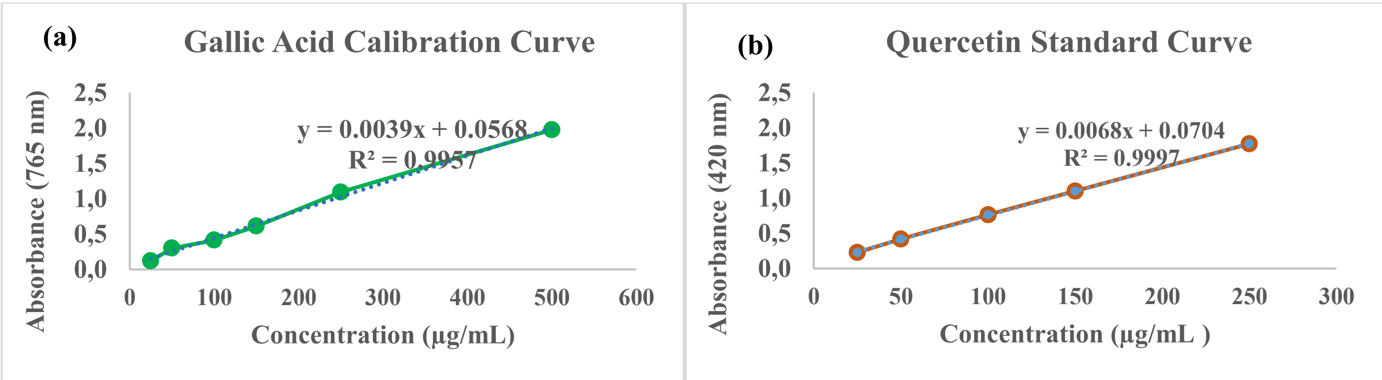

Figure 4S. Standard calibration curve of **(a)** Gallic acid and **(b)** Quercetin solution.
Source: Elaborated by the authors.

Comparative and figurative presentation of antioxidant potential of extract fractions

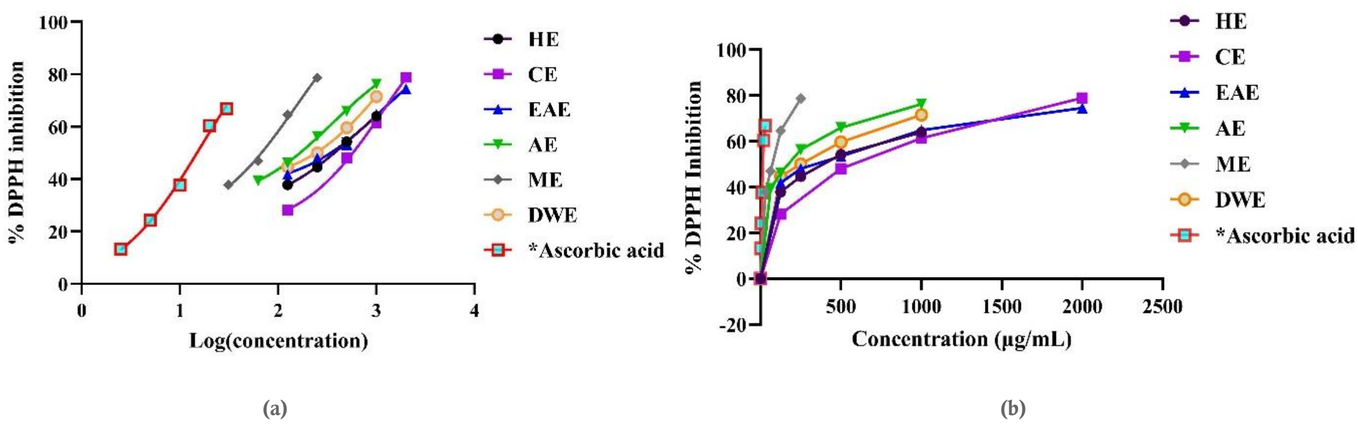

Figure 5S. The comparative trending curve of DPPH assay of extracts in dose-inhibition response with **(a)** Log(concentration) vs % scavenging of DPPH and **(b)** concentration vs % scavenging with positive control ascorbic acid.
Source: Elaborated by the authors.

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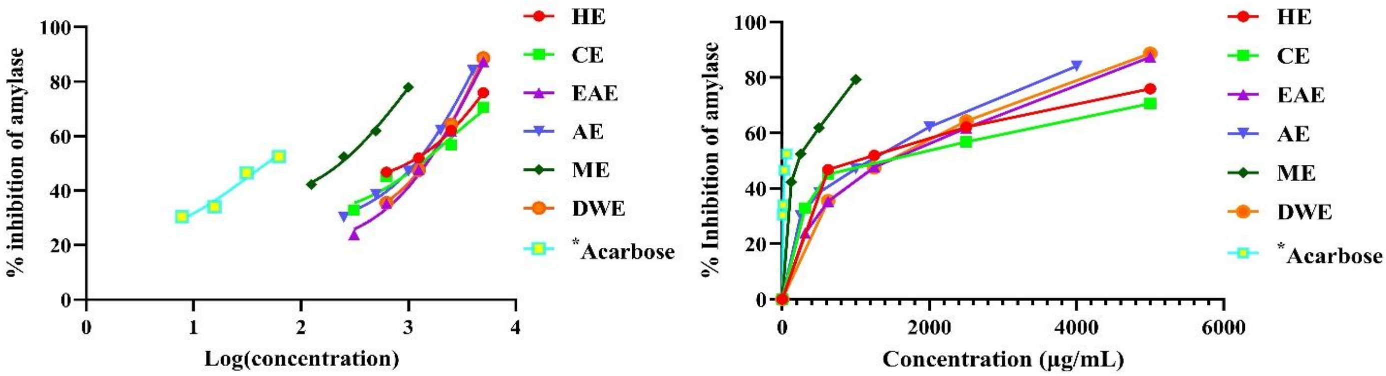
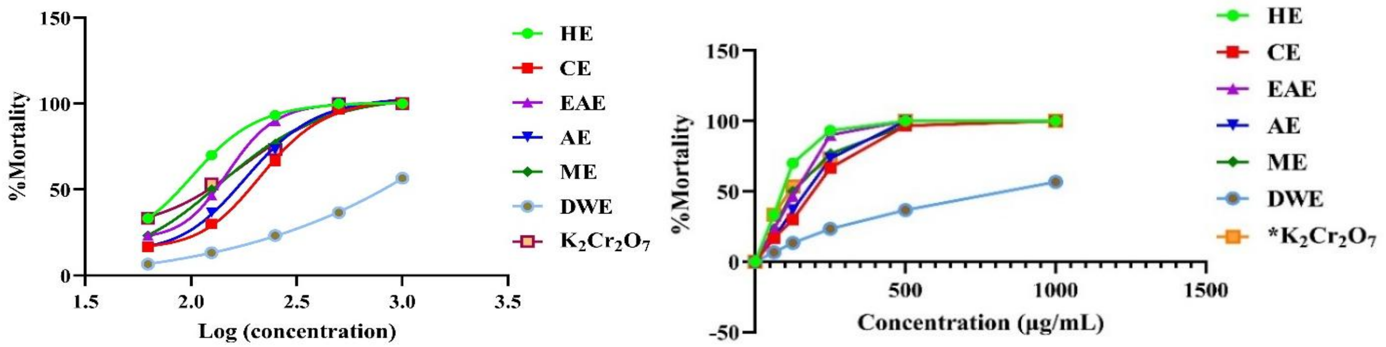
Alpha amylase inhibition assay



(a) (b)

Figure 6S. Nonlinear regression graphic presentation of **(a)** log(concentration) vs percentage inhibition of enzyme **(b)** Concentration vs percentage inhibition of enzyme with positive control acarbose.
Source: Elaborated by the authors.

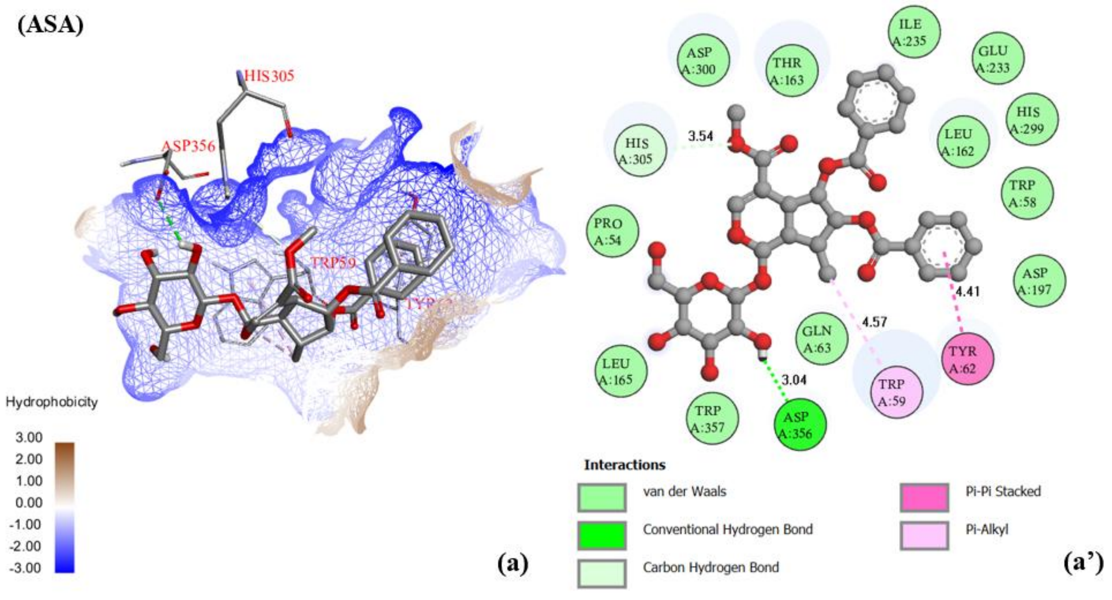
Brine shrimp lethality assay



(a) (b)

Figure 7S. Nonlinear regression comparative curves of **(a)** log (concentration) vs % mortality and **(b)** concentration vs % mortality of triplicate with control, the plot is drawn from GraphPad Prism in dose-inhibition response plot.
Note: **HE** (Hexane extract); **CE** (Chloroform extract); **EAE** (Ethyl acetate extract); **AE** (Acetone extract); **ME** (Methanol extract); **DWE** (Distilled water extract).
Source: Elaborated by the authors.

Protein-ligand Interactions



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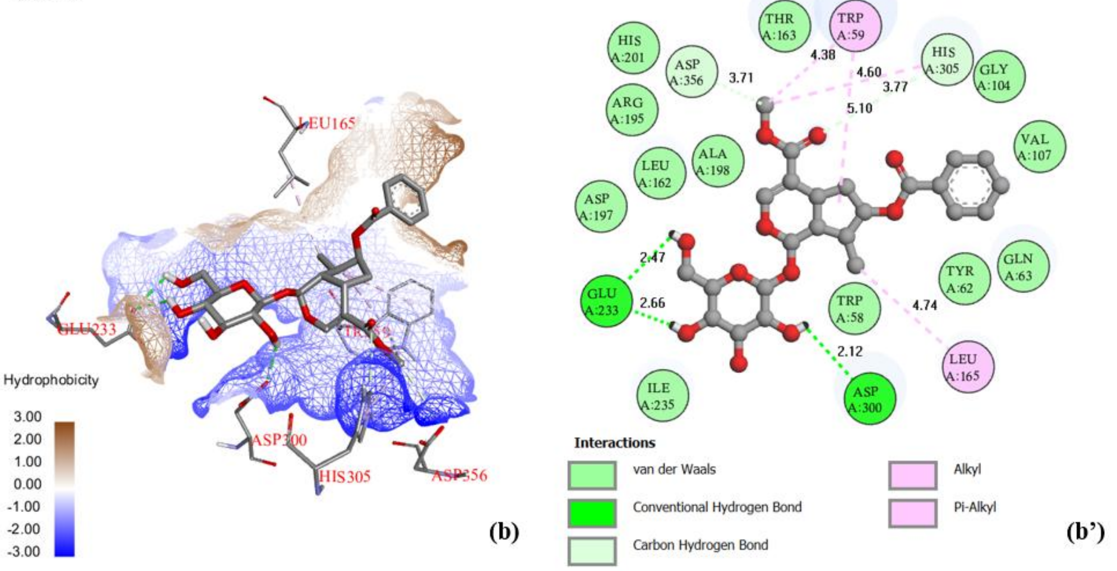
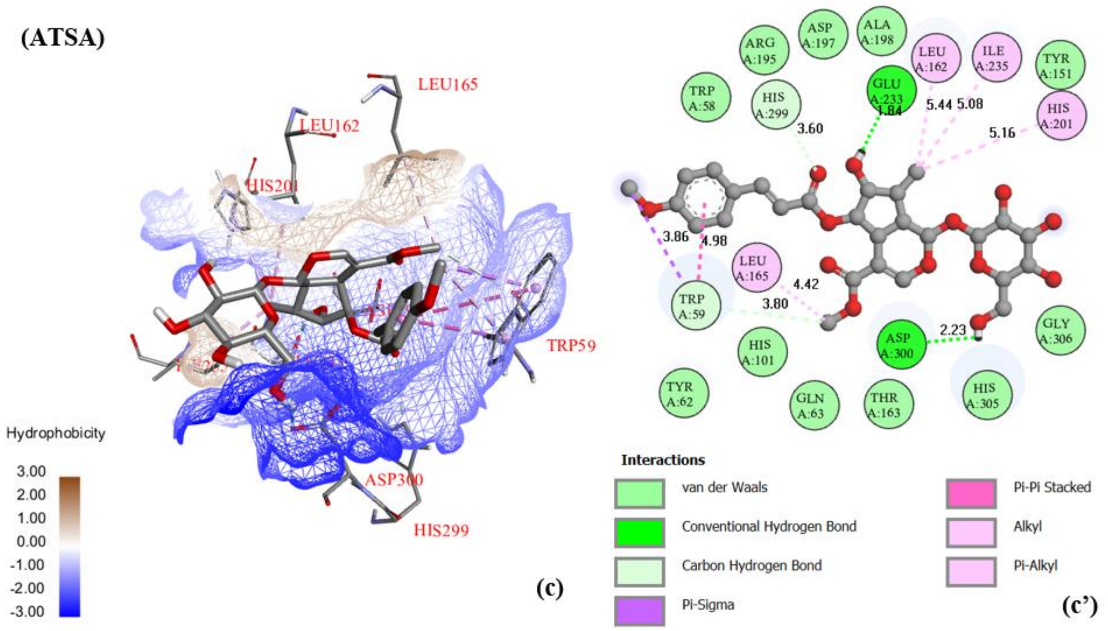
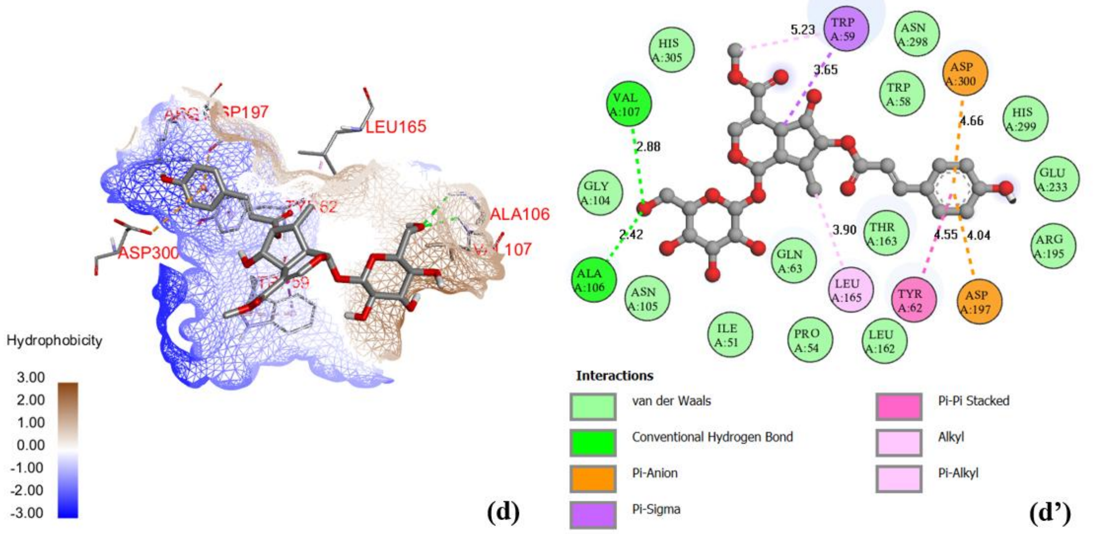
(ASB)



(ATSA)



(ATSC)



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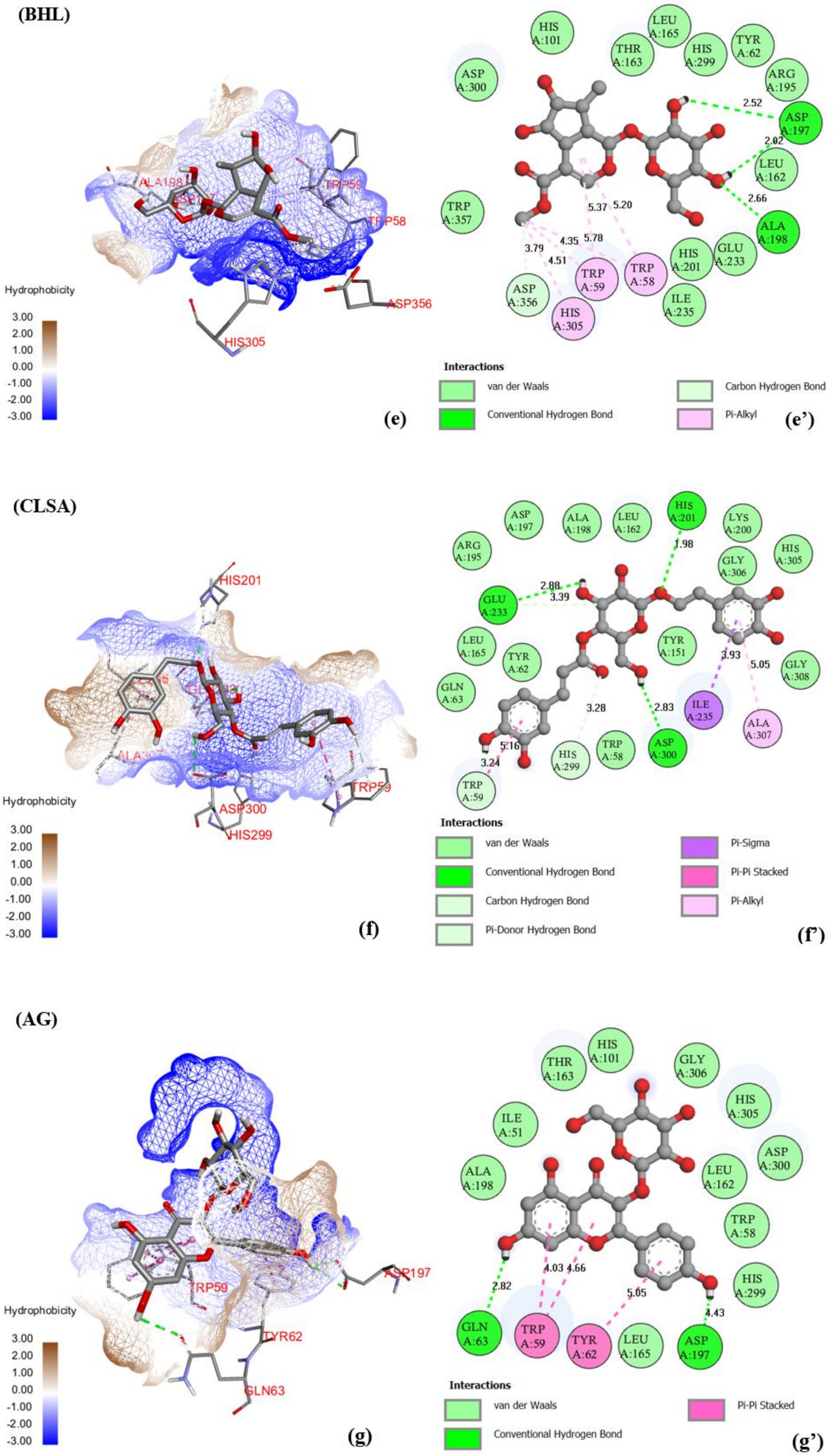

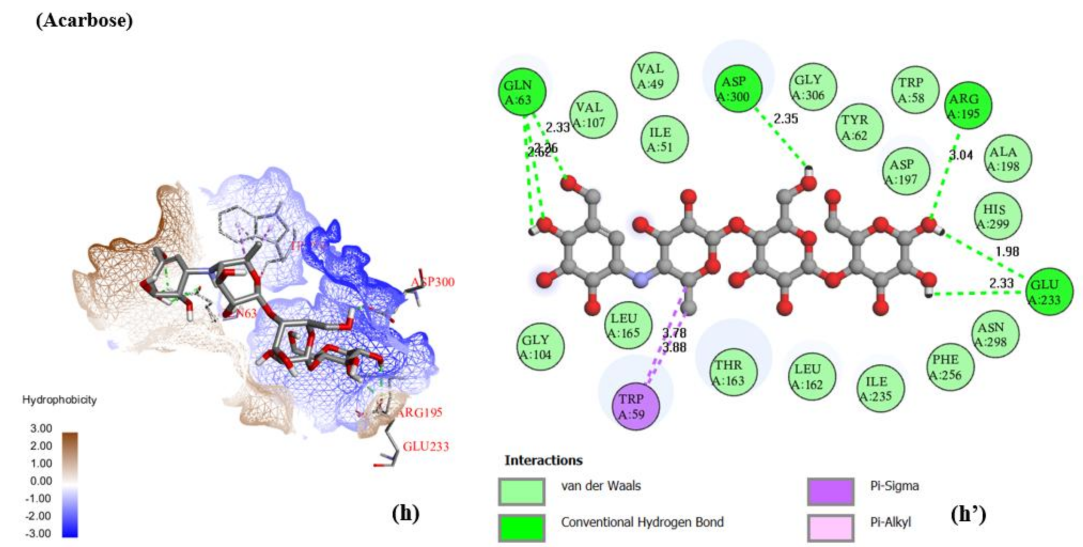

Figure 8S. 3D and 2D interactive presentations of {ASA **(a, a')**, ASB **(b, b')**, ASTA **(c, c')**, ASTC **(d, d')**, BHL **(e, e')**, CLSA **(f, f')**, AG **(g, g')**, and Acarbose **(h, h')**} with hydrophobicity in receptor protein in molecular docking.
Source: Elaborated by the authors.

