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In vitro and *in silico* evaluation of the antimicrobial potential of *Celtis zenkeri* roots volatile metabolites

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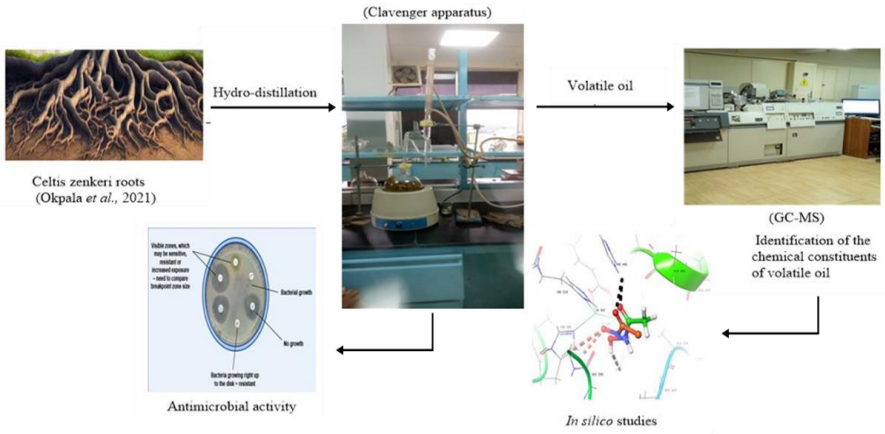
Abstract

This study is aimed at investigating the volatile constituents of the air-dried roots of *Celtis zenkeri*. The volatile oil was extracted using hydro-distillation method and characterised using gas chromatography-mass spectrometry (GC-MS). The volatile oil was screened against six selected bacteria and four fungi strains using the agar diffusion method. The molecular docking study of the identified compounds was conducted to investigate their binding pattern with the substrate and nucleotide complexes of *Enterococcus faecium* aminoglycoside-2''-phosphotransferase-IIa [APH(2'')-IIa] (PDB ID: 3HAM) and full-length Lanosterol 14 alpha-Demethylases of Prominent fungal pathogens *Candida albicans* (PDB ID: 5V5Z). The yield of the volatile oil (% w/w) root of *C. zenkeri* was 0.79%. Six compounds were identified in the root essential oil representing 80.07% of the volatile oil. 2-methyl-1-pentene (40.01%) was the most abundant compound in the root essential oil. The volatile oil from roots of the *C. zenkeri* exhibited good activity against all the screened bacteria and fungi strains at a concentration of 12.5-100 mg/mL when compared with Gentamicin for bacteria and Tioconazole for fungi.



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1. *Celtis zenkeri*;
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3. gas chromatography-mass spectrometry;
4. antimicrobial activity.

Section Editor

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Highlights

- The chemical components of *Celtis zenkeri* roots' volatile oil are first reported.
- The antimicrobial potential of the identified chemical constituents was evaluated.
- Molecular docking studies were made on the oil volatile identified compounds-STEM is a new model.

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1. Introduction

About 80% of the world's population, according to the World Health Organisation (WHO, 2008), primarily receives their primary healthcare from traditional medicine. Plant-based products are also a major component of the healthcare systems of the remaining 20% of the world's population, which is mostly found in wealthy nations. The 44th World Health Assembly adopted a resolution in 1991 that promoted the use of "traditional, safe, effective, and scientifically approved medicines" (Pamplona-Roger, 2014).

The WHO encourages the study of medicinal plants throughout all its geographic zones. WHO funds and organizes seminars on the development of drugs from medicinal plants to accomplish this. The African Union has independently established centres in Cairo, Dakar, Ile-Ife, Kampala, and Tananarive for the coordination of research on African medicinal plants. Furthermore, several African nations have contributed to the development of traditional medicine in distinctive ways (WHO, 2008). The Universal Education, Social, and Cultural Organisation (UNESCO) stated that one of the safest ways to guarantee complete health care for the world's rapidly growing population is through conventional treatments (UNESCO, 1994). In essence, chemicals derived from plant extracts have remained the focus of natural products.

As per the findings of Hamburger and Hostettmann (1991), the crude extract is a diverse mixture comprising various compounds that are by-products of metabolism. They are metabolites, either primary or secondary. It has been proposed that secondary metabolites in plants are what give them their therapeutic properties because they comprise most biologically active compounds (Fabeku, 2006; Neumann and Hirsch, 2000).