Apo structure Dynamics and characterization

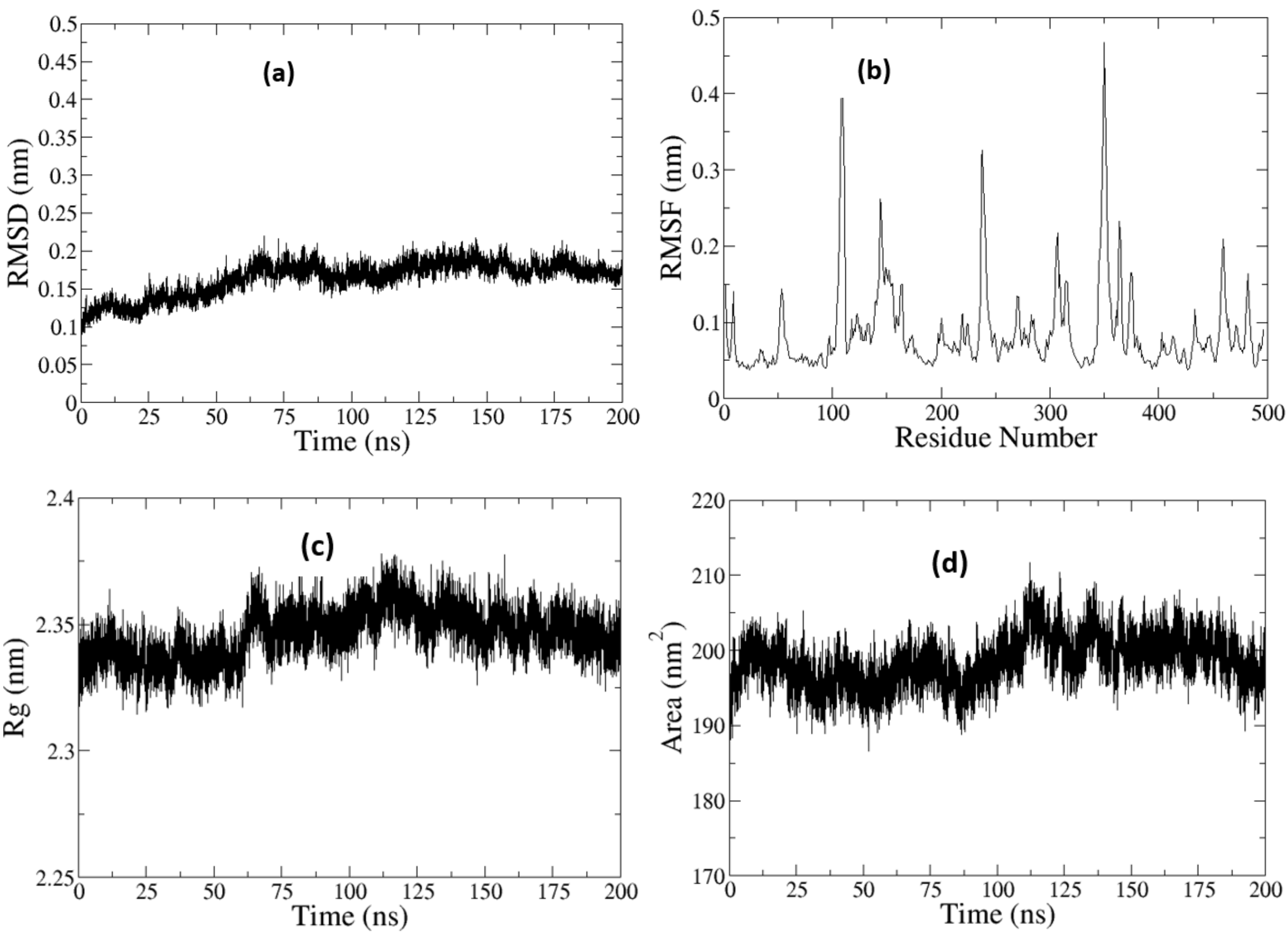

Figure 9S. Presentation of MDS of the apo structure of amylase protein (4GQR) of **(a)** RMSD **(b)** RMSF **(c)** R_g **(d)** SASA.
Source: Elaborated by the authors.

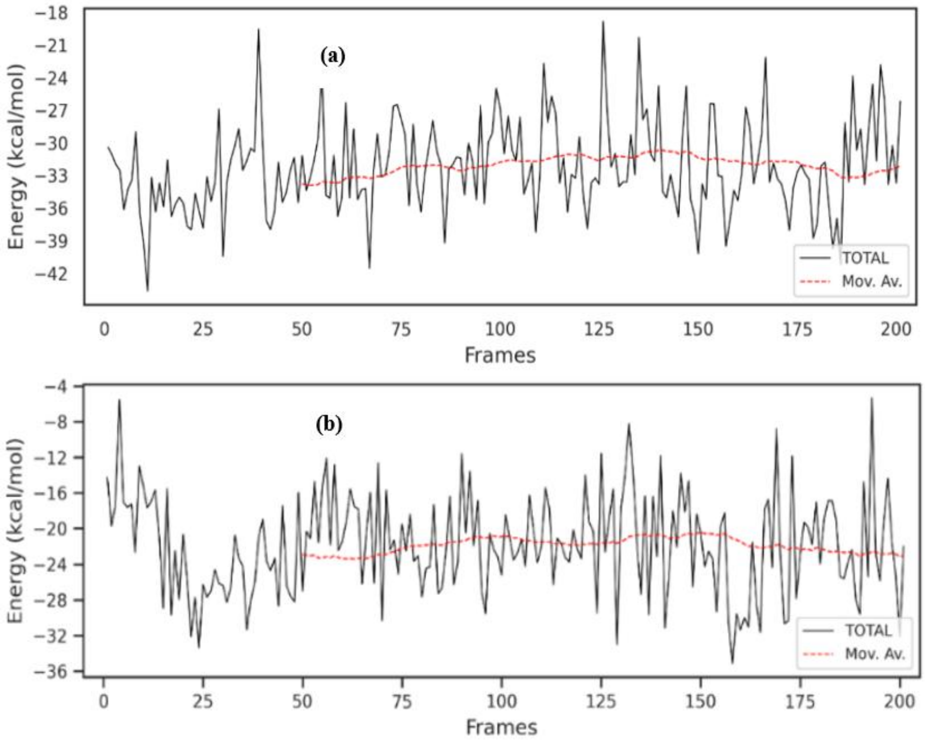

Figure 10S. Binding free energy change with respect to the time frame of the complex with ligand **(a)** ASC **(b)** ASD.
Source: Elaborated by the authors.

Table 1S. Ligands with their docking scores.

Ligands	Ligand ID	Molecular weight (g/mol)	PubChem ID	Docking score (kJ/mol)	References
6-β-Hydroxyloganin	BHL	406.4	341846	−29.7	Rathore *et al.*, (1989)
Calceolarioside-A	CLSA	478.4	5273566	−32.6	Agrawal and Pal (2013); Dewi *et al.* (2022)
Ascorbic acid	ABA	176.12	54670067	−22.6	Agrawal and Pal (2013); Sah and Verma (2012)
Methyl salicylate	MSC	152.15	4133	−23.0	
Arbortristoside D	ATSD	584.5	14632886	−32.6	Agrawal and Pal (2013)
Vanillic acid	VA	168.15	8468	−33.0	Agrawal and Pal (2013); Sah and Verma (2012)
Syringic acid	SA	198.17	10742	−24.3	

Source: Elaborated by the authors.

Table 2S. Detailed information about some ligands containing docking scores, type of interactions and active site residues involved.

Candidates	Docking score (kJ/mol)	Interactions	Active site residues (Distance Å)
ASA	−32.6	Conventional H-bond	ASP356 (3.04)
		Pi-Pi Stacked	TYR62 (4.41)
		Pi-Alkyl	TRP59 (4.57)
		Carbon-Hydrogen Bond	HIS305 (3.54)
		van der Waals	PRO54, TRP58, GLN63, LEU162, THR163, LEU165, ASP197, GLU233, ILE235, HIS299
CLSA	−32.6	Conventional Hydrogen Bond	HIS201 (1.98), GLU233 (2.88), ASP300 (2.83)
		Pi-Alkyl	ALA307 (5.05)
		Carbon Hydrogen bond	TRP59 (3.24)
		Pi-Pi Stacked	TRP59 (5.16)
		Pi-sigma	ILE235 (3.93)
		Pi-Donor Hydrogen bond	HIS299 (3.28)
		van der Waals	TRP58, TYR62, GLN63, TYR151, LEU162, ARG195, ASP197, ALA198, LYS200, HIS305, GLY306, GLY308
ATSC	−31.4	Conventional Hydrogen Bond	ALA106 (2.42), VAL107 (2.88)
		Pi-Alkyl	TRP59 (5.23)
		Pi-Anion	ASP197 (4.04), ASP300 (4.66)
		Pi-Pi Stacked	TYR62 (4.55)
		Pi-sigma	TRP59 (3.65)
		Alkyl	LEU165 (3.90)
		van der Waals	ILE51, PRO54, TRP58, GLN63, LEU162, THR163, LEU165, ARG195, GLU233, HIS299, ASN298, HIS305

Continue...

BHL	−29.7	Conventional H-bond	ASP197 (2.02, 2.52), ALA198 (2.66)
		Pi-Alkyl	TRP58 (5.20, 5.78), TRP59 (4.35, 5.37), HIS305 (4.51)
		Carbon-Hydrogen Bond	ASP300 (3.79)
		van der Waals	TYR62, HIS101, LEU162, THR163, LEU165, ARG195, HIS201, GLU233 ILE235, ASP300, TRP356, TRP357

Note: **ASA** (arborside-A); **CLSA** (Calceolarioside-A); **ATSC** (arbortristoside-C); **BHL** (6-β-Hydroxyloganin).
Source: Elaborated by the authors.

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Comparison of chemistry students in solving algorithmic, conceptual and open-ended problems

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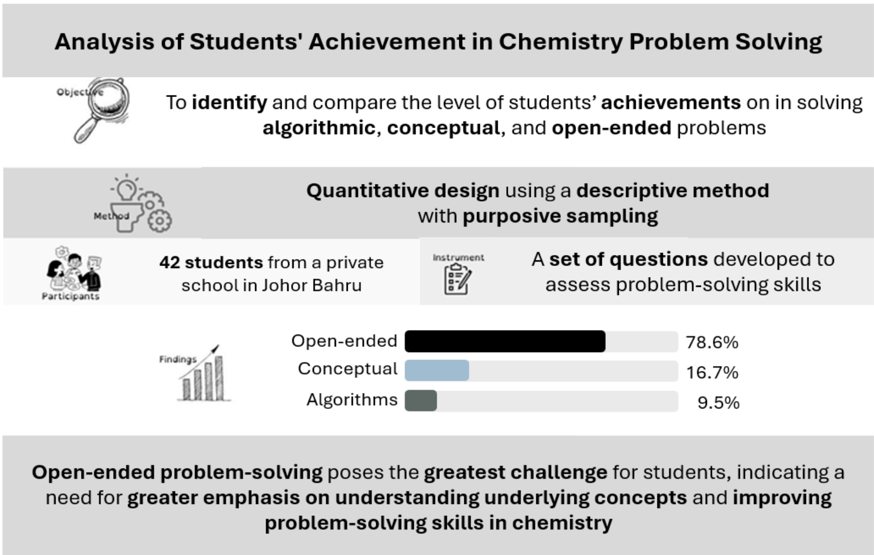
Abstract

This study analyses students' achievements level in solving chemistry problems. Questions were designed in algorithm, conceptual and open-ended formats. The limited research on students' effectiveness in different problem types highlights the need to examine their grasp fundamental chemistry concepts. This research aims to identify and compare achievements levels across the three problems types. A quantitative design using a paper-and-pencil test was employed. The instrument, covering mole concept, acid base, and polymer topics, was administered to 42 high school chemistry students from private school in Johor Bahru through purposive sampling. Descriptive statistics including frequency and percentage, were used to summarize the achievement level. Data were analyzed using SPSS 27.0. Findings shows that open-ended problems recorded the highest low achievement rate (78.6%), compared to algorithmic (9.52%) and conceptual problems (16.7%). These results indicate that open ended problems are the most difficult for students to master, reflecting weaknesses in understanding underlying concepts. Greater emphasis on cultivating problem solving culture in classrooms is recommended.



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Keywords

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2. chemistry;
3. students' achievement.

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Habiddin **Habiddin**

Highlights

- Students showed strong procedural skills in algorithmic problems.
- Performance declined in conceptual problems, reflecting reliance on memorization.
- Open-ended problems pose challenges but limit creativity and application.

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1. Introduction

Chemistry can be defined as the branch of science that relates to the properties of composition, element and compound structure, how they change and the release or absorption of energy when they change. Chemistry is an important subject as it discusses the composition and structure of things surrounding us (Sausan *et al.*, 2018). It also explains the natural phenomena that occur in our daily life, and helps us to solve our problems scientifically. However, it is an unpopular subject among students. This subject requires the students to give an effort in comprehending the concepts. Compared to language and other subjects, chemistry is more abstract because it comprises macroscopic, microscopic, and symbolic domains that describe chemical behaviors in different ways. The majority of secondary students think that chemistry is a difficult subject (Sausan *et al.*, 2018). The difficulties of learning chemistry make students have a negative perception towards chemistry. Studies show they are less interested and less motivated in learning chemistry concepts (Sausan *et al.*, 2018).

Based on results released by the Organisation for Economic Cooperation and Development (OECD), Malaysia scored 409 in Mathematics, 388 in Reading and 416 in Scientific Literacy in the Programme for International Student Assessment (PISA) 2022 (Ly *et al.,* 2022). Plus, according to Azahar and Cheng (2024), Malaysia's PISA scores in 2022 were 18% lower in reading, 14% lower in science, and 13% lower in mathematics compared with the OECD average. These differences are larger than they were a decade ago. The advancement of science and technology depends on the professional and well-skilled people who excel in science understanding and can apply scientific knowledge in our daily life (Kinge *et al.,* 2020).

Students have not been given the idea of the method to solve chemistry problems effectively. Students are not adequately equipped with the cognitive and affective skills necessary to apply their learning effectively in academic and real-life situations (Surif *et al.*, 2014; Sarwari and Kakar, 2023). To foster a greater interest in acquiring knowledge of chemistry among students, measures need to be taken. Recent studies were conducted to emphasize the importance of integrating different problem-solving approaches. These approaches include solving the problem of algorithmic, conceptual, and open-ended, which each play unique roles in enhancing students' understanding and engagement (Surif *et al.*, 2014; Sarwari and Kakar, 2023). However, there is limited evidence on how integrating these approaches could lead to improved outcomes, particularly in chemistry education.

Algorithmic problem-solving only focuses on step-by-step procedures and formulas. It is often criticized for promoting rote learning without encouraging deep understanding even it helps students solve structured problems such as stoichiometric problems. Conceptual problem-solving approaches emphasize understanding the underlying scientific principles rather than just applying formulas. This method helps students grasp fundamental concepts in chemistry, including acids and bases, atomic structure, and chemical bonding (Hagos, 2025). The last problem is open-ended which has no single correct answer and requires students to evaluate various solutions. It can encourage creativity, higher-order thinking, and the application of knowledge in real-world contexts. Plus, it also demonstrates the effectiveness of open-ended problem-solving in fostering students' abilities to tackle complex, real-life chemistry scenarios (Raman *et al.*, 2024).

Therefore, this study aims to fill these gaps by identifying the achievement levels of high school chemistry students in three types of problem-solving (algorithmic, conceptual, and open-ended), focusing on three critical areas, namely the mole concept, acid-base reactions, and polymers. These topics were selected due to their importance in understanding both foundational and advanced chemistry concepts. The mole concept addresses algorithmic skills in quantifying chemical reactions; acid-base reactions require both calculation and conceptual understanding, and polymers encourage open-ended thinking related to real-world applications and sustainability (Tsaparlis, 2021). By focusing on these areas, the study seeks to enhance chemistry education and improve student performance to meet international standards.

1.1. Problem-solving

A problem can be described as a situation that arises when there is a disparity between the current position and the desired goal. Problem-solving plays a crucial role in all scientific disciplines and solving problems can reveal fundamental concepts underlying those disciplines (Frey *et al.*, 2022). Problem-solving can be defined as the action taken when there is no solution to a problem. Through problem-solving, students can develop a deep understanding of chemical concepts and connect them to practical applications. These skills are also relevant in career preparation, enabling students to design experiments, analyse data, and overcome challenges. With these skills, students become proficient problem-solvers in the field of chemistry and science. However, problem-solving skills in chemistry have been identified as important skills that are lacking among students in current education. Students often encounter difficulties when solving problems as it involves critical thinking and in-depth analysis (Jamil *et al.*, 2024).

In the context of chemistry, problem-solving is crucial because it enables students to apply theoretical knowledge to real-world situations. For example, in a stoichiometry problem, students need to determine the amounts of reactants and products in a chemical reaction. This requires both algorithmic problem-solving (applying formulas and procedures) and conceptual understanding, which requires grasping the law of mass conservation. As for open-ended problem solving, such as chemical equilibrium topics, students must analyse reaction rates and the dynamic nature of reversible reactions. Here, open-ended problem-solving becomes important as students explore different conditions (such as temperature or concentration) that can influence the outcome of a reaction. This approach fosters deeper understanding and prepares students to handle real-world applications. The use of problem-solving in these areas supports students' ability to think critically and apply their chemistry knowledge to complex open-ended problems. It can bridge the gap between theoretical learning and practical application. This approach not only enhances learning outcomes but also prepares students for future careers in STEM fields.

In this research, different topics are chosen to solve algorithmic, conceptual, and open-ended problems in chemistry, assessing a range of cognitive skills and knowledge. Algorithmic problems focus on students' ability to apply formulas and perform calculations, emphasizing procedural skills in areas like stoichiometry or chemical equations. Conceptual problems, on the other hand, assess understanding of core principles and theories, such as atomic structure or thermodynamics, requiring deeper comprehension of how and why processes occur. Open-ended problems involve applying chemical knowledge to complex, real-world scenarios, thereby fostering critical thinking, creativity, and problem-solving skills. By using a variety of topics, teachers can evaluate both the practical and theoretical understanding of students across different cognitive domains.

1.2. Algorithmic problem

Algorithmic type of problem solving can be defined as a step-by-step process to resolve equations or numerical calculation problems. The correct formula or equations are needed to calculate this type of problem. According to Sangguroa and Surifa (2021), an algorithmic problem is a problem in which the required data are given, the solution method is known, and the objective is clearly defined. This type of problem does not assess the student's problem-solving skills but rather emphasizes their capacity to apply acquired knowledge consistently. According to Frank *et al.* (1987), algorithmic problems serve as helpful shortcuts for practicing exercises, but one disadvantage is that they can impede understanding when students encounter real-world problems. Algorithmic problems often discourage the development of students' conceptual understanding. When a student's initial approach is merely to select which algorithm to apply, it shows that the problem has not been genuinely solved (Frank *et al.,* 1987). To reduce dependence on rote learning, students must develop a strong conceptual understanding of chemistry.

A few topics in chemistry that are often difficult for students to grasp include chemical equations, stoichiometry, mole concepts, and acid-base reactions (Rosa *et al*., 2022). For instance, the mole concept is a fundamental building block in stoichiometry, which is critical for solving various quantitative problems in chemistry. The urgency in using the mole concept as test material stems from its role in bridging theoretical knowledge with practical applications. A study by Diaz and Aizman (2024), found that students in a control group struggled with the mole concept in a pretest for stoichiometry, and despite a post-test intervention, many still had trouble. This demonstrates the persistence of misunderstandings, making it an essential area to focus on for improving student learning outcomes.

1.3. Conceptual problem

Conceptual questions typically present students with chemical scenarios that they are unfamiliar with. This question necessitates that students justify their choices, predict future occurrences, explain processes, establish connections between various topics, recognize questions presented in a novel manner, and extract valuable information from multiple sources or broad scopes. Students are required to synthesize their answers and evaluate the problem in order to determine an appropriate mathematical tool for solving it.

According to Cracolice *et al.* (2008), a conceptual problem necessitates that students focus on enhancing their understanding of the underlying concept rather than relying on memorized procedures. A strong conceptual understanding of the relevant topics is essential for students to successfully solve conceptual questions (Haláková and Proksa, 2007; Hurrel, 2021).

One of the problems that students find difficult to understand in chemistry is acid and base concepts. A study by Yaman *et al.* (2019) showed that the main problem faced by students is the understanding of acid and base concepts. They tend to only associate acids with H^+ ions and bases with OH^- ions, without a deep understanding of the characteristics and reactions of acids and bases. Another study by Tóthová *et al.* (2021) and Tóthová and Rusek (2021) found that students lack the skills necessary to solve conceptual chemistry problems.

Conceptual problem-solving is essential for students, as it assists students in attaining a more comprehensive and in-depth learning experience. It also helps to strengthen their comprehension and proficiency in applying the knowledge they have acquired to novel situations. Students' critical thinking abilities would be enhanced through improved conceptual problem-solving skills. It also fosters students' enthusiasm for science and the pursuit of knowledge. This type of problem-solving evaluates students' understanding of a subject matter, rather than solely assessing their knowledge and problem-solving abilities. It also offers a means to identify and address students' misconceptions through diagnostic assessment.

1.4. Open-ended problem

An open-ended problem is a problem that does not have a singular correct answer; instead, it has the possibility of multiple correct answers (Fitriana *et al.*, 2018). Critical thinking skills, creativity, and problem-solving skills are needed to solve this type of problem (Fitriana *et al.*, 2018). According to Authority (2010) and Aziza (2021), open-ended questions in chemistry are designed to evaluate a student's understanding of fundamental concepts rather than their ability to memorize specific facts. This approach ensures that students are not impeded from answering the questions solely due to a lack of recall, as there are always multiple alternative paths to arrive at the ultimate solution. It is important to have open-ended problem-solving skills (Bayarcal and Tan, 2023). This is because creative and adaptable solutions are required to solve many challenging real-world problems in an effective and practical way. One will come out with new ideas and innovations that can improve the world once they have mastered open-ended problem-solving skills (Bayarcal and Tan, 2023). Furthermore, collaboration and teamwork are required to solve open-ended problems which are indirectly able to learn others' perspectives and experiences.

Students must acquire problem-solving skills to develop conceptual understanding and the ability to solve real-life problems with scientific skills. Specifically, this research aims to examine students' level of ability in solving these types of problems, which include algorithmic problems, conceptual problems and open-ended problems. According to the Curriculum Development Department, in Malaysia education, the KSSM curriculum emphasizes active learning and critical thinking. Students are taught to analyze problems, collect data, identify the causes of problems, and formulate solutions in the subject of Chemistry. Thus, the KSSM curriculum helps students to develop problem-solving problems related to chemistry concepts. The change in curriculum from KBSM to KSSM may potentially affect students' performance in solving real-life problems. Therefore, a new study is needed to examine how the KSSM curriculum influences students' ability to solve problems. In response to these challenges, a study has proposed to examine the students' level of achievement in solving various types of problems in chemistry (algorithmic, conceptual, and open-ended). Thus, this study aims:

- **i)** To identify students' level of achievement in algorithmic problems
- **ii)** To identify students' level of achievement in conceptual problems
- **iii)** To identify students' level of achievement in open-ended problems
- **iv)** To compare students' level of achievement in algorithmic problems, conceptual problems and open-ended problems

2. Methodology

The study focuses on identifying and comparing the chemistry students' level of achievement in solving algorithmic, conceptual, and open-ended problems. This methodology section covers aspects such as research design, population and sampling, data collection instruments, and data analysis techniques.

2.1. Research design

A quantitative study utilizing a descriptive research design was conducted to address the research objectives. This approach involved systematically collecting numerical data to describe and assess key characteristics related to the research topic. The data collection process was executed using a paper-and-pencil test, where participants were asked to complete a series of standardized questions designed to measure their knowledge, skills, or understanding of the subject matter. This method of data collection allowed for straightforward administration and reliable quantification of results, ensuring that the data could be analysed effectively to meet the study's goals.