Organic compounds play a major role in the drug research initiatives of the pharmaceutical industry (Ata *et al.*, 2007; Hanazki *et al.*, 2000). There are hundreds of plant species that have not yet been investigated for their phytochemicals and, consequently, their biological potential. For this reason, the importance of continued research into plant phytochemicals cannot be overemphasized (Fadipe, 2014; Rates, 2001). Likewise, the development of resistance to most known antimicrobial drugs and the resulting high cost of treatment have led to the search for novel, safe, efficient, and effective ways to manage infectious diseases (El-Mahmood and Doughari, 2008).

Fragrant liquids with an oily uniformity, essential oils are extracted from a wide variety of plant parts, such as flowers, roots, leaves, seeds, fruits, and bark (Ibok *et al.*, 2023; Odeja *et al.*, 2023). They are also referred to as volatile odoriferous oils. They can be extracted from plant materials in a variety of ways, such as steam distillation, expression, and hydro-distillation. Among all the techniques, for example, steam distillation has been used a lot, especially for large-scale production (Cassel and Vargas 2006; Di Leo Lira *et al.*, 2009). According to Masango (2005), plant essential oils are frequently a complex mixture of polar and non-polar natural compounds. The main ingredients of various essential oils are terpenes (monoterpenes and sesquiterpenes), aromatic compounds (aldehydes, alcohols, phenols), and terpenoids (Bakkali *et al.*, 2008; Mohamed *et al.*, 2010).

According to ethnobotanical reports, *Celtis zenkeri*, a member of the Ulmaceae family, has a variety of medicinal uses, including the treatment of skin infections, cancer, arthritis, and coughs. It is acknowledged that ligand- and structure-based computational studies are useful tools for hastening the drug design process (Lapa *et al.*, 2012). This study reports the volatile secondary metabolites from the roots of *Celtis zenkeri*, its antimicrobial activity, and molecular docking analysis of the identified compounds in the roots of the volatile oil of *C. zenkeri* as part of our continuous investigation of this medicinal plant (Okpala *et al.*, 2021; 2022).

2. Material and methods

2.1. Plant collection and preparation

In August 2016, *Celtis zenkeri* was collected at an elevation of 305 metres in Ikire, Osun State, Nigeria (7º22′20''N; 4º11'14''E), identified, and authenticated at the Ibadan, Nigeria. Forestry Research Institute Herbarium, where a voucher specimen was placed under the accession number FHI-110554. The fresh plant materials were air-dried for a while to shield them from the sun's direct rays. The plant materials were first ground up to increase surface area, and then, to keep any volatile components from evaporating before use, they were sealed in airtight bags.

2.2. Extraction of volatile oils

Hydro-distillation methods were employed using the Clevenger apparatus for the extraction of essential oil from the roots of the *Celtis zenkeri*. The pulverised sample was weighed and carefully loaded into a 10 L round-bottomed flask, and water was added until the sample was fully immersed. The flask was placed on a heating mantle and fitted with the all-glass Clevenger distillation unit designed according to the British pharmacopoeia specification (Paterson, 1982). The extraction process was carried out for a minimum of 3 h at a temperature of 50 ºC. The volatile oils trapped in 2.0 ml of hexane were carefully collected using a syringe and put in a pre-weighed sample vial. The weight of the volatile oil was recorded, and the yield was calculated for each of the samples. The sample vial containing the oil was carefully corked and stored in the refrigerator for further analysis.

The percentage yield for the oil sample was calculated using the **Eq. 1**:

$$\% \text{ Yield} = \frac{Mass\ of\ oil}{Mass\ of\ sample} \text{ X } 100 \quad (1)$$

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

The volatile oil was analysed using a GC-MS Agilent Technologies, Model-7890A Gas Chromatograph, coupled with a 5975C mass spectrometer. The gas chromatograph capillary column type was an HP-5MS, with a column length of 30m, an internal diameter of 0.320 mm, and a film thickness of 0.25 µm. The volume of the sample injected was 1 µL, the split ratio was 50:1, and the split flow was 70.615 mL/min. The carrier gas, helium, had a flow rate of 1 mL/min. The pressure, linear velocity, and injection volume were set at 56.2 kPa, 362 cm/s, and 1.0 µL, respectively. The oven temperature was adjusted to 60 ºC, held for 1 minute, increased to 180 ºC for 3 min at 10 ºC/min, and finally reached 280 ºC for 2 min at 10 ºC/min. The temperatures of the injector and detector were set at 250 ºC. The constituents were identified by comparing the published mass spectral database (NIST 11.L) and literature data with the total chromatogram that had been auto integrated.