2.2. Population and sample

For this study, purposive sampling was employed by choosing 42 high school chemistry students from a private school in Johor Bahru. This school was chosen due to its diverse student population, which reflects a range of academic proficiencies and backgrounds, making it an ideal setting to examine different problem-solving abilities in chemistry. The purposive sampling method was selected to ensure that the participants included students with varying levels of proficiency in chemistry, from high achievers to those who struggle with the subject. According to Emara *et al*. (2021). This diversity is crucial for a comprehensive comparison across different cognitive domains related to algorithmic, conceptual, and open-ended problem-solving.

The sample size of 42 chemistry students was determined based on the feasibility of conducting in-depth analyses while achieving statistically meaningful comparisons. All the students selected for this study were 17 years old and had been exposed to chemistry from the same year, ensuring they had an equal foundation in the subject. This uniform exposure to chemistry provides a consistent basis for assessing their problem-solving abilities, minimizing any confounding variables related to differing levels of prior knowledge or experience. The sample was evenly distributed across gender, ensuring that the study's findings are not biased toward a particular demographic group.

2.3. Instruments

To collect the necessary data, a series of tests was administered to the students. These tests comprised three types of problem-solving questions specifically designed to assess students' abilities in solving algorithmic, conceptual, and open-ended problems. Each test was carefully crafted to ensure clarity and relevance to the chemistry curriculum, allowing students to demonstrate their understanding of key concepts and problem-solving strategies.

Plus, it was developed based on research objectives to ensure it effectively addressed the key areas of interest. Following its development, these tests were validated by two experts who have experience in teaching chemistry for more than 10 years. The experts provided several recommendations, such as revising certain items. Despite these suggestions, the experts confirmed that these tests met the requirements and achieved the study's objectives.

2.3.1. Algorithmic problems

The algorithmic problems focused on numerical calculations and the application of formulas, requiring students to follow step-by-step procedures. Each of these questions requires students to follow a defined set of steps, which is a hallmark of algorithmic problem-solving. Table 1 below presents a set of algorithmic questions used in the study to evaluate students' problem-solving abilities in chemistry. Each question is designed to test the application of specific formulas and procedures, reflecting the systematic and procedural nature of algorithmic problem-solving. Table 1 includes explanations for how each question relates to the development of these skills, emphasizing the role of algorithmic learning in reinforcing students' understanding of fundamental chemical concepts.

Table 1. Algorithmic questions in chemistry: assessment of problem-solving skills.

Questions	Explanation
1. A gas jar contains 300 cm^3 of ammonia gas at room condition. Calculate: a) The number of mole of ammonia gas.	This question typically uses the formula $n=\frac{V}{M}$, where n is the number of moles, V is the volume of gas, and M is the molar volume. Students must identify the relevant values and apply the formula to find the solution. This type of calculation emphasizes the procedural aspect of problem-solving.
b) The mass of ammonia gas.	This question relates to question (a). After students get the number of moles, students need to calculate the mass of ammonia using formula, Mass (g) = number of mole x Molar Mass.
c) The number of atoms in ammonia gas.	This question involves Avogadro's Constant (6.02×10^{23}) to find the total number of atoms. Students need to calculate the number of atoms using a formula. (number of particles = number of mole x Avogadro's Constant x number of ammonia atom in one molecule of ammonia gas).
d) Nitrogen gas can react with hydrogen gas to produce ammonia gas under certain conditions. I) Write the balanced chemical equation for the reaction.	I) This task assesses students' understanding of the stoichiometry relationships in chemical reactions, requiring them to recognize the reactants and products involved and ensure that mass is conserved in the equation. This is a critical skill in chemistry that connects algorithmic problem-solving with conceptual understanding.
II) Calculate the volume of hydrogen gas needed in this reaction if 300 cm^3 of nitrogen gas is used and measured under the same reaction condition.	II) This question typically uses stoichiometry to determine the volume of hydrogen gas required to produce a specific amount of ammonia. It emphasizes the application of theoretical knowledge to practical situations.

Source: Elaborated by the authors.

Algorithmic questions in chemistry are essential because they require students to follow established procedures, reinforcing their procedural knowledge and enhancing their problem-solving abilities. By applying systematic approaches to solve specific types of problems, students learn to navigate the complexities of chemical calculations with confidence (Vo *et al.*, 2022). These questions often involve mathematical skills, allowing students to practice and strengthen their ability to perform calculations relevant to chemical concepts. Furthermore, mastering algorithmic problem-solving fosters confidence, as students become adept at tackling quantitative problems, which serves as a foundation for developing higher-order thinking skills. Additionally, these questions are valuable for educators, as they provide insight into students' understanding of fundamental concepts and their capacity to apply that knowledge in practical scenarios. Overall, algorithmic questions play a crucial role in chemistry education by enabling students to effectively solve quantitative problems and prepare for more complex conceptual challenges (Rau *et al.*, 2021; Tsaparlis, 2021).

2.3.2. Conceptual problems

The conceptual problems presented scenarios that necessitated a deeper understanding of chemical principles, encouraging students to connect different topics and apply their knowledge creatively (Rahmawati *et al.*, 2022). Each question targets key aspects of chemical theory, encouraging students to engage in deeper thinking rather than simply recalling memorized facts. **Table 2** outlines a series of conceptual questions used to evaluate students' comprehension of fundamental chemical concepts related to acids. Each question is crafted to challenge students to think critically and apply their knowledge in new contexts, moving beyond simple recall to engage in deeper analytical thinking. **Table 2** also provides explanations for how each question relates to conceptual problem-solving, emphasizing the integration of knowledge and the application of scientific principles.

Table 2. Conceptual questions in chemistry: assessment of understanding and application of acid-related concepts.

Questions	Explanation
I) What is meant by acid?	It prompts students to articulate their understanding of acids in terms of their properties and characteristics, such as their ability to produce H^+ ions in aqueous solutions. This requires students to synthesize knowledge from different areas of chemistry.
II) State the ion that enables an acid to show its acidic properties.	This question encourages students to connect the concept of acidity with the ionization process which produce hydrogen ion that can show the acidic properties.
III) Suggest the name of acid P and acid Q.	It requires students to apply their knowledge of chemical nomenclature. This not only tests their ability to identify specific acids but also assesses their familiarity with common acids and their formulas.
IV) Acid P and acid R have the same concentration. It was found that the pH value of acid P is lower than acid R. Explain why the pH value of both acids are different.	It challenges them to apply their understanding of concepts such as dissociation and strength of acids. This question emphasizes the importance of understanding that pH is influenced by factors beyond concentration, such as the acid's inherent strength and degree of ionization.

Source: Elaborated by the authors.

These questions exemplify conceptual problem-solving in chemistry because they require students to integrate their knowledge and apply it to new scenarios. They encourage critical thinking and analysis, as students must justify their answers based on scientific principles rather than relying solely on rote memorization. By engaging with these conceptual questions, students deepen their comprehension of acid-related concepts, which is essential for their overall success in chemistry.

2.3.3. Open-ended problems

The open-ended problems require students to explore multiple pathways to arrive at solutions, fostering critical thinking and creativity (Shanta and Wells, 2022). The open-ended questions presented here are designed to encourage students to explore complex concepts related to the use of polyvinyl chloride (PVC) in water pipe manufacturing and its environmental implications. **Table 3** presents a set of open-ended questions designed to assess students' ability to analyse and articulate complex issues related to the use of PVC in water pipe manufacturing and its environmental impact. Each question encourages students to explore practical applications, environmental considerations, and ethical implications, fostering critical thinking, creativity, and problem-solving skills. **Table 3** contains explanations for how these questions relate to open-ended problem-solving, highlighting the importance of addressing multifaceted problems that allow for several valid responses.

Table 3. Open-ended questions in chemistry evaluating critical thinking and application in real-world contexts.

Question	Explanation
a) State and explain the importance of using PVC in the manufacture of water pipes.	It prompts students to consider the practical applications of PVC in real-world scenarios. This question encourages them to think critically about the material's properties, such as durability, resistance to corrosion, and cost-effectiveness, which make it a popular choice in plumbing. Students must synthesize information and articulate their reasoning, demonstrating a deeper understanding of material science and engineering principles.
b) Explain how PVC can cause environmental pollution. Suggest on ways to solve this problem.	It challenges students to examine the broader implications of using PVC, including its production, use, and disposal. This question invites students to engage in environmental science and ethics, requiring them to think about sustainability and the consequences of material choices. By asking for solutions, the question fosters creativity and problem-solving skills, as students must propose practical measures to mitigate PVC-related pollution.

Source: Elaborated by the authors.

These questions exemplify open-ended problem-solving because they do not have a singular correct answer, allowing for a range of responses based on students' perspectives and knowledge. They encourage students to analyse complex issues, consider multiple viewpoints, and express their thoughts coherently. Such questions are essential for developing students' higher-order thinking skills and their ability to apply scientific knowledge to real-world problems, thus enhancing their overall learning experience in chemistry and related fields.

Before administration, the items in each problem were validated by two expert chemistry teachers with ten years of experience each. The tests were pilot-tested with a small group of students to ensure that the questions were appropriate in terms of difficulty and clarity. The tests were designed to be completed within 40 minutes, providing students with sufficient time to think critically and demonstrate their problem-solving abilities. Students were instructed to complete the tests independently, providing their responses in writing. The data obtained from the tests were meticulously recorded for further analysis, ensuring that each student's performance could be accurately assessed and compared.

2.4. Data analysis techniques

Quantitative analysis techniques were applied to analyse the collected data. A set of questions consisting of three types of questions that related to algorithmic, conceptual and open-ended problems was given to the students. The students' answers were marked, and the marks were referred to the answer scheme. The mark achievement for each problem-solving category was then analysed using SPSS 27.0. **Table 4** shows the specification table for each item.

Table 4. The specification table for each item.

No	Types of question	Topic	Total marks
1	Algorithmic	Mole concept	10
2	Conceptual	Acid and base	10
3	Open-ended	Polymer	10

Source: Elaborated by the authors.

Descriptive statistics such as frequency and percentage were used to summarize the achievement level of students. The Malaysian Ministry of Education classifies students' chemistry achievement into two categories based on their assessment scores. **Table 5** shows the students' achievement level. This score level of achievement was used to provide clarity and ease of assessment.

Table 5. Level of achievement.

Marks	Level of Achievement	Description
0-39	Low	Unable to solve problem
40-100	High	Able to solve problem

Source: Adapted from Surif *et al.*, 2014.

Based on **Table 5** a score of 40% or higher is regarded as a passing mark, indicating a higher level of achievement in the subject. Conversely, students who score below 40% are considered to have a lower level of achievement in problem-solving skills. The "low" range can help teachers identify areas for improvement, while the "high" range can reinforce proficiency and foster motivation for the students.

This score also enables teachers to quickly identify students who may need additional support, making it an effective tool for promoting skill development in Chemistry (Ely, 2019). This research was supported by interviewing five students to provide deeper insights into the students' experiences and perceptions regarding problem-solving.

3. Results and discussion

Table 6 shows the frequency and percentage of students' achievement across three types of questions, which are algorithm problems, conceptual problems and open-ended problems. The data illustrates how students performed in each category.

Table 6. The frequency and percentage of students' achievement on the three types of questions.

	Marks	Level of achievement	Frequency	Percentage (%)
Algorithmic	0-39	Low	4	9.52
	40-100	High	38	90.5
		Total	42	100.00
Conceptual	0-39	Low	7	16.7
	40-100	High	35	83.3
		Total	42	100.00
Open-ended	0-39	Low	33	78.6
	40-100	High	5	11.9
		Total	42	100.00

Source: Elaborated by the authors.

3.1. Students' level of achievement in algorithmic problems

The findings reveal that the majority of students (90.05%) achieved scores above 40% when solving algorithmic problems, indicating a strong performance in this area. Only a small percentage (9.52%) struggled with these problems, suggesting that most students are proficient in applying the required techniques. This is particularly evident in question number one, which assessed students' understanding of calculations related to the mole concept a fundamental topic in chemistry.

The high level of achievement in algorithmic problems can be attributed to students' reliance on structured problem-solving techniques and consistent practice. These results are consistent with Olubunmi (2016) findings, which emphasize that the algorithmic learning method focusing on the three steps of analysis, calculation, and evaluation effectively supports students in mastering mathematical concepts in chemistry. By adhering to these steps, students can systematically approach and solve problems, thereby improving their performance.

Algorithmic learning methods enhance students' problem-solving skills and deepen their understanding of fundamental chemical concepts by promoting critical thinking and application over rote memorization. This approach breaks down complex problems into manageable steps, encouraging students to analyze, evaluate, and synthesize information to find solutions. By emphasizing the relationships between different chemical concepts and processes, algorithmic learning helps students grasp underlying principles and apply their knowledge to new situations, fostering a more comprehensive and adaptable understanding of chemistry (Vo *et al.*, 2022).

This study contributes to existing knowledge by reinforcing the importance of teaching problem-solving techniques in a structured and methodical manner. Furthermore, it suggests that when teachers emphasize the process ensuring students demonstrate in writing how they apply specific techniques students are more likely to internalize these methods, leading to better outcomes in examinations. This insight could inform future pedagogical strategies, particularly in designing curricula that prioritize skill development through algorithmic problem-solving.

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There are five questions provided based on the information in **Fig. 1**.

1. A gas jar contains 300 cm^3 of ammonia gas at room condition. Calculate: [Relative atomic mass: N = 14, H = 1; Avogadro constant = 6.02 x 10^{23}; Molar volume = 24 dm^3 mol^{-1}]
   a) The number of mol of ammonia gas. [2 marks]
   b) The mass of ammonia gas. [2 marks]
   c) The number of atoms in ammonia gas. [2 marks]
   d) Nitrogen gas can react with hydrogen gas to produce ammonia gas.
      i) Write the balanced chemical equation for the reaction. [2 marks]
      ii) Calculate the volume of hydrogen gas needed in this reaction if 300 cm^3 of nitrogen gas used. [2 marks]

Figure 1. Algorithmic question.
Source: Elaborated by the authors.

All students were able to answer question numbers for (a), 1(b), 1(d)(i) and 1(d)(ii). However, only 14 students can answer question number 1(c) correctly. Most of the students answered the question the same as the students' sample answers. Students' misconceptions about the types of particles because they can't differentiate between atom, molecule and ions. Plus, this misconception also occurs due to students unable to master the chemical formula (Chophel, 2022). **Box 1** show the sample students answers for question 1(c).

Box 1. Sample students' answer for question 1(c)

Student 1: $N(NH_3) = 0.0125 \times 6.02 \times 10^{23} = 7.525 \times 10^{21}$

Student 2: $N = 0.0125 \times 6.02 \times 10^{23} = 7.525 \times 10^{22}$

Source: Elaborated by the authors.

3.2 Students' level of achievement in conceptual problem

Secondly, for conceptual problem questions, 83.3% of the students scored a high level of achievement between 40 to 100 marks. Only 16.7% of students scored a low level of achievement (0 to 39%). From these findings, we can conclude that most students can solve conceptual problems. For this section, four questions were provided based on the information in **Fig. 2**.

Table 1 shows information of three different acids.

Acid	Degree of ionisation in water	Basicity
P	High	Monoprotic
Q	High	Diprotic
R	Low	Monoprotic

Table 1

Based on table 1

(i) What is meant by acid? [1 mark]

(ii) State the ion that enables an acid to show its acidic properties [1 mark]

(iii) Name acid P and acid Q [2 marks]

(iv) Acid P and acid Q have the same concentration. It was found that the pH value of acid P is lower than acid R. Explain why the pH value of both acids are different. [3 marks]

Figure 2. Question for the conceptual problem.
Source: Elaborated by the authors.

The main findings of this research reveal several important aspects of students' understanding and misconceptions in chemistry. First, all students were able to successfully answer the first question [2(i)]. It demonstrates that they are familiar with the basic concept and can effectively elaborate on it. This indicates a strong foundational knowledge in this area, suggesting that the initial teaching strategies or prior exposure to similar questions have been effective. However, the second question [2(ii)] exposed a misconception among students, with one student incorrectly identifying OH^- ion instead of H^+ ion. This mistake likely stems from confusion between different types of ions or a lack of understanding of their charges, highlighting a common issue in chemistry education where students may confuse similar concepts.

For the third question [2(iii)], many students answered correctly but some responded with chemical formulas (e.g., HCl, H_2SO_4) instead of the full names of the acids. This finding suggests that while students can recall chemical formulas, they may struggle with translating this knowledge into the required format, such as using the full chemical names. This points to a gap in instruction regarding the importance of chemical nomenclature. The final question [2(iv)] proved to be the most challenging, with many students unable to correctly address all three required points. This difficulty indicates that while students may grasp individual concepts, they struggle to synthesize these concepts into solving more complex, multi-step problems. This highlights a potential area where additional instructional support or practice might be necessary. **Box 2** shows the sample students answer for question [2(iv)].

Box 2. Sample students' answer for question 2(iv)

Student 1: This is because acid P is strong acid that its degree of ionization in water is higher than acid R, hence its pH value is lower.

Student 2: The pH of R will be lower as the degree of ionization of R in water is lower, it only partially dissociates in water. Thus, the pH of R will be lower.

Student 3: The pH value of both acids is different because acid P is strong acid which can dissociate fully while acid R is weak acid which can only dissociate partially. Thus, pH value of acid R is higher than acid P.

Student 4: Because acid P is monoprotic acid.

Source: Elaborated by the authors.

To score all the points, students must state that acid P ionizes completely in water to produce a high concentration of hydrogen ions. Next, they also need to mention that acid R ionizes partially in water to produce low concentrations of hydrogen ions. Then, summarize them in a sentence such as the higher the concentration of hydrogen ions, the lower the pH value. This question allowed teachers to analyze the students' conceptual understanding and their skills in elaboration. Based on the answers given by the students, there were slight misconceptions detected in explaining the topic.

These findings contribute uniquely to the existing knowledge in chemistry education by providing insights into specific areas where students commonly experience confusion, such as ion identification and the translation of chemical formulas into names. This research underscores the importance of not only testing students' knowledge but also their ability to apply that knowledge in various forms, suggesting a need for more comprehensive instructional strategies that address these issues. Additionally, the study highlights the significance of assessment design in capturing students' true understanding and the necessity of teaching students how to navigate between different representations of chemical knowledge. Overall, this research offers valuable implications for refining educational practices in chemistry, aiming to enhance student understanding and performance.

3.3 Students' level of achievement in open-ended problems

Based on the results in **Table 6** above, regarding the students' achievement level in solving open-ended problems, only 11.90% of the students achieved high-level marks, indicating that they are able to answer open-ended problems effectively. However, 78.60% of them were unable to solve open-ended chemistry problems. Open-ended questions not only allow teachers to assess their students' level of knowledge, but they also help students strengthen fundamental abilities like observation, analysis, and inference, as well as their creative potential in several contexts. Divergent thinking is associated with open-ended questions that permit multiple correct responses and various solution strategies. Open-ended questions also require students to construct unrestricted responses.

Polymeric which frequently seen in day-to-day life is also one of the chemistry topics that has a high volume of the notion of a low level of concept mastery (Hill, 2020). In addition, the majority of teachers disregarded the development of students' comprehension of polymer concepts. Regarding this research, **Fig. 3** shows the question of open-ended problems. It consists of two sub-questions, which are about polyvinyl chloride or vinyl (PVC), and this question primarily assesses mastery of factual knowledge rather than higher-order skills. PVC is a type of polymer in which chlorine contains more than 50% of the total component by weight. According to Osama, *et al*. (2020) research, it is made through the polymerization of the vinyl chloride monomer. In Malaysia, the current curriculum, which is the Secondary School Standards-based Curriculum (KSSM), includes polymer as one of the topics in the Form Five Chemistry subject syllabus.

3. Polyvinyl chloride (PVC) is a synthetic polymer commonly used in the production of water pipes. However, the use of PVC in water pipe manufacturing also has implications for the environment.

diagram 1

a) Explain the importance of using PVC in the manufacture of water pipes. [4 marks]

b) Explain how PVC can cause environmental pollution. Suggest on ways to solve this problem. [6 marks]

Figure 3. Open-ended question.
Source: Elaborated by the authors.

The main findings of this research indicate significant gaps in students' understanding and ability to articulate key concepts related to the composition and environmental impact of PVC (polyvinyl chloride). When faced with open-ended questions, many students were able to identify key points but failed to elaborate on them in detail. This lack of detailed explanation suggests that while students may have some surface-level understanding, they do not possess the deeper comprehension required to thoroughly explain the concepts. Furthermore, a considerable number of students provided incorrect answers, indicating that they have not yet mastered this essential topic, which is particularly concerning given the relevance of the compounds discussed to their everyday lives.

Specifically, question 3(a) asked students to state and explain the importance of using PVC in the manufacture of water pipes. The achievement levels of students were varied. This question, contributing 4 marks, revealed that while some students could state the importance of PVC, they struggled to provide a detailed explanation, further highlighting their limited understanding. **Table 7** shows the level of students' achievement for question 3(a).

Table 7. The level of students' achievement for question 3(a).

Level of Achievement	Explanation	Sample Students' Answer
Excellent *[4 marks]*	Demonstrates a comprehensive understanding of the importance of PVC in water pipe manufacturing, providing detailed explanations and mentioning multiple benefits.	*S9. PVC is vital for water pipe manufacturing due to its durability, corrosion resistance, cost-effectiveness, lightweight design and smooth flow. PVC pipes are long-lasting, resistant to corrosion and can withstand various temperature and pressure. They are lightweight, easy to handle and reduce transportation and labor costs. The smooth inner surface minimizes friction losses and prevents scaling or blockages.*
Adequate *[2-3 marks]*	Provides some relevant points and explanations, highlighting the durability, corrosion resistance, cost-effectiveness, and other advantages of using PVC in water pipe manufacturing.	*S4. The importance of using PVC in the manufacture of water pipes is that it pressures drinking water quality due to their high degree of inertness and resistance to corrosion.* *S5. PVC is a waterproof, isolator in heat and electric. So, it can be used as waterpipes. PVC is not degradable, so it can be using a long time and need not be replaced, PVC manufacturing is low-cos.t*
Limited *[1 mark]*	Provides limited or brief explanations, mentioning a few characteristics or properties of PVC without further elaboration.	*S2 & S3. PVC plastic is corrosion, resistance and chemical-resistant.* *S6. Stable polymer doesn't break easily. can be used for a long time* *S7. Structure of PVC plastic is stable it doesn't dissolve in water and it is an insulator of heat and electric* *S8. PVC is durable and stable, easy to change in shape* *S10. PVC helps to ensure energy and water by creating virtually leak-free pipes that are not corrosion and resist environmental stress* *S26 & S28. PVC is chemically stable and does not easily corrode. It is easy to be shaped.* *S29. PVC plastic is corrosion-resistant and chemical resistance* *S35. PVC pipes are durable, as the expected lifespan is 100 years or more.* *S42. The expected lifespan of a PVC pipe is 100 years or more for underground pipes. Besides, PVC water mains show a much lower failure rate than non-plastic materials. PVC pipes are safe and durable*
Incomplete *[0 mark]*	It does not provide an answer or explanation for the importance of using PVC in water pipe manufacturing	*S1. PVC is a polymerized substance. It has a better characteristic than other substances. It is more suitable for the manufacture of water pipes.* *S11. No idea* *S21. It is a high-strength thermoplastic material*

Source: Elaborated by the authors.