2.4. Antimicrobial assay

2.4.1. Preparation of graded concentration of the samples

A solution of volatile oil was prepared by dissolving 200 µg of the oil in 2.0 mL of dimethylsulfoxide (DMSO) to give 100 µg/mL. From the 100 mg/mL solution, 1 mL was taken into another sample bottle and 1 mL of solvent (DMSO) was added to give 50 µg/mL. This was serially diluted until a 6.25 µg/mL concentration was obtained. Two other sample bottles contained the negative control (DMSO solvent) and the positive control (gentamicin 10 µg/mL for bacteria and tioconazole 0.07 µg/mL for fungi).

2.4.2. Agar diffusion: pour plate method for bacteria

The volatile oil was screened against two gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*; four gram-negative bacteria: *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia,* and *Salmonella typhi*; obtained from the Department of Pharmaceutical Microbiology, University of Ibadan. An overnight culture of each microorganism was prepared by taking a loop full of the microorganisms from stock (slope) and inoculating each into a sterile nutrient broth of 5 mL each, incubated for 18-24 h at 37 ºC. From overnight culture, 0.1 mL of each microorganism was taken and put into 9.9 mL of sterile distilled water to get 1:100 (10^{-2}) dilution of the microorganisms.

From the molten diluent (10^{-2}), 0.2 mL was taken into the 20 mL of freshly prepared sterile nutrient agar, then shaken gently for uniformity and aseptically poured into sterile Petri dishes, allowed to solidify for about 30-50 minutes. Using a sterile cork borer of 6 mm diameter, the wells were made in the set nutrient agar plate according to the number of graded concentrations of the samples.

In each well, 0.02 mL of the different graded concentrations of the sample were introduced using a Pasteur pipette. This was done in triplicate. The plates were allowed to stay on the bench for 1 h for pre-diffusion. The plates were incubated uprightly in the incubator for 18-24 h at 37 ºC. Then the observed zones of inhibition were measured.

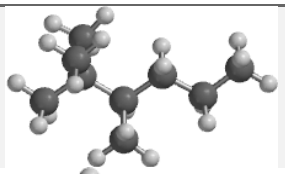
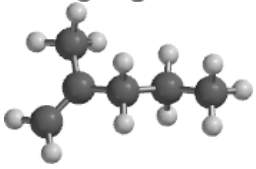
2.4.3. Agar diffusion: surface plate method for fungi

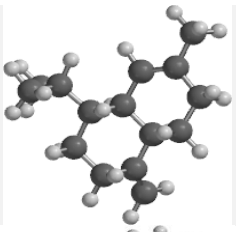
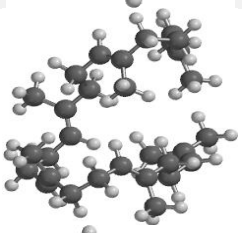
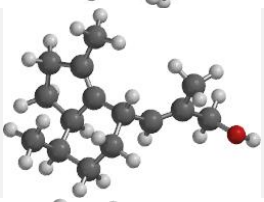
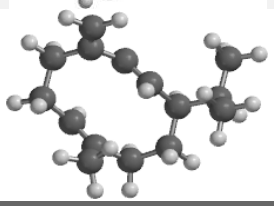
The antifungal potential of the volatile oil was determined against four fungi strains: *Candida albicans, Aspergillus niger, Rhizopus stolonifer,* and *Penicillum notatum;* obtained from the Department of Pharmaceutical Microbiology. A sterile Sabouraud Dextrose Agar (62 g/L) was prepared accordingly and aseptically poured into the sterile plate in triplicates and allowed to set properly, 0.2 mL of the 10^{-2} of the agar was then spread using a sterile spreader to cover the surface of the agar. The wells were made using a sterile cork borer 8 mm in diameter. In each well, the graded concentrations of the oil were introduced, including the controls. The plates were left on the bench for 120 min to allow the oil to diffuse properly into the agar, i.e., pre-diffusion. The plates were incubated uprightly in the incubator for 48 h at 26-28 ºC.

2.5. Molecular docking of the identified compounds in the volatile oil

The AutoDockTools (ADT), a free graphic user interface (GUI) for the AutoDockVina program, was used to conduct molecular docking investigations (Tanchuk *et al.*, 2015). The compounds were docked against the protein's active site (PDB ID: 3HAM and 5V5Z) using AutoDockVina using the usual methodology (Bottomley *et al.,* 2007; Narramore *et al.,* 2019; Tanchuk *et al.*, 2015). With suitable 2D orientation assigned, the ChemOffice program (ChemDraw 16.0) was used to build the chemical structures of the compounds. Spartan 14's graphical user interface was used to reduce the energy of each molecule (**Table 1**). The docking simulation was then performed using the input provided by the energy-minimized ligand molecules to AutoDock Vina (Zeleke *et al.*, 2020). The crystal structures of substrate and nucleotide complexes of *Enterococcus faecium* aminoglycoside-2''-phosphotransferase-IIa [APH(2'')-IIa] (PDB ID: 3HAM) and the crystal structures of full-length Lanosterol 14 alpha-demethylases of the prominent fungal pathogen, *Candida albicans* (PDB ID: 5V5Z) were downloaded from the protein data bank. The target protein file was prepared by leaving the associated residue with the protein using auto preparation of the target protein file AutoDock 4.2 (MGLTools 1.5.6), and the protein preparation was carried out using the reported standard protocol (Narramore *et al.*, 2019). The grid box for the docking simulations was set using the graphical user interface application. The macromolecule's region of interest was put up in the grid so that it is encircled by it. The best-docked configuration between the ligand and protein was sought using the docking algorithm offered by AutoDock Vina (Narramore *et al.,* 2019; Seeliger and de Groot, 2010; Zeleke *et al.*, 2020). For each ligand, a maximum of 9 conformers were considered throughout the docking procedure. The post-docking evaluations were conducted using **PyMOL** and Discovery Studio.

Table 1. The chemical compounds identified in the volatile oil of C. *zenkeri.*

S/N	PubChem No.	Name	3D Structure
1	28021	2,2,3-trimethylhexane	
2	12986	2-methyl-1-pentene	

3	6432308	γ-murolene	
4	638072	Squalene	
5	91699505	Velerenol	
6	74764030	Gremacrene D	

3. Results and discussion

3.1. Yield of the oil

The weight of roots of *C. zenkeri* used during hydro-distillation extraction, and the weight of the essential oils obtained, and the corresponding percentage yield are presented in **Table 2**. **Figure 1** is the GC-MS chromatogram of the root volatile oil of *C. zenkeri.* Plant material (200 g) was used in the extraction process, resulting in 1.58 g of colourless oil, which equates to a yield of 0.79%.

3.2. Identification of the chemical constituents of the volatile oil

The GC-MS result of the chemical constituents of the volatile oil is given in **Table 3** and **Fig. 2**. The 6 identified compounds are responsible for 80.07% of the root's volatile oil. The major compounds in root volatile oil include 2-methyl-1-pentene (40.01%), Gremacrene D (19.68%) and Squalene (10.95%). The root volatile oil of *C. zenkeri* was of different classes of compounds: alkene (40.01%), sesquiterpenes (20.35%) and triterpene (10.95%), oxygenated sesquiterpenes (2.71%) and alkane (6.05%). Terpenoids are known for their antimicrobial activity (Ogunnusi *et al.*, 2010).

In the GC-MS analysis of the *n*-hexane extract of *Bambusa nrundinaceae* leaves and *Trigonell tehranica* essential oils, 2, 2, 3-trimethylhexane and 2-methyl-1-pentene were identified as one of the main chemical constituents in the extract and essential oils of both plants. The *B. nrundinaceae* leaf extract and *T. tehranica* oil exhibited strong antimicrobial properties (Kiashi *et al.*, 2017; Zubair *et al.*, 2013). In the *Hypericum perforatum* plant, volatile oils rich in germacrene D and other sesquiterpenes have been found to have anti-radical and anti-proliferative effects on tumour cell lines (Mockute, *et al.,* 2008; Casigilia *et al.*, 2017). Similarly, the germacrene D dominant essential oils of *Siparuna aspera, Siparuna macrotepala, piper leticianum, piper augustum* possess significant antimicrobial activity (Noriege *et al.*, 2019), *Ocotea silvestris* and *Ocotea indecora* leaves exhibit good antifungal activity against *candida parapsilosis* (Rambo *et al.*, 2022). Studies have shown that the squalene-rich acetone fraction of *Stichopus hermanni* extract is a potent antibacterial and antifungal agent. Squalene is a well-known dietary supplement that has been shown to be effective in the treatment of cancer (Nazemi *et al.*, 2022).

Table 2. Yields and properties of the volatile oil.