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Question 3(b) required students to explain how PVC can cause environmental pollution and suggest solutions. Table 8 shows the level of students' achievement for question 3(b) where it shows students faced even greater challenges. This question, contributing 6 marks, not only tested their knowledge of PVC's environmental impact but also their ability to propose practical solutions. The results indicated that students are not only struggling with the basic comprehension of the material but also with applying their knowledge to broader environmental contexts.

Based on Table 7 and 8, students' answers have already been arranged according to the level of students' achievement, which is excellent, adequate, limited and incomplete. This research found that most of the students can only achieve a level of limited and incomplete. Thus, the result shows that students not yet achieve the skill in open-ended questions. To overcome this ineptness, effective strategies must be implemented to ensure that students grasp the concept and are able to think critically and creatively (Black, 2018).

Table 8. The level of students' achievement for question 3(b).

Group	Explanation	Sample Students' Answer
Excellent *[4-6marks]*	Shows a comprehensive understanding of how PVC can cause environmental pollution, mentioning harmful chemicals, additives, and toxic substances associated with PVC production, use, and disposal. They also suggest several solutions to address the problem.	*S5. PVC is a kind of plastic, it is not degradable, and it needs a variety of processes to decompose PVC, so, if the PVC is disposed of it will cause pollution. Besides, when PVC is decomposed it will release toxin gas that also causes air pollution. To solve this problem, we can use degradable materials like plant base to manufacture water pipes to replace PVC.* *S9. PVC can cause environmental pollution through its production, use and disposal hazardous chemicals and additives used in PVC manufacturing can harm the environment. Improper disposal and incineration release toxic substances and microplastics, posing risks to ecosystems and human health. solution include sustainable manufacturing practices, PVC alternatives without harmful additives, improved recycling technologies, proper waste management, responsible consumption and reducing plastic waste at the source.*
Adequate *[3 marks]*	Mentions relevant points about how PVC can cause pollution, such as recycling difficulties and the release of harmful substances. However, their suggestions for solving the problem are limited or lack detailed explanations.	*S2. PVC release many harmful chemicals. PVC is difficult to recycle and it will remain in the environment for a long time. We should use recycled bags to replace PVC.* *S3. PVC is a stable polymer. It takes such a long period to disintegrate. The discarded PVC will accumulate and end up present in a large amount of unwanted trash that will cause environmental pollution. using natural rubber or latex as an alternative of PVC.* *S4. If PVC is landfilled, it leaches toxic addictive and releases toxin. To solve this problem, recycle PVC and reduce the usage of PVC.*
Limited *[1-2 marks]*	Provides incomplete or brief explanations, mentioning some characteristics or properties of PVC without further elaboration.	*S1. The production of PVC is not eco-friendly because it releases many harmful chemicals. Besides PVC is difficult to recycle and it will remain in the environment for a long time.* *S7. Releasing the manufacture of PVC plastic. Use other eco-friendly materials. PVC is hard to dissociate. So, it is hazardous to the environment.* *S10. The process of making PVC is harmful for the environment. It releases huge amounts of toxic chemicals into the environment. Discharged pollutants impact nearby drinking water supplies. Using natural rubber and latex be alternative to PVC.*
Incomplete *[0 mark]*	Does not provide an answer or explanation about how PVC can cause environmental pollution or suggestions to solve the problem.	*S6. –* *S8. PVC is a polymer. It is a stable molecule and it can last for a long time. recycle used PVC to make new products.*

Source: Elaborated by the authors.

3.4. Comparison of students' achievement in solving algorithm, conceptual and open-ended problems

Table 9 shows the minimum score (min), maximum score (max), mean and standard deviation of algorithmic, conceptual and open-ended questions. In algorithmic questions, the minimum score achieved by students is two out of ten, while the maximum score achieved by students is ten out of ten. The mean scores of students in algorithmic questions are 8.00, while the standard deviation of algorithmic questions is 1.87. In conceptual questions, the minimum score achieved by students is three out of ten, while the maximum score achieved by students is seven out of ten. The mean value of conceptual questions score among students is 5.60, while the standard deviation of algorithmic questions is 1.13. In open-ended questions, the minimum score of students is zero out of ten, and the maximum score of students is eight out of ten. The mean value of open-ended questions among students is 3.36, while the standard deviation of open-ended questions is 1.75.

Table 9. The minimum score, maximum score, mean and standard deviation of algorithmic, conceptual and open-ended questions.

	N	Min	Max	Mean	Std. Deviation
Algorithmic	42	2.00	10.00	8.00	1.87
Conceptual	42	3.00	7.00	5.60	1.13
Open-ended	42	0.00	8.00	3.36	1.75

Source: Elaborated by the authors.

Figure 4 shows the level of students' achievement in algorithmic questions, conceptual questions, and open-ended questions. Four students scored low marks on algorithmic questions. 38 students scored high marks in algorithmic questions. Seven students scored low marks on conceptual questions. 35 students scored high marks in conceptual questions. 33 students scored low marks in open-ended questions. Five students scored high marks on open-ended questions.

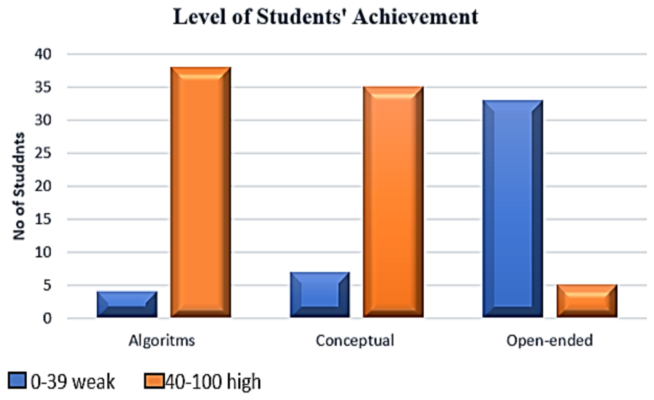

Figure 4. Level of students' achievement in algorithmic questions, conceptual questions, and open-ended questions.
Source: Elaborated by the authors.

Students are considered to have high marks for a particular type of question if they score between 40 to 100 marks, and low marks if they score between 0 and 39 marks. Among algorithmic, conceptual, and open-ended questions, it is evident that students faced the most difficulty with open-ended questions, as the mean of students' achievement in this category is the lowest.

Students tend to perform better on algorithmic questions because these questions typically require the application of a well-defined set of steps or procedures to arrive at a correct answer. The structured nature of algorithmic questions allows students to follow a clear path to the solution, reducing the cognitive load required to generate an answer. For example, in chemistry, algorithmic questions might involve calculating molar concentrations or balancing chemical equations, which are tasks that students can practice and master through repetition. Algorithmic questions often have a "yes" or "no" answer, or a specific numerical result, making them easier for students to tackle, especially those with lower proficiency (Csernoch *et al.*, 2021).

On the other hand, conceptual questions demand a deeper understanding of the underlying principles of chemistry. These questions test students' ability to apply their knowledge to new situations, requiring them to connect different concepts and think critically. While conceptual questions do not always have a straightforward answer, they still often involve a more guided thought process compared to open-ended questions. Students may struggle with these questions if they lack a strong foundational understanding or if they are not accustomed to applying concepts in varied contexts.

Open-ended questions, however, are the most challenging for students, as they require the generation of original responses based on the student's understanding and interpretation of the concepts learned. These questions do not have a simple "yes" or "no" answer; instead, they require students to express their opinions, synthesize information, and articulate their thought processes in a detailed manner (Komildjanovna, 2024). They often require a longer, more elaborated response, such as a paragraph or essay, which can be daunting for students, especially those with lower language proficiency or less confidence in their understanding of the material.

The difficulty in mastering open-ended questions lies in the need for students to not only recall information but also organize and communicate their reasoning effectively. As Nedjat-Haiem and Cooke (2021) noted, while students are encouraged to provide as much detail as possible, they often struggle to align their responses with the expected answers. This discrepancy might be due to a lack of practice in structuring their thoughts or a limited ability to apply their knowledge in a less structured, open format. Moreover, open-ended questions often assess higher-order thinking skills, such as analysis, synthesis, and evaluation, which can be challenging for students who have primarily been trained in rote memorization or procedural problem-solving.

To strengthen the research findings, it is essential to incorporate insights from the respondents' interviews. **Table 10** shows the response from five students via interview.

Table 10. The respondents' interviews.

Student	Response
Student 1	Algorithmic problems: "I like algorithmic questions since I can solve them step-by-step. They are straightforward, and I usually score well." Conceptual problems: "Conceptual questions challenge me because I need to think deeply. If I don't have a solid grasp of the concept, I feel lost." Open-ended problems: "Open-ended questions are the worst for me. I feel over helmed by having to come up with my own answers. I often write too little or get confused about what to include."
Student 2	Algorithmic problems: "I find algorithmic questions the easiest because they follow a clear procedure. I can memorize the steps and practice them, which makes me feel confident." Conceptual problems: "Conceptual questions are harder. I struggle to connect the ideas and apply them to new situations. Sometimes, I don't understand what the question is asking." Open-ended problems: "Open-ended questions make me anxious. I don't know how to start or what to write. I worry about not being detailed enough."
Student 3	Algorithmic problems: "Algorithmic questions are like puzzles; I enjoy solving them. I feel like I can rely on my skills and knowledge here." Conceptual problems: "Conceptual questions require me to think critically, which is tough. I need more practice relating different concepts." Open-ended problems: "I find open-ended questions intimidating. I can't express my thoughts clearly, and I'm afraid of getting it wrong since there's no right answer."
Student 4	Algorithmic problems: "I perform best in algorithmic questions. The rules are clear, and once I learn them, I can apply them easily." Conceptual problems: "Conceptual questions are tricky for me. Sometimes I can answer them if I remember the class discussions, but it's not always easy." Open-ended problems: "I struggle with open-ended questions. I want to provide detailed answers but often end up writing less because I can't think of what to say."
Student 5	Algorithmic problems: "I feel comfortable with algorithmic questions. They help me focus on calculations, which I'm good at." Conceptual problems: "Conceptual problems make me think differently, and that can be hard. I wish we practised these more." Open-ended problems: "Open-ended questions are challenging because they require creativity and articulation. I feel like I need more guidance on how to approach them."

Source: Elaborated by the authors.

From the response in Table 10, it is obvious that students expressed a sense of confidence with algorithmic problems due to their structured nature and clear procedures. Most felt they could master these through practice because they managed to answer the questions based on memorized procedures. Students generally found conceptual questions more challenging, often requiring a deeper understanding and connection of ideas, which they felt was lacking. Students managed to answer the questions only when they successfully related the concepts learned to real-life examples discussed in class. However, when it comes to open-ended questions, there was a consensus that open-ended questions were the most daunting. Students reported feelings of anxiety, confusion, and difficulty in articulating their thoughts, indicating a need for more practice and support in this area.

The main limitations of this research include potential sample size issues, where a small or non-representative group may limit the generalizability of the findings to a broader population (Hennink and Kaiser, 2022). Differences in problem difficulty, where algorithmic tasks might be easier or more familiar to students, can skew results, making it harder to assess true differences in problem-solving abilities. The influence of teaching methods, time constraints, and subjective grading of open-ended problems may also affect performance, leading to potential biases (Carpenter *et al.*, 2020). Moreover, factors such as student motivation, and prior knowledge may influence outcomes across problem types. It leads to complicated interpretations of their true achievement levels in algorithmic, conceptual, and open-ended problem-solving (Vo *et al.*, 2024).

In conclusion, the differences in student achievement across question types highlight the distinct cognitive demands each type imposes. Algorithmic questions were usually more procedural and accessible for students as they involved familiar steps and methods. While, open-ended questions require independent synthesis and expression of knowledge, which can present greater challenges (Broman, 2020). This indicates that students may struggle with the cognitive load and self-directed thinking needed for open-ended questions. Examining specific difficulties, such as challenges with critical thinking, applying concepts to new situations, or organizing responses, could yield valuable insights (Tornee *et al.*, 2019). By further analyzing these issues through interviews or targeted feedback, this research could pinpoint key areas where support is needed, informing interventions to strengthen students' problem-solving and critical thinking skills in Chemistry.

4. Conclusions

In summary, this study underscores the importance of strengthening students' conceptual understanding of chemistry and creating a curriculum that fosters balanced problem-solving skills through algorithmic, conceptual, and open-ended questions. The results suggest that KSSM assessments should incorporate more conceptual and open-ended questions to encourage students to not only grasp theoretical knowledge but also apply it to real-life scenarios. Additionally, the study emphasizes collaborative efforts between teachers and students to cultivate a problem-solving culture in the classroom, where teachers support a student-centered learning environment and provide academic guidance for students in need. Teachers are encouraged to explore diverse instructional approaches to enhance students' problem-solving abilities. By building a framework that promotes scientific creativity and problem-solving skills through these methods, it is anticipated that students will develop a deeper appreciation for chemistry and its applications in everyday life.

Authors' contribution

Conceptualization: Johari Surif; **Data curation**: Wan Nor Azlina Wan Abdullah; Syaida Ab Manaf; **Formal Analysis**: Wan Nor Azlina Wan Abdullah; Syaida Ab Manaf; **Funding acquisition**: Not applicable; **Investigation**: Wan Nor Azlina Wan Abdullah; Syaida Ab Manaf; Yam Pui Mun; **Methodology**: Yam Pui Mun; **Project administration**: Nor Hasniza Ibrahim; Abdul Hadi Bunyamin; Chee Ken Nee; **Resources**: Not applicable; **Software**: Not applicable; **Supervision**: Nor Hasniza Ibrahim; **Validation**: Nor Hasniza Ibrahim; **Visualization**: Yam Pui Mun; **Writing – original draft**: Wan Nor Azlina Wan Abdullah; Syaida Ab Manaf; **Writing – review & editing**: Wan Nor Azlina Wan Abdullah; Syaida Ab Manaf.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Improving students' critical thinking abilities and environmental sensitivity through project-based learning integrated with green chemistry principles

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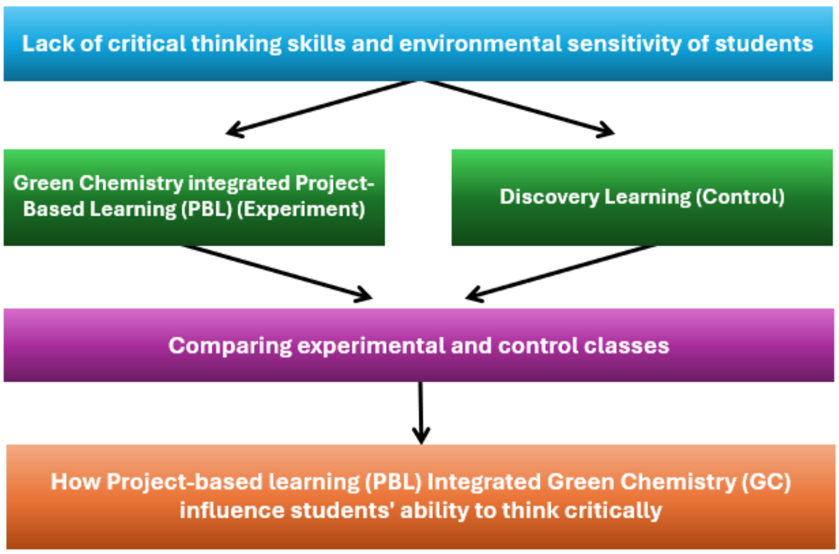
Abstract

This quasi-experimental research seeks to examine the impact of the Project-Based Learning (PBL) model integrated with Green Chemistry (GC) on students' critical thinking abilities (CTA) and environmental sensitivity (ES) at chemical equilibrium. A Randomized Post-test-Only Control Group design was used in four classes of Class XI F students at SMAN 1 Gowa, South Sulawesi as the population. The samples were classes XI F1 and XI F4, each consisting of 36 students, taken randomly. CTA data was obtained with seven essay questions which have been tested with quite high reliability (0.68). The ES questionnaire consists of 25 statements. Testing with the Independent Sample t-test shows that the integrated PBL GC Model influences increasing student CTA. The Wilcoxon test results also show that the application of the GC-integrated PBL model affects increasing students' ES. This research implies that it is hoped that teachers will continue to integrate green chemistry in learning continuously to support sustainability programs.



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Highlights

- Developing Critical Thinking Abilities through complex problem-solving.
- Developing CTA through critical analysis of environmental impacts.
- Environmental sensitivity: green chemistry principles in the learning process.
- Project-based learning (PBL) integrated with green chemistry (GC) enhances ES.
- PBL with GC encourages active involvement in understanding concepts.

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1. Introduction

One of the competencies students need to face 21st-century challenges is critical thinking skills (Mitarlis *et al.*, 2023). This ability prepares students to compete in the world of work (Thornhill-Miller *et al.*, 2023). Critical thinking skills help students evaluate and reflect on facts and data; they are not limited to solving academic problems (Huber and Kuncel, 2016; Tabanelli *et al.*, 2021). The same thing was also expressed by Van Brederode *et al.* (2020), where students' critical thinking skills in chemistry are related to understanding information in depth and making decisions based on careful consideration. Critical thinking skills help students become independent and responsible learners to help prepare students for changes that occur in society (Rimienė, 2002).

Students' critical thinking skills can foster environmental sensitivity to support sustainability programs. Students' environmental sensitivity can be fostered by providing understanding and familiarization related to the principles of green chemistry (GC) (Pluess, 2015). Green chemistry practices in everyday life can be instilled through a habituation process, for example reducing the use of plastic packaging, choosing safer materials, reducing waste, and using energy efficiently in chemistry learning/experiments (Aubrecht *et al.*, 2019). This contributes to environmental sustainability and the achievement of SDGs (Koulougliotis *et al.*, 2021).

Several studies have explored the link between learning materials and environmental issues, especially the need to apply green chemistry principles, thereby increasing environmental sensitivity (Santosa, 2023). Green chemistry principles integrated into education can increase students' sensitivity to environmental issues and encourage environmentally conscious behavior (Koulougliotis *et al.*, 2021). Green chemistry is integrated into the school curriculum, primarily through practical activities to increase students' awareness and involvement in improving environmental sustainability (Chen *et al.*, 2020). Integrating green chemistry into education not only enhances students' environmental awareness but also fosters their critical thinking skills. Through this approach, students learn about the importance of sustainable practices in chemistry and develop critical evaluation, reflection, and decision-making skills. Education that combines green chemistry and critical thinking skills can help create a more environmentally conscious generation that can face future challenges and contribute to sustainable development.

The development of students' critical thinking skills is considered an important step in achieving educational goals holistically, including learning goals with the independent curriculum in Indonesia (Pahrudin *et al.*, 2021). However, in reality, the development of students' critical thinking skills in Indonesia is still not optimal in terms of the role of teachers and supporting infrastructure (Khalid *et al.*, 2021). The learning paradigm does not emphasize students' ability to find out, formulate problems, think analytically, work together, and collaborate in solving problems. Critical thinking skills are very important for students because they enable them to solve problems (Basri *et al.*, 2019); and explain the reasons for solving problems (Shanta and Wells, 2020). Critical thinking will make students open-minded (Abrami *et al.*, 2015) and reflective thinking (Ennis, 2015).

Factors contributing to low critical thinking skills include the use of learning methods that do not actively involve students and emphasize memorization rather than investigation (Solihati and Hikmat, 2018). Direct teaching strategies, place the teacher as the focus (Sarwanto *et al.*, 2021). Students' self-efficacy in expressing ideas and low interest in learning (Hyytinen *et al.*, 2018). The low critical thinking skills of students in chemistry material were also reported by Purwanto *et al.* (2022). Furthermore, research indicates that Indonesian students' environmental sensitivity also requires significant improvement (Situmorang *et al.,* 2020).

Environmental sensitivity is an important aspect that needs to be studied because it plays a significant role in determining an individual's response to changes in environmental conditions. Research shows that environmental sensitivity affects how individuals process and respond to information from the environment (Pluess, 2015). In addition, it plays a vital role in shaping pro-environmental attitudes and behaviors. Individuals with high environmental sensitivity tend to be more aware of the impact of their actions on the environment and are more motivated to take responsible actions, such as recycling, saving energy, or reducing plastic use. Research shows that environmental sensitivity mediates the relationship between environmental knowledge and intention to behave pro-environmentally (Bala *et al.*, 2023). Environmental sensitivity can provide insights into developing more effective educational policies and intervention programs. By understanding the factors that influence environmental sensitivity, policymakers can design more focused and impactful educational programs, which will encourage future generations to be more actively involved in environmental protection (Kyriakopoulos *et al.*, 2020).

Efforts to overcome the low critical thinking skills and environmental sensitivity in students have been made, including by using a student-centered learning model that can provide meaningful learning experiences (Minan *et al.*, 2021). Chemistry learning is very possible by contextualizing the concepts being learned (Abdurrahman *et al.*, 2019). The PBL model was chosen as one of the learning models because it can improve student understanding and contribute to increasing environmental sensitivity (Hanifha *et al.*, 2023).

The PBL model is a student-centered learning model that provides meaningful learning experiences and produces products (Suradika *et al.*, 2023). PBL allows students to plan learning activities, carry out collaborative projects, and ultimately produce work products that can be presented. Experiences and concepts are built based on products produced in the project-based learning process. PBL is an innovative learning model that emphasizes creativity and problem-solving and provides students with opportunities to improve critical thinking and collaborative skills (Grossman *et al.*, 2019). PBL has been shown to increase student motivation and engagement if learning is integrated with the principles of GC (Mitarlis *et al.*, 2023). PBL, compared to conventional methods, is effective in increasing class participation and providing a real-world learning context (Almulla, 2020). Implementing PBL can improve students' abilities in terms of critical thinking, collaboration, environmental awareness, critical and creative thinking, social, emotional, collaborative, and problem-solving abilities (Mora *et al.*, 2020).

Critical thinking skills are not only applied in the learning process but can be reflected in students' environmental care attitudes. In the context of everyday life, critical thinking skills are needed to maintain environmental sustainability. The contextual meaning of critical thinking is how to maintain the environment so that it is useful for the present and the future (Amin, *et al.*, 2020).

This study analyzes the effect of implementing the GC-integrated PBL model on the critical thinking skills and environmental sensitivity of high school students. The main material of the study is the concept of chemical equilibrium by considering its characteristics related to everyday concepts. For example, the concept of dynamic equilibrium; factors that affect the chemical equilibrium system; the human blood equilibrium system related to blood pH regulation. This study has a novelty, namely integrating the principles of green chemistry into chemistry learning through teaching modules. This makes a significant contribution because it is very relevant to global issues, namely the issue of environmental quality which is currently a concern. To deal with this issue, of course, a positive attitude is needed from students including environmental sensitivity and critical thinking. Both are closely related because it is hoped that students who have high critical thinking skills will implement them in dealing with environmental problems. In Indonesia, several studies on PBL integrated with green chemistry in the learning process. However, there are still few that link it to students' environmental sensitivity (Amin *et al.,* 2020). Thus, it is very urgent to explore. On the other hand, the curriculum related to the application of green chemistry in schools is inadequate. Through this PBL, by inserting the habituation of attitudes related to the principles of GC, this is urgently built-in for students considering the phenomenon of shifting student characters including student insensitivity to the environment. This supports global efforts to promote sustainability (Sustainable Development Goals (SDGs) through the education or learning process.