S/N	Plants name & parts	Weight of plant material (g)	Weight of oil (g)	Percentage (%) Yield of oil	Colour of oil
1	*C. zenkeri*, root	200	1.58	0.79	Colourless

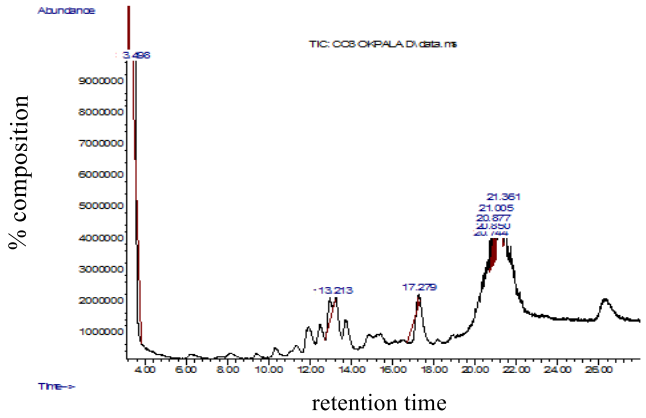


Figure 1. GC-MS Chromatogram of components of *Celtis zenkeri* roots volatile oil.

Table 3. Chemical composition of the roots volatile oil of *C. zenkeri.*

S/N	RI	Compounds	% Composition
1	91	2,2,3-trimethylhexane	6.05
2	321	2-methyl-1-pentene	40.01
3	447	Germacrene D	19.68
4	1302	γ- muurolene	0.67
5	1725	Velerenol	2.71
6	1825	Squalene	10.95
		Total % Composition	80.07

Note: RI = Calculated Retention Index; Compounds = Compounds listed in order of elution from a HP-5MS column; % Composition = Percentage composition.

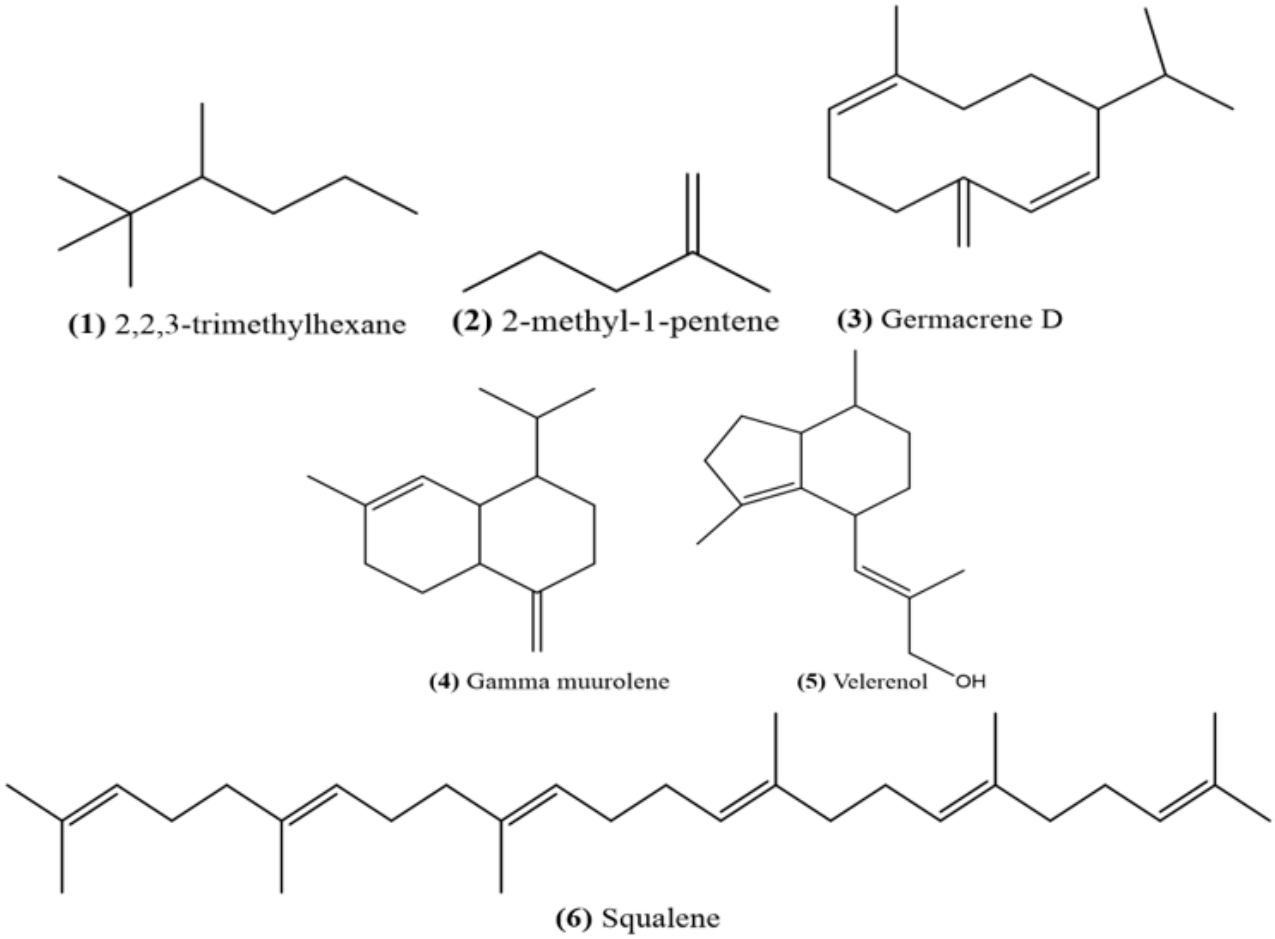


Figure 2. Structures of the compounds identified in roots volatile oil of *C. zenkeri.*

3.3. Antimicrobial activity of the volatile oil

The antimicrobial activity of the volatile oil from the roots of *C. zenkeri* is presented in **Table 4**. Comparing the volatile oil to the positive control (gentamicin 10 µg/mL for bacteria and tioconazole 0.07 µg/mL for fungi), the volatile oil showed a broad spectrum of activity against all microorganisms at 125-1000 µg/mL concentrations. Both gentamicin and ticonazole are well-known antibiotics that are frequently prescribed in healthcare facilities to treat infections (Ngoupayo *et al.*, 2015; Okpala *et al.*, 2019). In every tested concentration, they demonstrated better growth inhibition than the volatile oil. This investigation revealed that, despite significant variations in the recorded zone of inhibition when compared to the controls, which are well-known antibacterial and antifungal medications, the volatile oil exhibits moderate to good activity at higher concentrations (Lima-Filho *et al.*, 2002; Obame *et al.*, 2008; Sohail *et al.*, 2018).

The oil's zone of inhibition against gram-positive and gram-negative bacteria ranged from 24-10 mm, while Gentamicin's ranged from 40-38 mm at concentrations of 125-1000 µg/mL. The most susceptible bacteria strains to volatile oil were *Pseudomonas aeruginosa and Staphylococcus aureus*. At concentrations ranging from 125 to 1000 µg/mL, the oil

demonstrated growth inhibition against the fungi with a zone of inhibition between 18 and 10 mm whereas, the zone of inhibition of tioconazole was between 28 and 26 mm. At lower concentrations, there is either no activity or no significant inhibition. According to Kaur *et al.* (2011), a combination of compounds in essential oils is responsible for the antifungal activity of essential oils.

The volatile oil was generally found to be more effective against fungi than bacteria. This finding supports the traditional use of *C. zenkeri* for treating skin infections, including scabies and eczema, particularly those caused by *Rhizopus stolonifer*, *Penicillum notatum,* and *Candida albicans* (Burkill, 1995; Olaoluwa and Olapeju, 2015). Essential oils containing aldehydes or phenols as major constituents have been reported to exhibit the highest level of antimicrobial activity, with terpene alcohol-containing essential oils following closely behind. Other essential oils with ketones or ester had far less activity than volatile oils with terpene-hydrocarbons, which are usually inactive. However, the interaction of the different components may result in negative, positive, or advantageous effects (Janssen *et al.*, 1987).

3.4. Molecular docking analysis

The binding affinities calculated from the docking simulation of compounds found in the volatile oil of *C. zenkeri* are presented in **Table 5**. The identified compounds in the volatile oil were docked against the substrate and nucleotide complexes of *Enterococcus faecium* aminoglycoside-2''-phosphotransferase-IIa [APH(2'')-IIa] (PDB ID: 3HAM) and full-length Lanosterol 14 alpha-demethylases of the prominent fungal pathogen, *Candida albicans* (PDB ID: 5V5Z), and the interacting modes (hydrogen bonding, hydrophobic and Van der Waal interactions) of the compounds with promising affinities are displayed in **Tables 6** and **7**.

Molecular docking results of the substrate and nucleotide complexes of *Enterococcus faecium* aminoglycoside-2''-phosphotransferase-IIa [APH(2'')-IIa] (PDB ID: 3HAM) compared with the clinical drugs (Gentamicin) docked with compounds identified in the roots oil of *Celtis zenkeri.* The compounds were found to have minimum binding energies ranging from –17.6 to –29.7 kJ/mol (**Table 5**), with the best results achieved using compounds gremacrene D and γ-muurolene (–29.7 kJ/mol). According to Adepoju *et al.* (2022), the lower the binding affinity value of any compound, the better the inhibiting ability of such compound; thus, compounds: gremacrene D and γ-muurolene were observed to have the highest tendency to inhibit the studied receptor than other identified compounds.