2. Experimental methods

2.1. Method

The research design used was a comparative study of two independent groups. The design used was a Randomized post-test Control Group. The population of the study was four classes of students in grade XI F. The sample was taken randomly using the lottery technique, and two classes, XI F1 and XI F4 were selected, each with 36 students. The experimental class implemented the PBL model, while the control class implemented the discovery learning (DL) learning model. The instrument used was a structured essay test with seven items on the concept of chemical equilibrium. This instrument reveals critical thinking skills in the aspects of interpreting, analyzing, deducting, inferring, and evaluating. Content validation of the instrument device was carried out by two chemical education experts. Gregory (2007) stated that a content validity examination was carried out to test the accuracy of critical thinking ability indicators for the items that had been created. The data were analyzed using the independent sample t-test because the two samples were independent of each other. This test was chosen because based on the prerequisite test results, the normality test with Kolmogorov Smirnov showed that both experimental groups had normal distribution (the value of 0.2 is greater than the significance value of 0.05). The homogeneity test with Levene statistic obtained a significance value of 0.054, meaning that the variance of both groups was homogeneous.

The Environmental Sensitivity Questionnaire (ESQ) employed in this study is based on the instrument developed by Jusniar *et al.* (2023). The questionnaire comprises four indicators, totaling 25 items, and demonstrates a reliability coefficient of 0.792, indicating a high level of internal consistency. The four aspects assessed by the ESQ are:

1. Harmony of life and diversity
2. Environmental balance
3. Interdependence
4. Sustainability

Three of the twelve Green Chemistry Principles (GCP) are associated with these aspects:

- Preventing waste (GCP 1)
- Increasing energy efficiency (GCP 6)
- Minimizing the potential for accidents (GCP 12)

Responses to the ESQ were measured using a four-point Likert scale:

- Strongly Agree (SA)
- Agree (A)
- Disagree (DA)
- Strongly Disagree (SDA)

2.2. Research procedure

Pre-test: A pre-test was conducted to determine students' initial abilities before being given treatment.

Treatment: The experimental class was given project-based learning (PBL) integrated with Green Chemistry, while the control class received learning with the DL Model. Implementation of control and experimental class learning as in **Table 1** and **Table 2**.

Table 1. Control and experimental class learning syntax.

Integrated PBL Stages GC Principles	DL Stages
Basic questions (Integrating GC principles)	Stimulation
Design a plan for the project (Integrating GC principles)	Formulating problems
Develop a schedule	Collecting data
Monitor Progress	Processing and analyzing data
Present and test the design results (Integrating GC Principles)	Verifying
Evaluate the learning experience	Generalizing

Source: Elaborated by the authors.

Table 2. Synthesis of critical thinking skills from several theories.

Ennis (2015)	Krulik *et al*. (1995)	Facione (1990)	Synthesis Result
Providing simple explanations Building basic skills	Identifying and Interpreting information	Interpretation: clarifying meaning through categorization and translation	Interpretation
Summarizing	Analyzing information	Analysis: Identifying and examining	Analysis
Providing advanced explanations Organizing strategies and technique	Evaluating evidence and arguments	ideas, arguments, or procedures Self-regulation: Self-assessment and reflection	Inferences Deduction/Evaluation

Source: Elaborated by the authors.

The instruments used in this study to assess critical thinking ability (CTA) comprise five core components (Ennis, 2015):

Interpretation: involves the selection of relevant information or knowledge and the clarification of meanings within specific provisions, facts, and informational content.

Analysis: refers to the identification of the constituent elements of a situation and the examination of their interrelationships.

Inference: encompasses the synthesis of available evidence, the formulation of alternatives, and the drawing of logical conclusions.

Evaluation: pertains to the assessment of the credibility of information sources, the validity of claims, and the critical appraisal of arguments and assumptions.

Deduction: involves the formulation of reasoned judgments based on logical processes in which conclusions depend on sound deductive reasoning skills.

In the experimental class, the project designed in the form of posters is embedded with slogans that can accustom students to care and be sensitive to the environment. For example, the words "save energy"; "save our earth" and others. In the learning process, teachers instill habits that can have an impact on environmental sustainability. For example, in the initial motivation section, the teacher appeals to use drinking water tumblers to minimize plastic waste.

Post-test: After treatment, a CTA test is given to measure critical thinking abilities. Apart from that, students' environmental sensitivity questionnaires were also given after four learning meetings were completed.

2.3. Data analysis

The critical thinking ability data obtained were analyzed using parametric statistical tests, namely the Independent Sample t-test to compare the average between the experimental group using the Green Chemistry integrated PBL model and the control group with the DL model. The normality test was carried out using the Kolmogorov-Smirnov method, and the homogeneity test was carried out using Levene Statistic to ensure that the data was normally distributed and the variance between groups was homogeneous.

3. Results and discussion

The instrument is tested for validity and reliability before use. Testing of content validity and construct validity is carried out and assessed by experts (validators). Content validity refers to the extent to which an instrument or test covers representatively all aspects of the domain to be measured. Meanwhile, construct validity involves the extent to which the instrument measures the intended construct or concept following the underlying theory or conceptualization. The results of the validity test by experts (two chemistry education experts and 2 practitioners) concluded that the critical thinking skills test instrument used met the criteria for being very valid, with an average inter-rater consistency of 97.6%. Thus, it can be used to measure students' critical thinking skills. Apart from the validity test, a reliability test was also carried out using internal consistency via Cronbach's Alpha. This test aims to assess the extent to which a test can provide consistent and reliable results. Reliability measures how well a measurement instrument can produce stable or consistent scores if measurements are taken repeatedly. The reliability testing technique on this CTA instrument test obtained was 0.68, which indicates that the reliability of the CTA instrument is quite good. This reliability value is considered sufficient with a moderate and quite reliable level (Creswell, 2000). The results of the study are presented sequentially, namely the CTA test prerequisite test, the Hypothesis test to determine the effect of the PBL model on students' CTA, and the hypothesis test to determine the effect of implementing the PBL model on environmental sensitivity.

3.1. CTA prerequisite test results

The results of the analysis requirement test consist of a normality test and a homogeneity test. The results of the normality test are shown in Table 3.

Table 3. Results of normality test.

Class	sig	Mean	SD
Control	0.200	67.89	8.23
Experiment (PBL)	0.200	75.39	5.95

Source: Elaborated by the authors.

Based on the data in the table above, the sig value (2-tailed) is 0.200, because sig > 0.05, we do not have enough evidence to reject the null hypothesis. Therefore, we can assume that the data from the student's critical thinking ability test results are normally distributed. With the results of this normality test, data analysis can be continued using parametric statistical tests, in this case, the independent t-test to compare the averages between different groups. Homogeneity testing as a prerequisite test is then carried out using Levene statistics. The test results obtained a significance value of 0.054, meaning that the variance of the two groups is homogeneous.

3.2. Hypothesis test results

The calculation results for the hypothesis test can be seen in Table 4.

Table 4. Independent sample t-test.

CTA PBL-DL	F	sig	t	df	Sig.	Mean difference	SE
Equal variance assume	3.833	0.054	4.430	70	0.000	7.50	1.69
Equal variance not assume			4.430	63.71	0.000	7.50	1.69

Source: Elaborated by the authors.

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Based on the table of t-test results above, it can be concluded that there is an influence of the PBL model on students' critical thinking skills in chemistry subjects. This is proven by the probability value that gets a score below 0.05 and the average value of the critical thinking test score in the class using the PBL model is significantly different compared to the control class using the DL model.

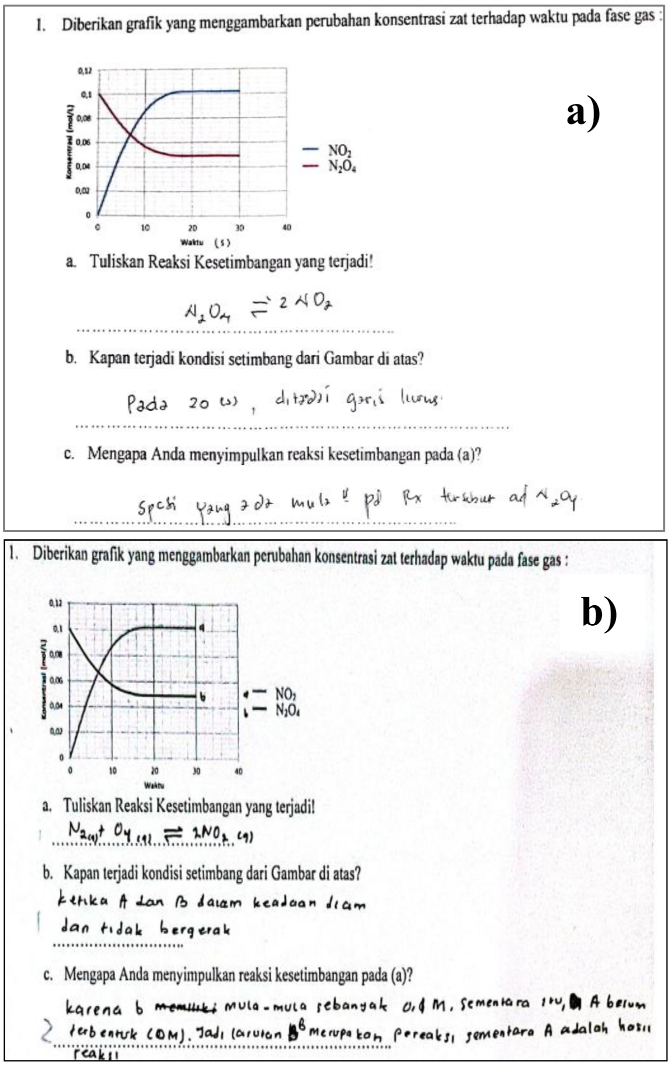

Figure 1. Documentation of Student Answers to Question No. 1. **(a)** correct answer **(b)** wrong answer.
Source: Elaborated by the authors.

Data interpretation is an important process that involves analyzing, organizing, and understanding data to draw meaningful conclusions and make informed decisions. The answer shown in **Fig. 1a** shows that the student was able to interpret the graph that the initial species N_2O_4 and NO_2 are equilibrium products. Equilibrium conditions can be determined at 20 minutes with the characteristic that the graph is flat, meaning that there is no more change over time. The interpretation for the answer to point c is that the equilibrium reaction occurs because macroscopically, there is no more change, but microscopically, the reaction is still ongoing; only the rate towards the formation of NO_2 gas is the same as the rate of decomposition back into N_2O_4 gas. This includes extracting important information, identifying patterns and understanding the implications of the data. Through this process, individuals and organizations can gain valuable insights, identify opportunities, detect potential risks, and evaluate the effectiveness of strategies or interventions.

Data interpretation is not only a technical process but also a social process because it requires access to experiences that enable the use of all meaning-making features (Zagallo *et al.*, 2016). In the picture above on the right, you can see the students' ability to interpret and analyze data in the form of a graph of the equilibrium reaction given. This thinking ability is a key aspect of critical thinking. From the answers given, not only can we see the students' ability to understand the concept of chemical equilibrium but also their ability to analyze information, solve problems, interpret information carefully, and make logical conclusions. In contrast to the student's answer shown in the picture on the left, it can be seen that the student was unable to write the reaction correctly and was unable to describe and interpret the graph related to the conditions for equilibrium to occur. Several studies have shown that students who do not have a good level of scientific reasoning may have difficulty with tasks such as data interpretation and presentation (Coll *et al.*, 2006; Hartmann *et al.*, 2015).

The interpretation of the answer on the right is not entirely correct for point (c) when students are asked to conclude why the reaction at time (b) is categorized as an equilibrium reaction. The correct answer should be that in the sixth minute of the reaction, the reaction rate towards the product (SO3) is the same as the reaction rate of the formation of the reactants (SO_2 and O_2). The ability to draw conclusions is an important component of critical thinking. In the context of critical thinking, inference refers to the ability to draw logical conclusions based on available information and evidence.

This skill is essential for evaluating arguments, making decisions, and solving problems. Several studies have highlighted the importance of inference as one of the main indicators of critical thinking skills. For example, a study that emphasizes the importance of inference as one of the five indicators of critical thinking skills, alongside interpretation, analysis, explanation, and evaluation (Sutama *et al.*, 2022). Likewise, this study identifies inference as one of the six indicators of critical thinking skills, along with interpretation, analysis, evaluation, explanation, and self-regulation. Furthermore, it underlines the inclusion of inference as part of the indicators of critical thinking skills, along with the ability to analyze, evaluate, and make decisions (Hall and Barnes, 2016).

These findings collectively highlight the consensus on the importance of inference as a fundamental aspect of critical thinking skills. From **Fig. 2a**, the evaluation and inference skills of students can be seen. From the answers given, it can be seen that students can understand concepts well and connect various concepts and ideas to create strong arguments. It is also seen that students have good analytical and interpretation skills, indicating that students' critical thinking skills are well-honed. This is different from **Fig. 2b**, where students' evaluation and inference skills are lacking. Several studies have shown that students' poor evaluation and inference skills can be caused by various factors, including cognitive aspects, lack of problem-solving skills related to scientific reasoning skills, poor memory, integration failure, and inability to integrate mathematical constructions into scientific content (Coleman *et al.*, 2023).

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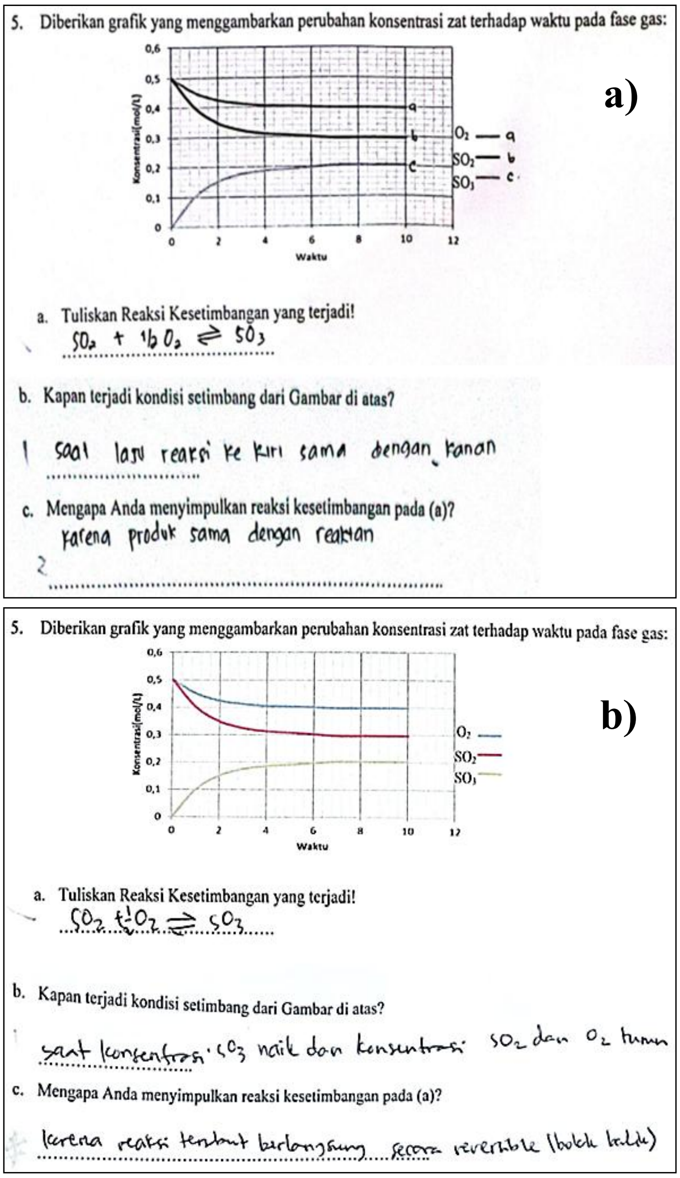

Figure 2. Documentation of Student Answers to Question No. 5. **(a)** correct answer and **(b)** wrong answer.
Source: Elaborated by the authors.

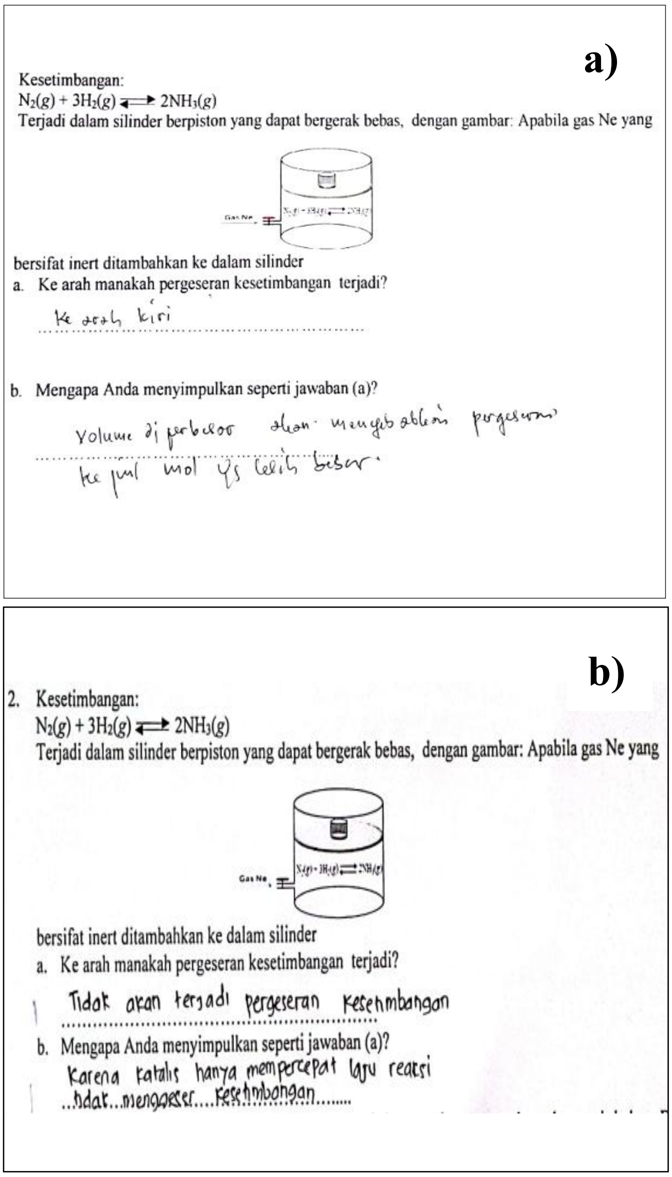

Figure 3. Documentation of Student Answers to Question No. 2. **(a)** correct answer and **(b)** wrong answer.
Source: Elaborated by the authors.

In **Fig. 3a**, students' ability to interpret and analyze data in the form of a given equilibrium reaction graph can be seen. This thinking ability is a key aspect of critical thinking. From the answers given, not only can students' ability to understand the concept of chemical equilibrium be seen, but also their ability to analyze information, solve problems, interpret information carefully, and make logical conclusions. In addition to interpretation skills, students' inference skills can also be seen in the students' answers above. It is proven that students can make logical conclusions based on existing information and understanding related to the concept of chemical equilibrium. Connecting various information and evidence that then underlies decision-making is an integral part of critical thinking skills.

Figure 3b shows students' lacking abilities in terms of interpretation, inference, and customary analysis. Several source indicate that poor data interpretation and analysis by students can be caused by various factors, including lack of strategic knowledge, differences in text or information processing, test anxiety, and the impact of internal and external factors on students' academic performance. as well as scientific research skills (Pols *et al.*, 2021; Wu *et al.*, 2022).

The effectiveness of the implementation of the PBL model can also be proven through documentation of student work results after completing the critical thinking ability test. From the documentation results above, we can see the answer patterns that show their ability to analyze information, link different relevant concepts, and formulate argumentative thinking or problem-solving. The image above also shows students' ability to organize and communicate their ideas clearly and structure.

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Through this documentation, teachers can use it as a very useful evaluation tool to detail and describe students' achievements in developing their critical thinking skills during a certain learning period.

The results above prove the effectiveness of the PBL model in improving students' critical thinking skills. Students who receive learning using the PBL model, their learning patterns tend to make students think more broadly. This is inseparable from the advantages of the PBL model, which hones creative thinking skills, self-regulated learning skills, and self-evaluation (Jansson *et al.*, 2015).

Project learning involves investigation and problem-solving. By assigning projects, students are trained to analyze, synthesize, and critically evaluate a problem so that the peak of the activity is that students can create work (Sutama *et al.*, 2022). Project-based learning makes learning more independent, improves critical thinking and analytical skills, and increases learning interest (Cortázar *et al.*, 2021). The PBL model also fosters collaboration, engages and motivates students, enhances content knowledge, and meets the needs of students with different skills and learning styles, thereby improving student engagement and chemistry learning outcomes (Mebert *et al.*, 2020; Domenici, 2022).

In addition, PBL has been shown to have a positive influence on the development of soft skills and environmental awareness in students, indicating its holistic impact on students' professional and personal growth (López and Palacios, 2024). Furthermore, integrating micro-project-based learning in chemistry has been shown to improve conceptual understanding and essential learning skills across disciplines, highlighting the interdisciplinary benefits of PBL (Tian *et al.*, 2023).

The Green Chemistry integrated PBL model not only provides students with a better understanding of chemical concepts but also broadens their horizons about Green Chemistry and its principles. This model supports sustainable development goals through education (Mitarlis *et al.*, 2023). This article makes a major contribution to improving critical thinking skills and forming environmental sensitivity characters for students who certainly take part in the goals of sustainable development.

3.3. Environmental sensitivity

The Wilcoxon test for Overall and each of the four aspects of environmental sensitivity are presented in Table 5.

Table 5. Environmental sensitivity test results.

Test Results	Environmental Sensitivity	Aspect 1 (Harmony of life and diversity)	Aspect 2 (Environmental balance)	Aspect 3 (Interdependence)	Aspect 4 (Sustainability)
Z	−4.800	−4.128	−4.234	−2.762	−3.976
Sig. (2-tailed)	0.000	0.000	0.000	0.006	0.000

Source: Elaborated by the authors.

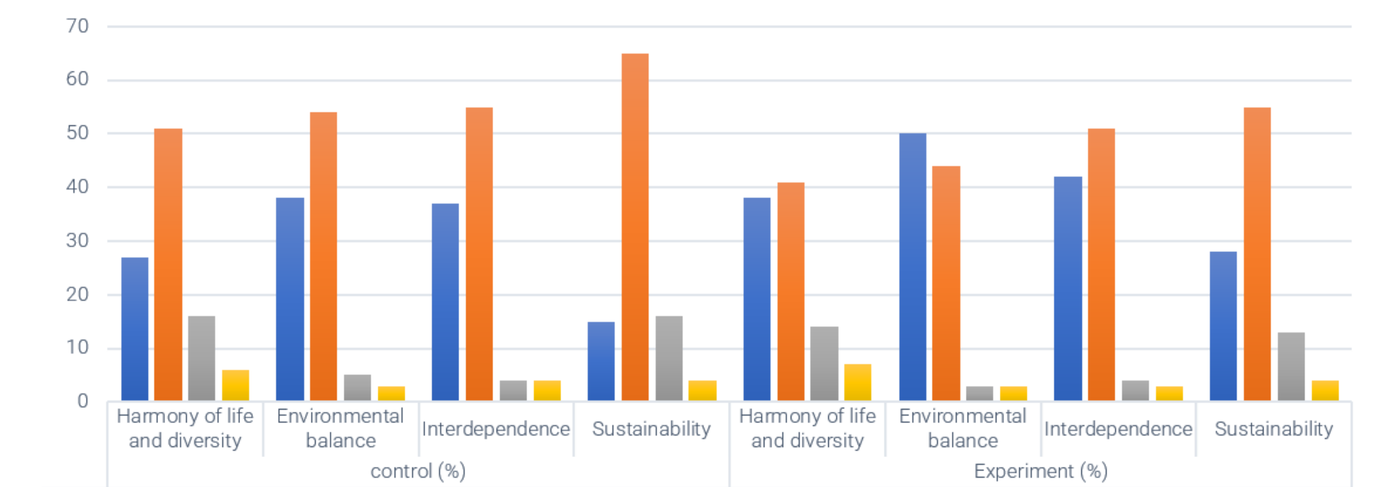
The diagram in Fig. 4 shows the results of a survey or study comparing two groups of students, the control group and the experimental group, using Project Based Learning integrated with Green Chemistry. Some aspects measured in this environmental sensitivity survey include Harmony of Life, Diversity, Environmental Balance, Interdependence, and Sustainability measured using a Likert scale, namely Strongly Agree (SA), Agree (A), Disagree (DA), and Strongly Disagree (SDA). In the aspect of harmony of life and diversity in the experimental class, there was a significant increase in the categories of 'Strongly Agree' (SA) and 'Agree' (A) for this aspect, with values reaching 38% and 54% respectively for "Harmony of Life," and 50% and 44% for "Diversity." This shows that students involved in PBL integrated with Green Chemistry have a better understanding of the importance of harmony in life and environmental diversity. This finding is in line with research conducted by Amin *et al.* (2022) where the PBL model integrated with the Green Chemistry vision not only improves their conceptual understanding of chemistry but also strengthens their awareness of the need for harmony between human activities and environmental sustainability. Learning activities such as observing and taking real actions, especially in the surrounding environment, can increase environmental sensitivity and student involvement in environmental problems that occur (Amin *et al.*, 2022; Hanifha *et al.*, 2023).

In contrast, in the control group, the percentage of 'Strongly Agree' (SS) only reached 27% for "Harmony of Life" and 38% for "Diversity," indicating a lower understanding of these concepts compared to the experimental group. The increase in understanding in the experimental group was due to students gaining direct experience in environmental-based projects that were relevant to real contexts, which is a characteristic of the PBL model.

In the aspect of environmental balance and interdependence, the 'Agree' (S) response in the experimental group for "Environmental Balance" reached 55%, higher than the control group, which was only 51%. This increase reflects that project-based learning allows students to better understand the interdependence between humans and the environment and the importance of maintaining ecological balance. The increase in the percentage of 'Strongly Agree' and 'Agree' in the experimental group also shows that students are becoming more aware of the importance of sustainable behavior and how individual actions can affect the balance of the ecosystem as a whole.

In the aspect of "Sustainability," there was an increase in environmental awareness in the experimental group, with 42% of students answering, 'Strongly Agree' and 51% answering 'Agree'. In contrast, the control group only had 28% who answered, 'Strongly Agree' and 55% who answered 'Agree'. This indicates that the integration of Green Chemistry in PBL can improve students' understanding of sustainable development goals (SDGs) and the importance of protecting the environment for future generations.

This finding is in line with Mitarlis *et al.* (2023), which shows that the integration of green chemistry principles in basic chemistry learning can support the achievement of SDGs. This approach has been shown to contribute to students' understanding of the concept of sustainability and has a positive impact on students' behavior in protecting the environment (Amin *et al.*, 2020). The results of the study showed that the experimental group involved in PBL integrated with Green Chemistry was better able to understand and apply critical concepts related to the environment, such as harmony of life, diversity, environmental balance, and sustainability. This is because PBL requires students to think critically, analyze problems, evaluate various options, and find innovative, data-based solutions to real environmental challenges. In line with these findings, Issa and Khataibeh (2021) stated that PBL could not only improve conceptual understanding and critical thinking but can also make students creative and communicative, dare to make decisions and have learning independence. The integration of Green Chemistry in PBL allows students to learn through direct experience, which increases their sensitivity to the environmental impacts of various human activities. The data in the diagram shows that students who learn through PBL have more positive responses to aspects of sustainability, indicating increased sensitivity to environmental issues.



	control (%)				Experiment (%)			
	Harmony of life and diversity	Environmental balance	Interdependence	Sustainability	Harmony of life and diversity	Environmental balance	Interdependence	Sustainability
SS	27	38	37	15	38	50	42	28
S	51	54	55	65	41	44	51	55
TS	16	5	4	16	14	3	4	13
STS	6	3	4	4	7	3	3	4

Figure 4. Description of environmental sensitivity in the control and experimental classes.
Source: Elaborated by the authors.

3.4. Research limitations

This research was conducted limitedly in one school so that other researchers could continue it on a larger scale. The project-based learning model applies the habituation method to integrate GC principles into learning. Further researchers can apply other learning that integrates green chemistry to socialize and support sustainability through education. Further researchers can explore the same thing at various levels of education or integrate technology to support the GC-integrated PBL learning model.

4. Conclusions

In this research, it was found that there was an influence of implementing the PBL model integrated with GC principles on critical thinking skills and environmental sensitivity. These two aspects of ability and character of environmental sensitivity are very important in dealing with global problems related to the environment in the world. Environmental conditions that are not good certainly need to receive serious attention in various fields, especially the education sector, to support sustainability. Thus, it is recommended for teachers and policymakers to always integrate green chemistry into learning and the curriculum to support sustainability.

Authors' contribution

Conceptualization: Jusniar Jusniar; Dewiyanti Fadly; **Data curation**: Army Auliah; Jusniar Jusniar; **Formal Analysis**: Jusniar Jusniar; Army Auliah; **Funding acquisition**: Jusniar Jusniar; Syamsidah Syamsidah; **Investigation**: Jusniar Jusniar; Dewiyanty Fadly; **Methodology**: Syamsidah Syamsidah; Jusniar Jusniar; **Project administration**: Jusniar Jusniar; Dewiyanti Fadly; **Resources**: Jusniar Jusniar; Army Auliah; **Software**: Not applicable. **Supervision**: Jusniar Jusniar; Syamsidah Syamsidah; **Validation**: Jusniar Jusniar; Army Auliah; **Visualization**: Dewiyanti Fadly; **Writing – original draft**: Jusniar Jusniar; Dewiyanti Fadly; **Writing – review & editing**: Jusniar Jusniar; Dewiyanti Fadly.

Data availability statement

The data will be available upon request.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Short Review

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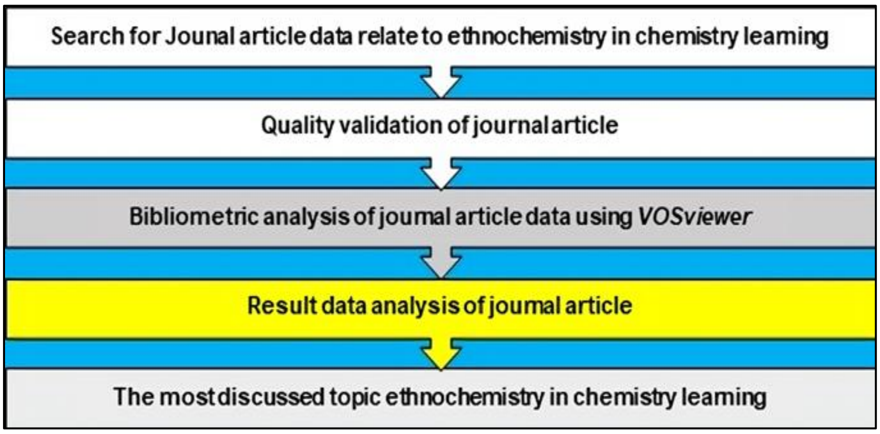
Bibliometric analysis: most discussed topics ethnochemistry in chemistry learning

Resty **Utami**[1+], Trining Puji **Astutik**[2]

Abstract

Ethnochemistry, integrating local culture with chemistry education, enhances students' comprehension by relating lessons to everyday life. This study utilizes bibliometric analysis to identify prevalent topics in ethnochemistry research within chemistry learning from February 2018 to January 2023. Using Publish or Perish (PoP) software, Google Scholar, and the VOSviewer application, 31 journal articles were analyzed to reveal key themes and trends. The findings indicate a strong association of ethnochemistry with terms like "guidelines," "development," "implementation," "local wisdom," "ability achieved," and "learning process". Ethnochemistry has shown significant potential to make chemistry learning more engaging and relevant by linking abstract concepts to cultural and everyday contexts. However, the integration of ethnochemistry into curricula is still limited, and practical applications in modern education remain underexplored. This study highlights the importance of developing strategies to incorporate ethnochemistry into teaching and research, fostering critical thinking, scientific literacy, and cultural appreciation among students. The findings offer a framework for future studies, encouraging innovative approaches that bridge science education with cultural heritage to create more meaningful learning experiences.



CONTENTS

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Highlights

- Bibliometric analysis is to map and identify research trends in ethnochemistry.
- Ethnochemistry integrates local culture with chemistry education.
- This enhances students' understanding and makes learning contextual and meaningful.
- VOSviewer visualizes data relationships, revealing key points in ethnochemistry.
- These are important in chemistry learning and their evolution between 2018-2023.

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1. Introduction

Ethnoscience is an interdisciplinary field that connects cultural anthropology with scientific study. It focuses on understanding scientific knowledge by exploring the local knowledge embedded in the culture of a society or ethnic group (Dewi *et al.*, 2019; 2021). According to Sutrisno *et al.* (2020, p. 7833) "ethno refers to group members in cultural environments identified by cultural traditions, codes, symbols, and myths to consider and conclude concepts". Authentic scientific knowledge encompasses the facts and understandings within a society. This knowledge is passed down through generations, often in an informal, unstructured, and non-systematic manner, reflecting people's perceptions of natural phenomena (Dewi *et al.*, 2019).

Ethnochemistry is part of ethnoscience and views a culture based on a chemistry perspective involving cross-disciplines of social sciences and humanities. Ethnochemistry studies chemical materials from a local cultural perspective (Rahmawati *et al.*, 2018a), both chemistry shapes culture and culture contribute to chemistry and its changes (Gani *et al.*, 2022) and ethnochemistry can be applied to the learning process (Seprianto and Hasibuan, 2021). Implementation of ethnochemistry in the learning process either from strategies, models, learning resources or scientific investigations can develop critical thinking skills, scientific attitudes, and scientific processes and improve students' cognitive, affective and psychomotor abilities (Wahyudiati and Qurniati, 2023). Students would prefer learning if the topics studied were related to everyday life (Zidny and Eilks, 2020; Abumchukwu *et al.*, 2021), have conducted research in the Onitsha Education Zone, Anambra State University in Nigeria, and received the data showing implementation of ethnochemical learning methodologies can considerably boost students' understanding of concepts and learning outcomes.

Irawati and Sofianto (2019) explained that the application of ethnochemistry in learning is carried out through the utilization of local cultural or traditional products through the learning process or included in learning resources. The application of ethnochemistry in learning is carried out through the integration stage, and students' understanding of chemical concepts becomes more meaningful. Wahyudiati (2022a) explained that the application of ethnochemistry in learning can help students formulate research problems and prove hypotheses so that chemistry learning objectives can be achieved optimally. Azizah and Premono (2021) stated that the integration in question is a reciprocal relationship between culture and chemistry so that learning becomes more contextual.

Heliawati *et al.* (2022) and Wahyudiati (2022a; 2022b) show that the implementation of ethnochemistry in the learning process can improve students' understanding of chemical concepts. Despite its potential, there are still significant gaps in this field. Ehnochemistry has not been widely integrated into the learning process, and there is a lack of comprehensive studies that analyze research trends, popular topics, and their practical relevance to modern education. Additionally, few studies have explored effective ways to incorporate ethnochemistry into chemistry curricula to make learning more meaningful and connected to real-life contexts. Addressing these gaps is essential to fully realizing ethnochemistry's potential to improve chemistry education. Attempts at the relevance of ethnochemistry in the chemistry learning process are still not widely carried out to increase the relevance it is necessary to analyze the development of literature related to ethnochemistry in chemistry learning. Increasing relevance will result in new ways of overcoming the problem of conveying chemistry concepts in future learning (Fadli, 2019; Sutrisno *et al.*, 2020). Literature analysis can help researchers in the field of ethnochemistry find research topics that have been widely researched in the last 5 years as an effort to improve the quality of learning.

Bibliometric analysis research is a measurement using a mathematical and statistical approach to analyze certain literature to help VOSviewer application (Nandiyanto and Husaeni, 2021). The VOSviewer application was chosen because it can display network data mapping visualizations, interesting analysis and investigations (Widiawati *et al.*, 2022). Article data searches can be carried out with the help of Publish or Perish (PoP) software because PoP can reach various metadata such as Google Scholar, Scopus, Web of Science, and others.

Ethnochemistry and *etnokimia* are the main keywords in the literature search (journal article data). Journal articles with ethnochemical topics in chemistry learning used in the analysis were selected based on several criteria, namely: **(1)** Literature in the form of journal articles where the contents include title, author, year, journal, keywords, abstract, and bibliography; **(2)** The publication deadline is at least the last 5 years with a timeframe from February 2018 – January 2023; **(3)** The literature discusses ethnochemistry/*etnokimia* in chemistry learning; **(4)** Literature is presented in easy-to-understand Indonesian and/or English grammar. Journal article data is relevant to the research topic and can be used if it meets all the specified criteria. Researchers can identify research mapping based on keywords (co-occurrence) to find out topics that are often discussed in research. Previous research on bibliometric analysis was carried out by Kamdem *et al.*, (2019) and Wati *et al.* (2021) to find out the trend of a study on the Scopus and Google Scholar databases.

Based on the description above, the importance of learning chemistry with local culture makes it easier for students to understand the concept of the material being taught. Efforts to make ethnochemistry relevant in the learning process have not been widely carried out, and analysis of literature development is needed to determine research opportunities and topics. Bibliometric analysis is a measurement with a mathematical and statistical approach to analyzing literature carried out with the help of the VOSviewer application, because it can display data mapping visualizations, interesting analysis and investigations. The purpose of this study is to analyze scientific articles that examine ethnochemistry that have been published from February 2018 – January 2023 on reputable scientific platforms.

2. Research methodology

This research uses descriptive qualitative research with a bibliometric approach to convey the results of review of journal articles. The data search method was carried out using PoP software with the main Google Scholar database and analyzed using the VOSviewer application (Rahayu *et al.*, 2022). This research was adapted from (Hudha *et al.*, 2020) through 5 stages (**Fig. 1**):

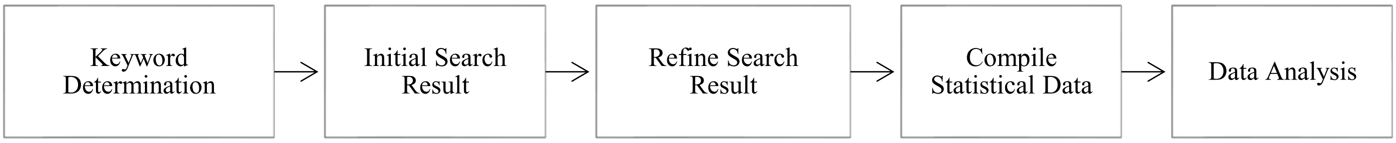

Figure 1. Research Stages.
Source: Adapted by the author using data from Hudha *et al.* (2020).

A systematic literature review on ethnochemistry was conducted in January 2023 using the keywords "ethnochemistry" and "*etnokimia*". The process involved five key stages. First, keyword determination was carried out using Google Scholar, supported by PoP software, to search for journal articles published between February 2018 and January 2023, yielding 43 articles stored in RIS format, containing essential bibliographic information. Second, the initial search results were refined by filtering data based on study topics and ensuring only journal articles were included, which were then transferred to Mendeley for enhancement. Third, a thorough validation process was conducted to check the completeness and quality of the articles, ensuring the inclusion of relevant and high-quality literature, resulting in a final dataset of 31 articles. Fourth, statistical data compilation was performed in Mendeley to ensure all bibliographic details, such as publication year, volume, and page numbers, were complete and accurate. Finally, bibliometric network analysis and visualization were conducted using VOSviewer software, enabling the creation of network maps that revealed literature clusters, historical connections, and potential research opportunities within the field. This structured approach ensured a focused and reliable analysis of the topic.

Data collection used a purposive method; the data were selected based on the special characteristics determined by the researcher Faizah *et al.* (2021) so that data relevant to the research topic was obtained. Research object data can be seen in **Fig. 2**. The process of data analysis for bibliometric analysis in research uses co-occurrence analysis as a description of the conceptual structure or knowledge from the literature and analysis based on keywords to see the development of research and the development of visualized data. Briefly, bibliometric data analysis techniques can be seen in the following flowchart in **Fig. 3**.

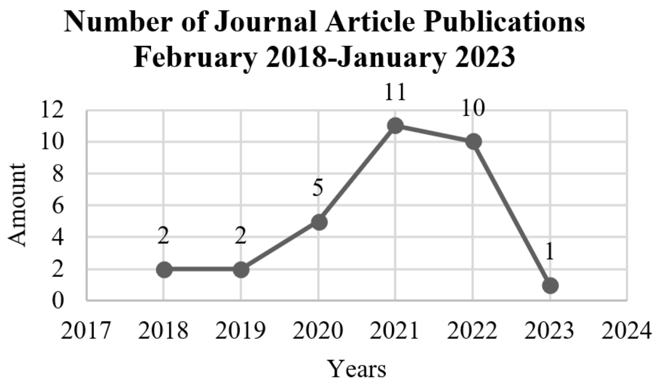

Figure 2. Journal article publication data February 2018 – January 2023.
Source: Elaborated by the authors.

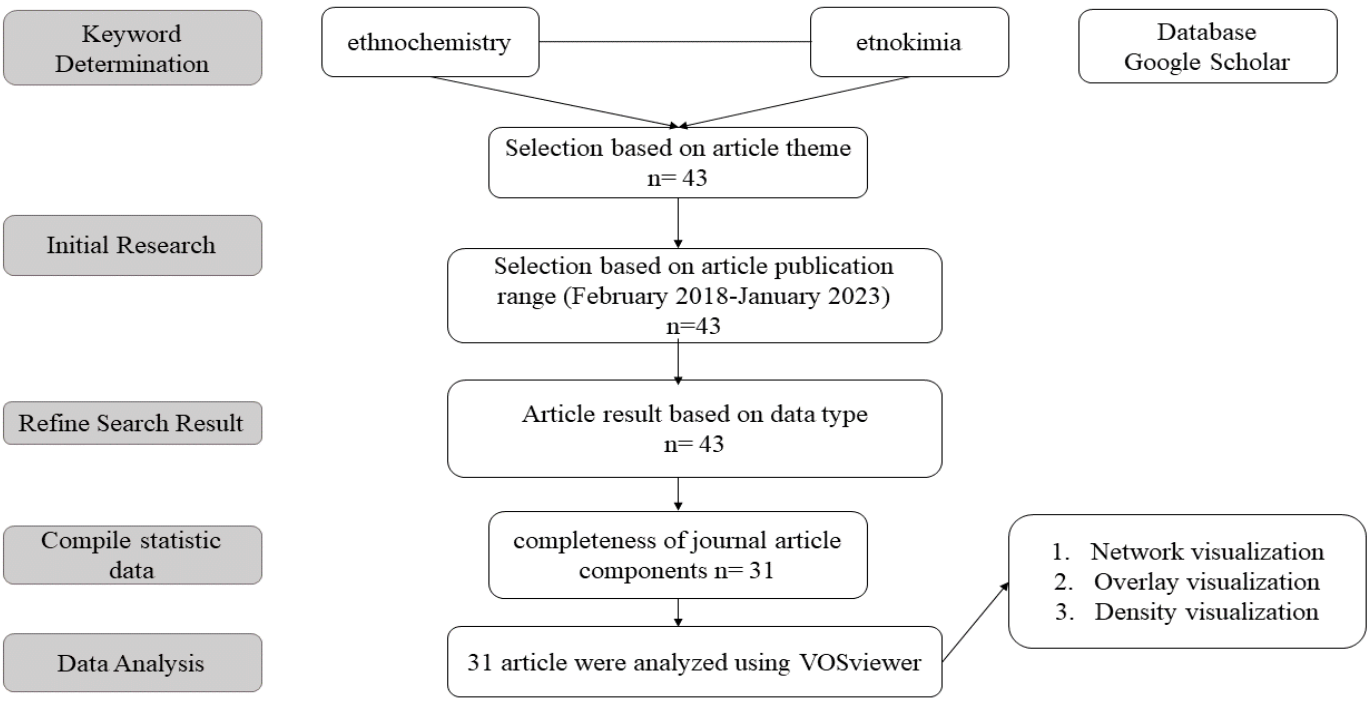

Figure 3. Data analysis technique.
Source: Elaborated by the authors.

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3. Results and discussion

3.1. General information and growth trends

Using Publish or Perish to search for journal articles from the Google Scholar database, 43 relevant articles were initially identified. These articles were then processed using the Mendeley application to complete missing information and undergo quality validation, resulting in 31 articles deemed useful and relevant to the research topic (**Table 1**). Validity was ensured by applying predefined inclusion and exclusion criteria, with articles classified by two chemistry educators. Overlay visualization was used to show the frequency of topics mentioned in research over a specific period, represented by color gradients.

So, it is known that the development of ethnochemistry research trends from 2018 to 2023 is developing well, where publications increased quite significantly in 2021 by 11 articles. There was a decrease in 2022 to 10 articles. As for the publication in 2023 of at least 1 article, this is due to limited data collection in January. Based on these data, it shows that research on ethnochemistry has become a popular topic among chemistry education researchers despite fluctuations in interest over the past 5 years and must be further developed through continuous research and publication.

Table 1. An article relevant to the research topic.

No	Reference	Source	Citations
1	Asmaningrum *et al.* (2018)	Jurnal Tadris Kimiya	17
2	Rahmawati *et al.* (2019)	Journal Of Physics: Conference Series	1
3	Rahmawati *et al.* (2018b)	International Conference of Chemistry	9
4	Jofrishal and Seprianto (2020)	Jurnal IPA dan Pembelajaran IPA	4
5	Hidayatussani *et al.* (2020)	Chemistry Education Practice	5
6	Ugwu (2018)	Journal of the Nigerian Academy of Education	2
7	Zidny and Eilks (2020)	Sustainable Chemistry and Pharmacy	28
8	Sutrisno *et al.* (2020)	Universal Journal of Educational Research	26
9	Riza *et al.* (2020)	Jurnal Pendidikan IPA Veteran	6
10	Zidny and Eilks (2021)	International Joint Conference on Science and Engineering	0
11	Seprianto *et al.* (2021)	Antlantis Press	0
12	Azizah and Premono (2021)	Journal of Tropical Chemistry Research and Education	7
13	Robo *et al.* (2021)	Jurnal Ilmiah Wahana Pendidikan	1
14	Arif *et al.* (2021)	Jurnal Ilmiah Wahana Pendidikan	1
15	Wahyudiati (2021a)	Jurnal Pendidikan Kimia Indonesia	10
16	Wahyudiati (2021b)	SPIN Jurnal Kimia & Pendidikan Kimia	4
17	Abumchukwu *et al.* (2021)	African Journal of Science, Technology & Mathematics	1
18	Seprianto and Hasibuan (2021)	Budapest International Research and Critics Institute	1
19	Asmaningrum *et al.* (2021)	Atlantis Press	2
20	Konyefa and Okigbo (2021)	International Journal of Education and Evaluation	2
21	Zahro *et al.* (2022)	International Conference on Science, Education and Technology	0
22	Wahyudiati (2022a)	Journal of Xi'an Shiyou University, Natural Science Edition	2
23	Heliawati *et al.* (2022)	Jurnal Pendidikan IPA Indonesia	2
24	Wahyudiati (2022b)	Jurnal Pendididkan Kimia Indonesia	0
25	Wahyudiati (2022c)	Jurnal Tarbiyatuna	6
26	Irfandi (2022)	Proceeding of International Conference on Science and Technology	0
27	Wahyudiati (2022d)	Journal of Xi'an Shiyou University, Natural Science Edition	0
28	Mudau and Tawanda (2022)	International e-Journal of Education Studies	0

Source: Elaborated by the authors.