Similarly, results from docking lanosterol 14 alpha-demethylases of the prominent fungal pathogen, *Candida albicans* (**PDB ID: 5V5Z**) with the ligands showed that two of the identified compounds in the oil have higher inhibitory activities than tioconazole (**Table 5**) vis-à-vis γ-muurolene (–32.2 kJ/mol) and squalene (–38.1 kJ/mol). Thus, these identified compounds can be better drug candidates than Tioconazole (–31.4 kJ/mol) for the treatment of fungi infections. When a compound's binding affinity decreases, its drug-likeness increases, and its inhibitory potency increases (Omotayo *et al.*, 2022; Oyewole *et al.*, 2020).

Table 4. Antimicrobial activity of the volatile root oil of *C. zenkeri.*

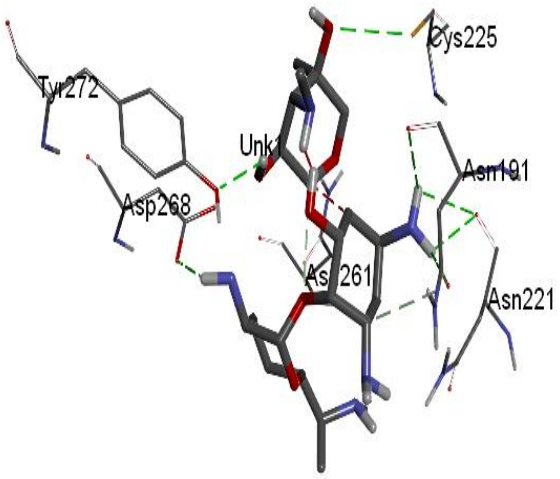
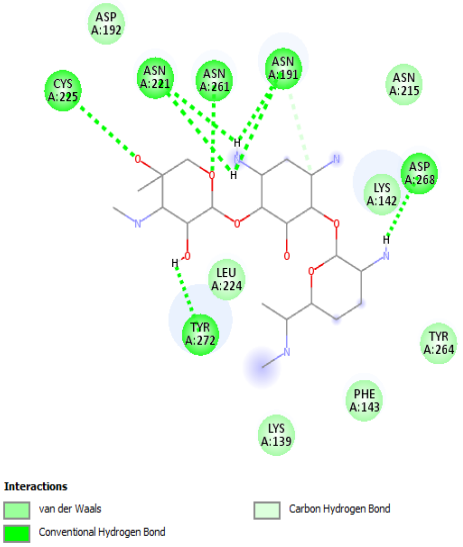
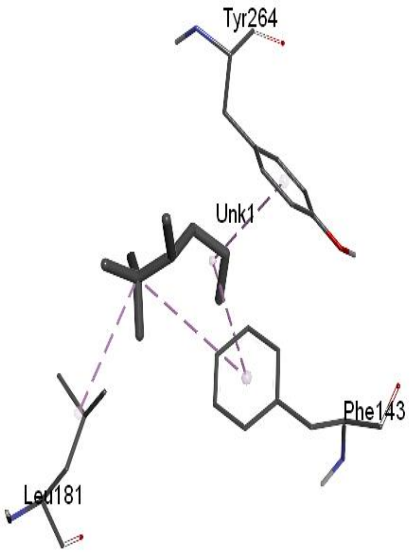
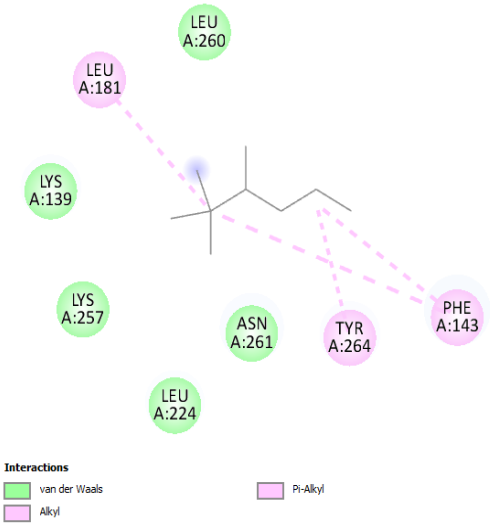
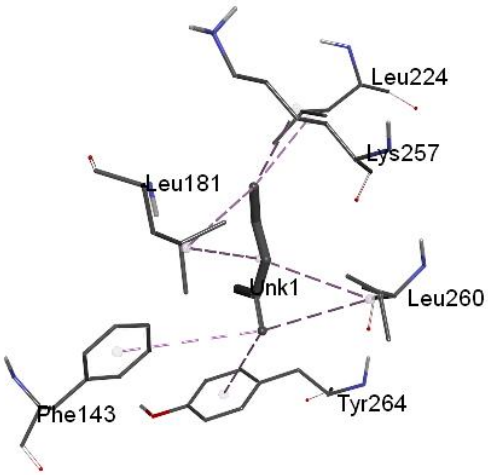
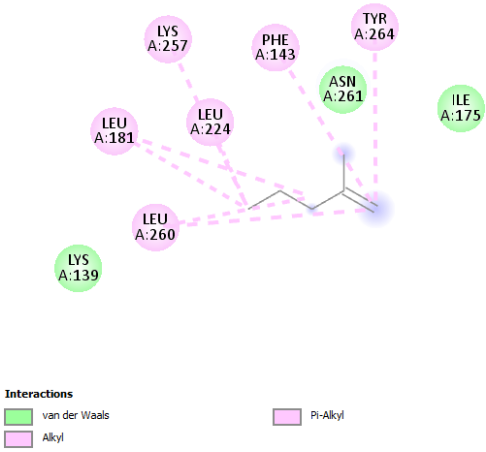
	Test microorganisms / Zones of Inhibition (mm)									
Conc. (µg/mL)	*S. auerus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhil*	*K. pneumona*	*C. albican*	*A. niger*	*P. notatum*	*R. stolonifer*
1000	24	18	20	24	22	20	18	16	16	18
500	20	16	18	20	18	18	16	14	14	14
250	18	14	16	18	16	16	14	12	12	12
125	14	12	12	14	14	14	12	10	10	10
62.5	10	10	10	12	12	12	10	-	-	-
31.25	-	-	-	10	10	10	-	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-
Gen. (+ve)	38	38	38	40	38	38	-	-	-	-
Tio. (+v)	-	-	-	-	-	-	28	28	26	28