3.2. Keywords and terms analysis

Journal article data was then analyzed using the VOSviewer application to be able to visualize and map bibliometric network data. The results of data analysis are in the form of a network visualization map display (**Fig. 4a**), an overlay visualization map display (**Fig. 4b**) and a density visualization map display (**Fig. 4c**). The data analyzed using VOSviewer generated an overlay visualization map (**Fig. 4b**) with 12 clusters, 111 links and 45 items (topics/terms). Ethnochemistry is the topic with the most links, namely 39.

Overlay visualization can represent the level of frequency of a topic mentioned in research throughout a specific period based on color levels. Ethnochemistry as the main topic has as many as 39 related links with the highest total link strength of 52 with an occurrence of 21. The brighter the color of the map area (yellow) the fewer items/topics are studied. Ethnochemistry is related to other keywords (**Fig. 4d**). According to Afandi *et al.* (2022) research in mapping research trends, "the brighter the color of the node, the more recent the topic is studied in research".

Figure 4b shows that old topics that are often discussed in a study are based on bibliometric analysis using VOSviewer. For example, in **Fig. 5a**, you can see the relationship between the topics of 'ethnochemistry' and 'guide' with purple nodes, which is one of the most discussed topic combinations in 2018. **Figure 5b** 'ethnochemistry' and 'development' with dark blue nodes because it is one of the most discussed topic combinations around 2019. **Figure 5c** 'ethnochemistry' and 'chemistry' with blue to dark green nodes because it is one of the most discussed topic combinations around 2020. **Figure 5d** 'ethnochemistry' with 'local wisdom' with nodes green because it is one of the most discussed topic combinations around 2021. **Figure 5e** 'ethnochemistry' with 'gender' with the node color light green because it is one of the most discussed topic combinations around 2022. **Figure 5f** 'ethnochemistry' with 'chemistry learning' with node yellow color because it is one of the most discussed topic combinations in around 2023.

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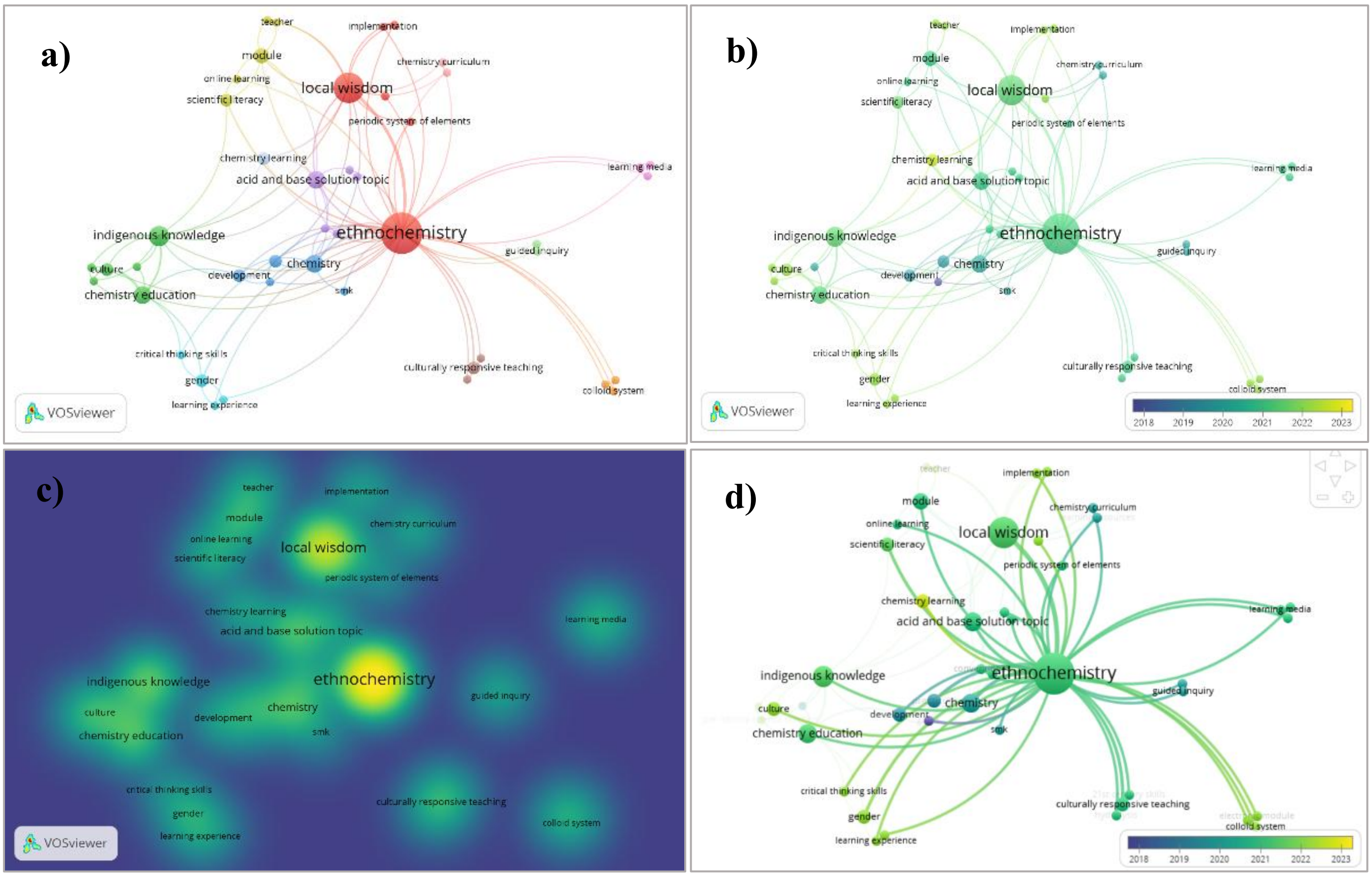

Figure 4. VOSviewer Data result. **(a)** Network visualization; **(b)** Overlay visualization; **(c)** Density visualization and **(d)** Topic related to ethnochemistry.
Source: Elaborated by the author using data from VOSviewer application (2023).

The latest topics that are often discussed in a study based on bibliometric analysis using VOSviewer are contained in color items light green and yellow: *student scientific attitudes, learning experience, gender, critical thinking skills, pre-service science teachers, chemistry metacognition, culture, training, teacher, implementation, islamic education concept, problem-solving, electronic module, colloid system, scientific literacy, teaching material* and *chemistry learning* which are widely researched in 2022 to 2023.

Ethnochemistry is intrinsically linked to local wisdom and indigenous knowledge because it fundamentally involves studying chemical materials from a local cultural perspective. Local wisdom is built upon the traditional knowledge of communities within specific regions. Contextual chemistry content is easier to learn when an ethnochemistry approach is applied. Students are better able to construct knowledge when they have prior knowledge or experiences to build upon. Ethnochemistry helps students understand chemical concepts by integrating local cultural knowledge with chemical principles. For example, traditional remedies like *jamu brotowali*, *jamu kunyit asam*, and *bunga bougainville* can be used as acid-base indicators (Riza *et al.*, 2020). By connecting science with cultural contexts, ethnochemistry fosters a deeper understanding of chemistry and enhances students' critical thinking and problem-solving skills.

Ethnochemistry exploration in the community environment is carried out first to identify which cultures are related to the chemical material to be taught (Azizah and Premono, 2021; Irfandi, 2022). Through the exploration carried out, it can provide convenience for researchers and teachers who will include ethnochemical topics in the learning process. For instance, in creating batik, the dye dissolution process demonstrates the application of chemical bonding principles. The batik dye itself can serve as a natural acid-base indicator, while other related chemical concepts such as moles, redox reactions, and compound nomenclature are also involved (Azizah and Premono, 2021). Several studies have shown that ethnochemically integrated learning can develop 21st-century skills in students (Arif *et al.*, 2021; Robo *et al.*, 2021).

The implementation of ethnochemistry in the chemistry learning process can improve critical thinking skills, learning experience, chemistry metacognition, problem-solving and scientific literacy of students (Heliawati *et al.*, 2022; Wahyudiati, 2024; 2022d). Efforts to improve students' abilities are carried out with the help of learning media, some media developed are integrated with ethnochemistry in the form of Adobe Flash Learning Media (Heliawati *et al.*, 2022), modules (Jofrishal and Seprianto, 2020; Riza *et al.*, 2020; Sanova *et al.*, 2022; Seprianto *et al.*, 2021; Zahroh Asna *et al.*, 2024) or other media such as creating vlog designs as chemistry learning media (Asmaningrum *et al.*, 2021). In its implementation, ethnochemistry is often combined with learning models such as PjBL, Inquiry and others (Asmaningrum *et al.*, 2021; Hidayatussani *et al.*, 2020) and combined with a learning approach such as culturally responsive teaching (CRT) (Arif *et al.*, 2021; Robo *et al.*, 2021; Wardani *et al.*, 2023; 2024) and Culturally Responsive Transformative Teaching (CRTT) (Najid *et al.*, 2021; Rahmawati *et al.*, 2018, 2023) according to the learning topic.

The application of ethnochemistry-based chemistry education can be influenced by gender, as seen in the research conducted by Wahyudiati (2023; 2022a) which indicates that female students tend to have higher critical thinking skills and more positive scientific attitudes compared to male students. This difference can be attributed to female students exhibiting greater motivation, perseverance, and self-confidence in their academic tasks, which directly enhances their critical thinking abilities. Furthermore, female students in the study were found to have a more positive attitude toward learning, especially in chemistry, when it is linked to real-life cultural contexts, such as the integration of ethnochemistry. These factors collectively contribute to their superior performance in critical thinking tasks compared to their male counterparts (the relationship between gender and ethnochemistry can be seen in **Fig. 5e**). Despite its potential, ethnochemistry is not widely integrated into current curricula, and there is a lack of comprehensive studies exploring its practical application in modern education. Addressing these gaps is crucial for leveraging ethnochemistry to improve chemistry learning outcomes. This study provides a new perspective on the development of ethnochemistry research, offering a foundation for future studies to explore effective methods for incorporating cultural contexts into chemistry education.

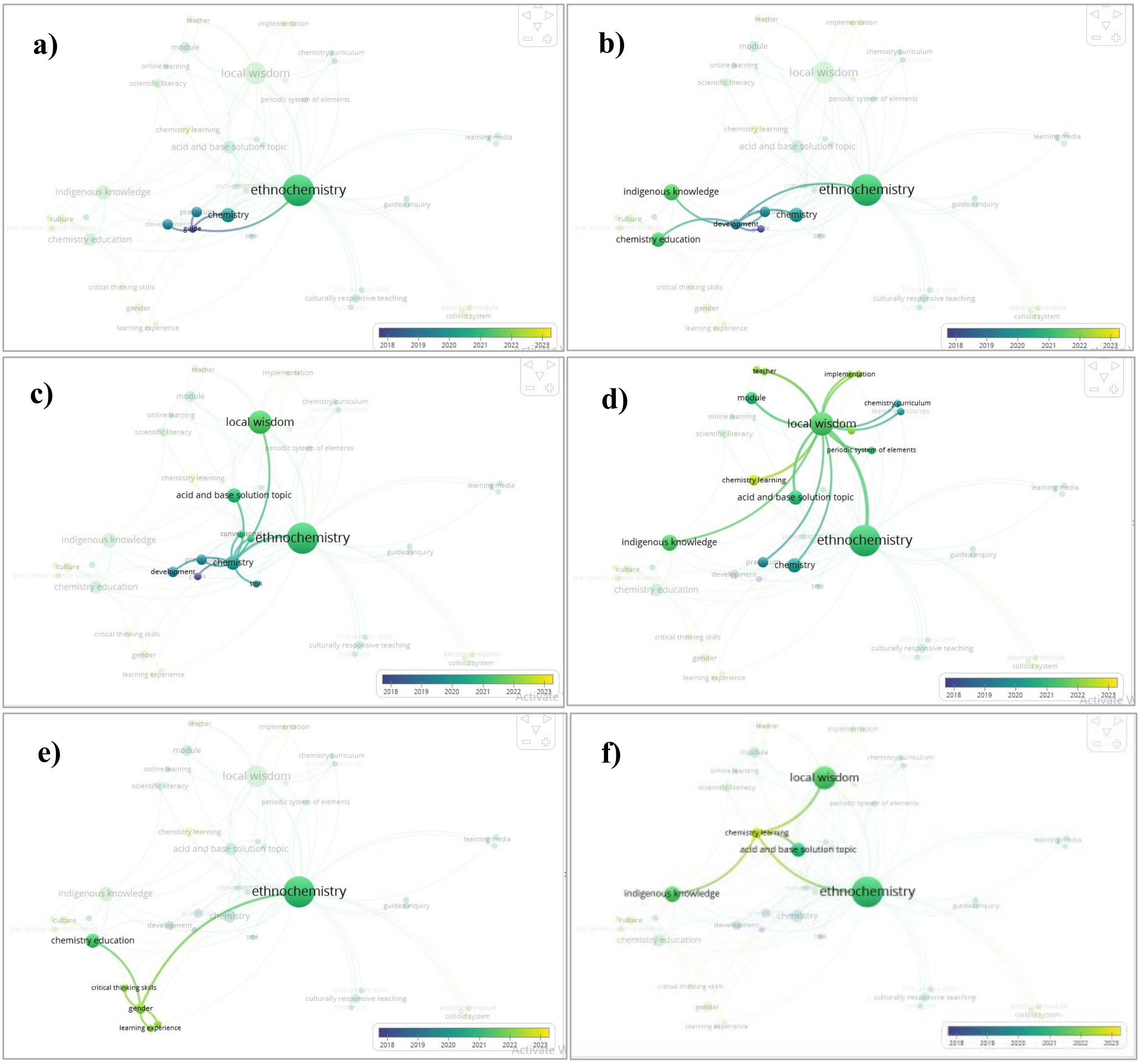

Figure 5. VOSviewer Data Result. **(a)** The Relationship Between Topic "Ethnochemistry" and "Guide"; **(b)** The Relationship Between Topic "Ethnochemistry" and "Development"; **(c)** The Relationship Between Topic "Ethnochemistry" and "Chemistry"; **(d)** The Relationship Between Topic "Ethnochemistry" and "Gender"; **(e)** The Relationship Between Topic "Ethnochemistry" and "Gender"; **(f)** The Relationship Between Topic "Ethnochemistry" and "Chemistry Learning".
Source: Elaborated by the author using data from VOSviewer application (2023).

5. Conclusions

The bibliometric analysis of 31 journal articles reveals that ethnochemistry is a frequently discussed topic within chemistry education. The research indicates that the integration of local culture into chemistry lessons significantly enhances students' understanding and engagement. Key terms associated with ethnochemistry in the article published from January 2018 to February 2023 include "guidelines", "development", "implementation", "local wisdom", "ability achieved," and "learning process". Despite its potential, ethnochemistry is not widely integrated into current curricula, and there is a lack of comprehensive studies exploring its practical application in modern education. Addressing these gaps is crucial for leveraging ethnochemistry to improve chemistry learning outcomes. By integrating local cultural contexts into chemistry learning, this approach enhances students' understanding of abstract concepts through relatable, everyday experiences. The bibliometric analysis highlights key research trends and identifies gaps, such as practical strategies for incorporating ethnochemistry into curricula. These findings contribute to teaching and research by offering innovative methods to make chemistry education more engaging and meaningful while promoting cultural preservation. This integration has the potential to improve critical thinking, scientific literacy, and 21st-century skills among students, fostering a more inclusive and contextually relevant approach to science education.

Supplementary material

Please visit **https://app.vosviewer.com/** and add the file from the link **https://revista.iq.unesp.br/ojs/index.php/ecletica/libraryFiles/downloadPublic/57** for visualization data from VOSviewer application

Authors' contribution

Conceptualization: Resty Utami; Trining Puji Astutik; **Data curation**: Trining Puji Astutik; Resty Utami; **Formal Analysis**: Resty Utami; Trining Puji Astutik; **Funding acquisition**: Not applicable; **Investigation**: Resty Utami; **Methodology**: Resty Utami; **Project administration**: Resty Utami; Trining Puji Astutik; **Resources**: Not applicable; **Software**: Resty Utami; **Supervision**: Trining Puji Astutik; **Validation**: Resty Utami; Trining Puji Astutik; **Visualization**: Resty Utami; **Writing – original draft**: Resty Utami; Trining Puji Astutik; **Writing – review & editing**: Resty Utami.

Data availability statement

The data will be available upon request.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

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Theoretical bio-investigation of 3-(benzo[d]thiazol-2-yl)-2-(substituted aryl)thiazolidin-4-one derivatives as potential *Mycobacterium tuberculosis* H37Rv inhibitors

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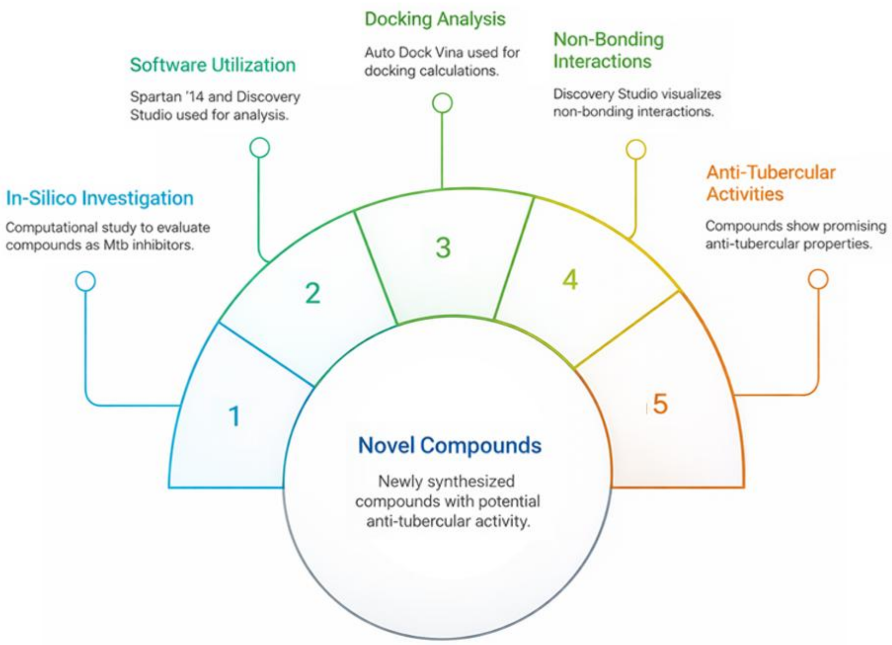
Abstract

3-(Benzo[d]thiazol-2-yl)-2-((substituted aryl) thiazolidin-4-one derivatives were recently synthesized with thioglycolic acid and evaluated for *in vitro* anti-tubercular activity. Two of the derivatives have good anti-tubercular activity. The present study evaluated an in-silico investigation of these ten novel compounds as potential *Mycobacterium tuberculosis* H37Rv inhibitors. The non-bonding interactions between the derivatives and the receptor were studied. Spartan '14 software was used for optimization. Discovery Studio software was used for the receptor treatment. The binding site in the downloaded protein was located using Autodock Tool software. Auto Dock Vina was used to calculate the docking, and Discovery Studio was used to view the non-bonding interactions between the docked complexes. Different other parameters were calculated to describe anti-tubercular activities of 3-(benzo[d]thiazol-2-yl)-2-(substituted aryl)thiazolidin-4-one derivatives. The findings demonstrated the potential anti-tubercular properties of all the substances under study and inhibited *Mycobacterium tuberculosis* (H37Rv). The calculated binding affinity of the docked compound showed improved inhibition against *Mycobacterium tuberculosis* (H37Rv) better than the standard drugs (Streptomycin and Pyrazinamide), with compound 6 being the best.



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Keywords

1. *Mycobacterium*
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4. docking
5. thiazolidin-4-one.

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Highlights

- Ten 3-(benzo[d]thiazol-2-yl)-2-((substituted aryl) thiazolidin-4-one was docked.
- The compounds were docked with a protein (PDB ID: 5zhv).
- The potential anti-tubercular properties of all docked substances were studied.
- All the docked compounds have significant anti-tubercular activities.
- Compound 6 activities inhibit *Mycobacterium tuberculosis* (H37RV) the most.

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1. Introduction

Tuberculosis (TB) has been described as a leading cause of mortality and a substantial cause of ill health (WHO, 2021). According to Trajman *et al.* (2022), TB has surpassed HIV/AIDS as the most common infectious disease that caused mortality before the coronavirus (COVID-19) pandemic. *Mycobacterium tuberculosis* is an infectious agent that causes tuberculosis, an illness primarily affecting the lungs (Moule and Cirillo, 2020). When infected individuals sneeze, spit or cough (Swalehe and Obeagu, 2024) the virus disperses through aerosolized particles. The illness has a significant worldwide economic and health impact. As one of the oldest infectious illnesses that may affect humans, research on the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex is ongoing worldwide (WHO, 2022). The development of therapy regimens has shown progress in the ongoing struggle to prevent and control TB (Dookie *et al.*, 2002). The worldwide health threat of *Mycobacterium tuberculosis* infections persists despite a lengthy history and ongoing efforts (Chandra *et al.*, 2022). Since its creation precisely a century ago, the Bacillus Calmette-Guérin (BCG) vaccine has been the most widely used globally and remains the sole approved treatment for tuberculosis. Tuberculosis is still quite high in areas where vaccination campaigns are widespread (Setiabudiawan *et al.*, 2022).

Human illnesses are caused by about 50 different mycobacterial species. The *Mycobacteriaceae* family contains *M. tuberculosis*, *M. canettii*, *M. africanum*, and other members that can infect other animal species with illness. All bacteria in the *Mycobacteriaceae* family have lipid-rich cell walls that provide them resistance to chemotherapeutic drugs but not to physical ones like heat and UV light (Behr *et al.*, 2019; Lerner *et al.*, 2020; Menzies *et al.*, 2018; WHO, 2021). Complex lipids make up almost 60% of the mycobacterium bulk, which makes it extremely hydrophobic. The strongly cross-linked peptidoglycan (PG) layer enveloping the plasma membrane forms covalent compounds with arabinogalactan. To drastically lower the number of new cases each year research breakthroughs are required, such as new medications, drugs and vaccines.

The prevalence of antibiotic-resistant, multidrug and extensively drug-resistant (MDR and XDR) strains as well as the co-occurrence of the HIV and diabetes epidemics, raise the risk of TB and make it an extreme concern (Ferrer *et al.*, 2009; Moule and Cirillo, 2020). The pathogen's capacity to produce cords and the bacteria's propensity for mutation have boosted both the pathogen's virulence and medication resistance (Behr *et al.*, 2019; Chandra *et al.*, 2022; Dookie *et al.*, 2002; Ferrer *et al.*, 2009; Lerner *et al.*, 2020; Menzies *et al.*, 2018; Setiabudiawan *et al.*, 2022; WHO, 2021). Apart from the respiratory complications of the disease, extrapulmonary TB and drug-resistant forms have emerged due to the changeable nature of the causative agent. Approximately 25% of the world's population currently has latent or active TB, with 1.2 million fatalities and 10 million new infections per year (WHO, 2022). A small percentage of tuberculosis cases, about 15%, take the extrapulmonary infections form, which can spread through organ or blood transfusions and affect organs other than lungs, such as the lymph nodes, kidneys, brain, bones, and joints. Such conditions are reportedly very challenging to identify and cure, and they spread quickly through organ transplants (Moule and Cirillo, 2020). However, its adverse effects are more obvious in middle-class and lower-class countries (WHO. Global Tuberculosis, 2021). The United Nation is trying to reduce TB fatalities by 90% by 2030, making the disease one of the key issues of the Sustainable Development Goals (SDGs).

An estimated 590,000 new cases of TB are reported each year in Nigeria, of which over 245,000 are HIV-positive. In Nigeria, more than 10% of fatalities are attributable to tuberculosis. Although there are effective treatments for the disease, around 30 people die from it every hour (Haider *et al.*, 2022). According to the data, around 181.1 million people were living in Nigeria as of 2015; however, six years later (2021), that number rose to 213, translating to a 17.8% rise. The prevalence of TB is rising along with the country's population. For instance, Nigeria, with its estimated 4.3% multidrug resistance in new cases, has the sixth-largest worldwide incidence of TB. Later research reveals that Nigeria rose from sixth to seventh place globally and to second place in Africa relative to 30 nations with a high TB burden (Fadare *et al.*, 2020; Umeokonkwo *et al.*, 2020).

A 1,3-thiazole ring is fused to a benzene ring to form the heterocyclic aromatic chemical class known as benzothiazoles (BTs). Drug developers are interested in benzothiazoles because of their extensive biological and pharmacological actions (Froes *et al.*, 2021). Benzothiazole (BTH), 2-hydroxy-BTH, 2-amino-BTH, 2-methyl benzothiazole (2-Me-BTH), 2-methylthio-BTH (2-Me-S-BTH), 2-mercapto-BTH (2-SH-BTH), 2-thiocyanomethylthio-BTH (2-SCNMeS-BTH), and 2-benzothiazole-sulfonic acid (2-SO_3H-BTH) are among the most frequently studied BHTs (Froes *et al.*, 2021; Shainyan *et al.*, 2022; Stremski *et al.*, 2021; Zhilitskaya *et al.*, 2021). Additionally, benzothiazoles are used as the main component of many different medications, including Zopolrestat and Viozan. The biological activities of benzothiazole derivatives include anticancer (Fadare *et al.*, 2020; Moule and Cirillo, 2020), antibacterial, tubercular (Haider *et al.*, 2022; Taghour *et al.*, 2022), antifungal (Haider *et al.*, 2021), antimicrobial (Levshin *et al.*, 2022), anti-inflammatory (Nasr *et al.*, 2022), antidiabetic (Dernovšek *et al.*, 2021), anticancer, analgesic, anticonvulsant, antimalarial, and anti-inflammatory properties (Dernovšek *et al.*, 2021; Kumar *et al.*, 2017), among others. Many different medicinal compounds are made using the benzothiazole nucleus. Due to these substances' exceptional pharmacological potentials, medicinal chemistry has a key function for them. Benzothiazole derivatives: production, structures, and biological activity have long been of interest to researchers in the medical field (Kumar *et al.*, 2017; Sucheta and Verma, 2017). There are recognized natural sources for BHTs, and tea leaves, and tobacco smoke are natural sources of BTH (Moodley *et al.*, 2022).

Mainly because of the function of benzothiazole in medicinal chemistry, where this moiety offers a more stable metabolic replacement for carboxylic acid functions as well as a more complimentary pharmacokinetic profile, interest in benzothiazole chemistry has been growing rapidly in recent decades. Excellent biological, pharmacological, and therapeutic applications can be achieved when the heterocyclic benzothiazole and thiazolidinedione are combined in a single molecular framework (Aziz, M. *et al.*, 2022a; Aziz, N. *et al.*, 2022b; Salina *et al.*, 2022; Shainyan *et al.*, 2022; Trotsko *et al.*, 2020; Zhilitskaya and Yarosh, 2021). The synthesis of benzothiazole derivatives has been performed using a few different synthetic routes. Condensation of 2-aminobenzenethiol with cyano or carbonyl is the most used technique for creating benzothiazole compounds (Lončarić *et al.*, 2022).

In drug development, docking is used as a screening method to find substances with higher inhibitory affinities. Molecular docking studies have been linked to several difficulties, including the flexibility of proteins and the protein-protein interface. This is because it can improve the interaction between any investigated pharmacophore and enzyme by locating the appropriate binding sites inside the investigated enzyme (receptor)

(Bahrami *et al.*, 2008). Additionally, the docking score aids in predicting the strength of the non-covalent interactions that occur between complexes after docking, the computed binding affinity between the investigated complexes might be given as a dock score (Abdullahi *et al.*, 2020). Furthermore, it reveals the position of the ligand in the active region of the receptor, which is crucial for optimization (Olasupo *et al.*, 2021; Oladipo *et al.*, 2021; Oyebamiji *et al.*, 2021). To design and develop a trustworthy anti-*Mycobacterium tuberculosis* (H37Rv) agent, it is expedient to investigate the roles of the derivatives attached to the parent compounds studied as potential anti-*Mycobacterium tuberculosis* (H37Rv) agents with efficient biological activities. This is the focus of this research.

2. Theoretical methodology

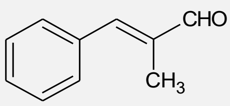
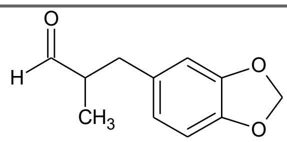
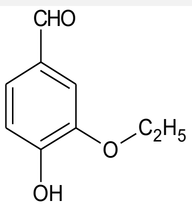
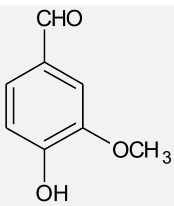
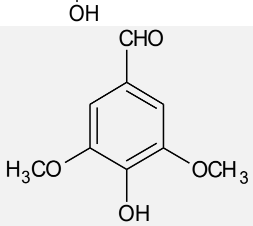
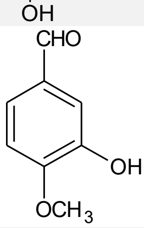
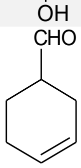
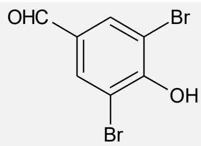
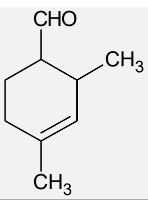
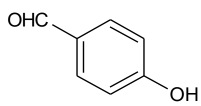
2.1. Studies on docking and optimization

Two-dimensional (2D) structures of ten derivatives of 3-(benzo[d]thiazol-2-yl)-2-(substituted aryl)thiazolidin-4-one (Table 1) were sketched using ChemDraw Professional version 16.0 (Olujinmi *et al.*, 2024; Oyebamiji *et al.*, 2024). Spartan '14 software was used to open the structures and save them as Spartan documents. The setting '6-31G* (B3LYP)' was used as the basis set to express the exchange correlation in the Density Functional Theory (DFT) energy equation. DFT was used to optimize all the derivatives (Oyebamiji *et al.*, 2024). The optimization generated descriptors that describe mycobacterium tuberculosis (H37RV) activities. In this work, a series of molecular descriptors were calculated, and eleven electronic descriptors were selected and used to describe the biological activities of the study compounds.

Protein with PDB ID: 5zhv was downloaded from the PDB bank, treated (cleaned) using Discovery Studio 2019 software (which was also used for viewing the non-bonding interaction between the docked complexes) and was resaved as '.pdbqt' file using Autodock tool software. The grid boxes (dimension and center) were centered (X = –11.899, Y = –6.275, Z = –17.836) and size (X = 30, Y = 30, Z = 30). The spacing was set to be 1.00 Å. The docking calculations (including binding affinities) were achieved using Autodock Vina (command tool) (Trott and Olson, 2010).

Molecular docking of the optimized compounds with the cleaned protein was carried out to calculate binding affinity for individual studied compounds and to observe the non-bonding interaction existing between the complexes. The Autodock Tool was also used for locating binding sites in the downloaded protein and for converting ligands and receptors to '.pdbqt' format from '.pdb' format (Olujinmi *et al.*, 2024).

Table 1. 2-Dimensional structures and IUPAC name of the studied compounds.

S/N	Ar	Molecular Formular/Name	S/N	Ar	Molecular Formular/Name
1		$C_{19}H_{16}N_2OS_2$ (2*E*)-2-methyl-3-phenylprop-2-enal	6		$C_{20}H_{18}N_2O_3S_2$ 3-(1,3-benzodioxol-5-yl)-2-methylpropanal
2		$C_{18}H_{16}N_2O_3S_2$ 3-ethoxy-4-hydroxybenzaldehyde	7		$C_{17}H_{14}N_2O_3S_2$ 4-hydroxy-3-methoxybenzaldehyde
3		$C_{18}H_{16}N_2O_4S_2$ 4-hydroxy-3,5-dimethoxybenzaldehyde	8		$C_{17}H_{14}N_2O_3S_2$ 3-hydroxy-4-methoxybenzaldehyde
4		$C_{16}H_{16}N_2OS_2$ cyclohex-3-ene-1-carbaldehyde	9		$C_{16}H_{10}Br_2N_2O_2S_2$ 3,5-dibromo-4-hydroxybenzaldehyde
5		$C_{18}H_{20}N_2OS_2$ 2,4-dimethylcyclohex-3-ene-1-carbaldehyde	10		$C_{16}H_{12}N_2O_2S_2$ 4-hydroxybenzaldehyde

Source: Elaborated by the authors.

3. Results and discussion

3.1. Molecular descriptors

The calculated molecular descriptors obtained from optimized studied compounds are the highest occupied molecular orbital energy (E_{HOMO}) and the lowest unoccupied molecular orbital energy (E_{LUMO}). The lowest energy orbital that is empty is denoted by LUMO, whereas the highest energy orbital that contains electrons is represented by HOMO. The HOMO-LUMO gap is the energy differential between HOMO and LUMO. A molecule's capacity to take and give electrons is correlated with its LUMO energy. A smaller HOMO-LUMO gap makes molecules more reactive, whereas a greater gap makes molecules more stable. The HOMO-LUMO energies can be used to anticipate how a chemical will interact with biological targets such as proteins or deoxyribonucleic acid (DNA), since many drug-target interactions entail electron transfer or sharing. Additionally, HOMO-LUMO energies have been linked to lipophilicity, which influences a drug's capacity to cross Bruch's membrane in terms of membrane permeability and lipophilicity.

Molecular parameters such as polarizability, dipole moment, hydrogen bond acceptor (HBA), energy band gap, area, volume, polar surface area (PSA), partition coefficient (log P), and hydrogen bond donor (HBD) are calculated and presented in **Table 2**. Higher membrane permeability, which can enhance drug absorption, is frequently correlated with higher polarizability (Tian *et al.*, 2021). On the other hand, very high polarizability could result in nonspecific binding and adverse effects. The dipole moment influences a molecule's solubility and protein binding, which are essential for therapeutic distribution and efficacy, as well as how well it interacts with polar environments like water and proteins. According to Lipinski's Rule of Five (Oyebamiji *et al.,* 2025), an excess of HBAs can impair membrane permeability and be detrimental to drug-target interactions and solubility.

Table 2. Calculated molecular parameters from 3-(benzo[d]thiazol-2-yl)-2-(substituted aryl)thiazolidin-4-one derivatives.

Compound	E_{HOMO}	E_{LUMO}	BG	MW/amu	log P	Vol/Å^3	PSA	Pol	HBD	HBA	NOR
1	−6.19	−1.27	−4.92	352.48	2.46	343.21	21.173	68.05	0	4	4
2	−5.68	−1.12	−4.56	372.47	0.05	346.51	44.272	68.41	1	6	4
3	−5.69	−1.19	−4.50	388.47	−1.27	355.24	50.223	69.13	1	7	4
4	−6.09	−1.15	−4.94	316.45	2.41	302.48	20.063	64.74	0	4	4
5	−6.11	−1.17	−4.94	344.50	2.91	338.81	19.133	67.69	0	4	4
6	−5.59	−1.17	−4.42	398.51	0.86	373.67	36.276	70.64	0	6	5
7	−5.69	−1.19	−4.50	388.47	−1.27	355.25	50.219	69.3	1	7	4
8	−5.98	−1.22	−4.76	358.44	−0.29	328.49	45.846	66.90	1	6	4
9	−6.26	−1.34	−4.92	486.21	0.96	336.96	37.831	67.55	1	5	4
10	−6.05	−1.15	−5.90	328.42	0.69	301.08	39.901	64.64	1	5	4
P	−7.24	−2.03	−5.21	123.12	−0.96	117.30	51.175	49.66	1	4	4
S	−5.90	−1.08	−4.82	581.58	−6.99	519.83	271.89	82.40	7	15	6

Note: **BG:** band gap, **MW:** molecular weight, **log P:** lipophilicity, **PSA:** polar surface area, **HBD:** hydrogen bond donor, **HBA:** hydrogen bond acceptor, **NOR:** number of rings, **P:** Pyrazinamide, **S:** Streptomycin.
Source: Elaborated by the authors.

The energy band gap is important for several drug classes, especially those that involve electron transport, and it can affect the stability and reactivity of a molecule. A compound's stability, reactivity, and capacity to engage in redox processes or function as a prodrug can all be impacted by its energy band gap.

A drug's ability to bind to target protein binding sites, as well as its ability to traverse membranes and distribute throughout the body, is influenced by its area and volume (Lawson *et al.*, 2021). The capacity of a drug to penetrate cell membranes is correlated with its PSA. Lower PSA frequently indicates better membrane permeability and oral bioavailability. Lipophilicity, which influences solubility, absorption, and distribution, is measured by the log P. For oral drugs, the ideal log P values are normally between 1 and 5 (Lipinski's Rule of Five). HBD is crucial for drug-target interactions and solubility, just as HBAs. Membrane permeability is limited to ≤ 5 by Lipinski's Rule of Five and can be decreased by excess HBDs.

The role of the dipole moment as one of the molecular non-bonded interactions in drug design cannot be over-emphasized (Lewis and Broughton, 2002; Shen *et al.*, 2010). As reported by Oyewole *et al.* (2020) and Oyebamiji *et al.* (2016), a molecular compound with a high dipole moment value is expected to have anomalous properties. As shown in **Table 2**, all the compounds studied have the potential to act as antifungal and antibacterial agents. 3-(1,3-benzodioxol-5-yl)-2-methyl propanal (Compound 6) showed the best ability to release electrons to the adjoining compounds, which are expected to translate into more inhibition than other derivatives, including the standard drugs. Also, the calculated E_{LUMO} obtained from the studied molecules reveal their tendency to receive electrons from the adjacent molecules (Abdul-Hammed *et al.*, 2020; Oyebamiji and Semire, 2020); thus, compound 9 possesses a higher ability to receive electrons from adjacent compounds than other studied ligands. This agrees with the *in vitro* assay (Bhoge *et al.*, 2024).

Other descriptors obtained are molecular weight, HBDs, HBA and log P; all these descriptors were found to fall within the standard range (molecular weight < 400 except for compound 9 (> 400), Streptomycin (> 500), HBD < 2, HBA ≤ 7 except for Streptomycin which is 15 and log P < 3). This showed that all the compounds studied possess drug potential.

3.2. Binding affinity

The degree of interaction between a ligand, like a drug molecule, and its target, usually a protein or receptor, is called binding affinity (Ren *et al.*, 2021). It measures the degree to which the ligand binds to its intended target.

It is commonly measured in molar concentrations (mol/L), with lower values indicating stronger binding. It is generally represented as the inhibition constant (Ki) or the dissociation constant (Kd). The ligand binds more firmly and remains bound for a longer period when it has a high binding affinity (low Kd or Ki). In this work, all the compounds studied show higher binding strengths (–24.3 to –31.0 kJ/mol) to inhibit *Mycobacterium tuberculosis* H37Rv better than Streptomycin (–20.9 kJ/mol) and Pyrazinamide (–16.3 kJ/mol) (which were used as standards), as shown in **Table 3**.

Since binding affinity frequently corresponds to a medicine's potency and efficacy, it is essential to drug research and influenced by several chemical interactions, including hydrophobic interactions, van der Waals forces, and hydrogen bonds.

Table 3. Calculated binding affinity.

Compound	Binding Affinity (kJ/mol)
1	−29.3
2	−25.1
3	−25.1
4	−25.5
5	−25.5
6	−31.0
7	−24.7
8	−27.6
9	−24.3
10	−25.1
Streptomycin	−20.9
Pyrazinamide	−16.3

Source: Elaborated by the authors.

3.3. Molecular docking results

The optimized compounds were subjected to docking studies (with a suitable receptor that has anti-tuberculosis inhibition activities (**Fig. 1**), via Discovery Studio, autodocking tool and autodocking vina software. Docking studies were carried out on the compounds studied to observe the non-bonding interaction present between 3-(benzo[d]thiazol-2-yl)-2-(substituted aryl) thiazolidin-4-one derivatives and a protein (PDB ID: 5zhv). According to Lewis and Broughton (2002), the compound with the lowest binding affinity value has the highest tendency to inhibit the target the most; thus, compound 6 (2D structure shown in **Fig. 2**) with –31.0 kJ/mol proved to be better than other studied compounds as an anti-tubercular agent, even than the standard drugs that were used.

The standard drugs i.e. Streptomycin and Pyrazinamide have a lower binding affinity compared to the binding affinity obtained for each of the studied 3-(benzo[d]thiazol-2-yl)-2-(substituted aryl)thiazolidin-4-one derivatives. The compounds under study showed different interactions with the protein studied (**Fig. 1**) and compound 6 (**Fig. 2**). The 2D structure of the docked 'compound 6' with receptor '5zhv' is displayed in **Fig. 3**.

4. Conclusions

The increasing knowledge of computational chemistry and its usage in the design and development of drugs and disease treatment has had a significant positive influence on mortality and morbidity globally. The study of anti-tuberculosis properties of 3-(benzo[d]thiazol-2-yl)-2-(substituted aryl)thiazolidin-4-one derivatives was observed in this work using density functional theory as well as molecular docking software (Autodock Tool, Auto dock vina and discovery studio). All the studied compounds can act as antifungal and antibacterial agents. The calculated E_{LUMO} obtained from the studied molecules reveals their tendency to receive electrons from the adjacent molecules, thus, compound 9 possesses a higher ability to receive electrons from adjacent compounds than other studied ligands. Other descriptors obtained are molecular weight, HBDs, HBA and log P; all these descriptors were found to fall within the standard range (molecular weight < 400 except for compound 9 (> 400) Streptomycin (> 500), HBD (< 2), HBA (≤ 7) except for Streptomycin which is 15 and log P (< 3)). This showed that all the compounds studied possess drug potential.

While all the docked compounds showed better anti-tubercular activities than the standard drugs, compound 6 was outstanding in its result. This *in silico* approach agrees with the *in vitro* assay earlier carried out by Bhoge *et al.* (2024). This research further strengthens the call that these derivatives could lead the path to the discovery of better anti-tubercular agents.

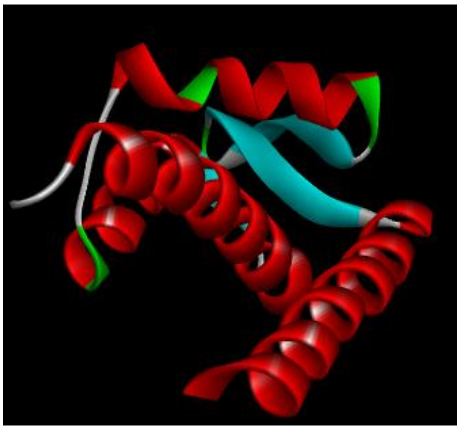


Figure 1. Receptor (5zhv) used for docking.
Source: Elaborated by the authors.

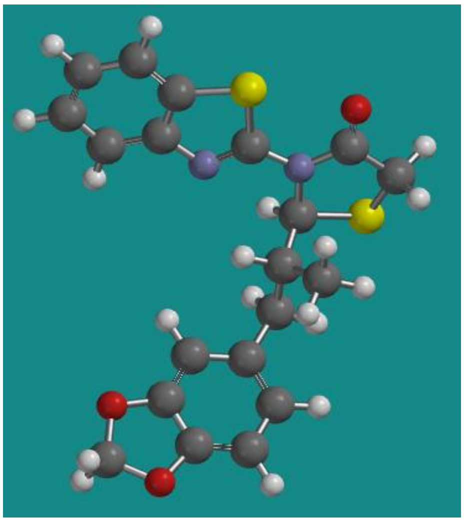


Figure 2. 2D structure of compound 6.
Source: Elaborated by the authors.

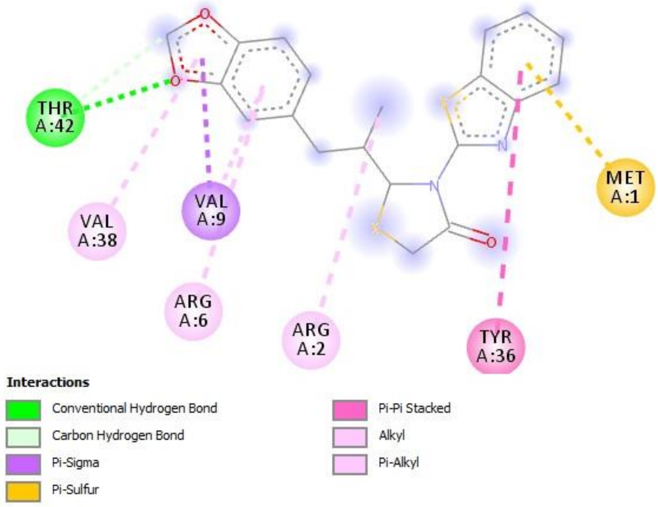


Figure 3. 2D structure of docked compound 6 with receptor 5zhv.
Source: Elaborated by the authors.

Authors' contribution

Conceptualization: Abel Kolawole Oyebamiji; **Data curation**: David Gbenga Oke; **Formal Analysis**: Faith Eniola Olujinmi; **Funding acquisition**: Juliana Oluwasayo Aworinde; **Investigation**: Olamide Adetunji Olalekan; **Methodology**: Abel Kolawole Oyebamiji, David Gbenga Oke; **Project administration**: Abel Kolawole Oyebamiji; **Resources**: David Gbenga Oke; **Software**: Abel Kolawole Oyebamiji; **Supervision**: Abel Kolawole Oyebamiji; **Validation**: Juliana Oluwasayo Aworinde; **Visualization**: Faith Eniola Olujinmi; Olamide Adetunji Olalekan; **Writing – original draft**: David Gbenga Oke; **Writing – review & editing**: David Gbenga Oke; Abel Kolawole Oyebamiji.

Data availability statement

All data sets were generated or analyzed in the current study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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