Note: DMSO: negative control (Dimethylsulphoxide); Gen. (+ve): positive control (Gentamicin at 10µg/ml for bacteria); Tio. (+ve): positive control (Tioconazole 70% for fungi); *S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; B. subtilis= Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. typhil: Salmonella typhi; K. pneumonae: Klebsiellae pneumona; C. albicans: Candida albicans; A. niger: Aspergillus niger; P. notatum= Penicillum notatum; R. stolonifer: Rhizopus stolonifer;* **-**: no inhibition.

Table 5. Binding Affinities of the Receptors PDB ID: 3HAM and PDB ID: 5V5Z with the identified compounds (ligands) in the volatile oil.

S/N	Ligand Number	PDB ID: 3HAM		PDB ID: 5V5Z	
		Binding Affinity (kJ/mol)	Inhibitory constant (Ki, µM)	Binding Affinity (kJ/mol)	Inhibitory constant (Ki, µM)
1	3467	−30.5	4.43		
2	5482	–	–	−31.4	3.16
3	28021	−19.7	357.40	−20.9	215.24
4	12986	−17.6	831.46	−17.2	984.41
5	6432308	−29.7	6.21	−32.2	2.25
6	638072	−23.8	66.00	−38.1	0.21
7	91699505	−27.6	14.44	−28.9	8.70
8	74764030	−29.7	6.21	−31.4	3.16

Table 6. Docking ligand-receptor complexes of selected ligands with the binding affinity (PDB ID: 3HAM).

	3D representation	2D representation
3467		
28021		
12986		

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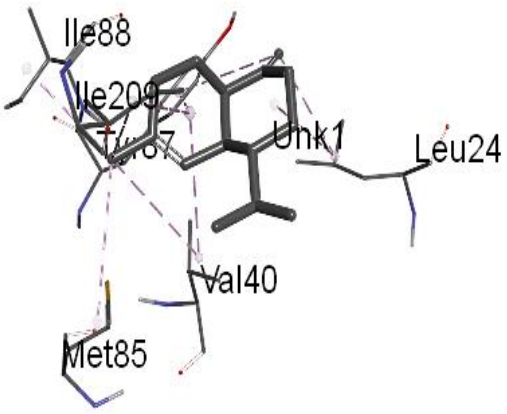

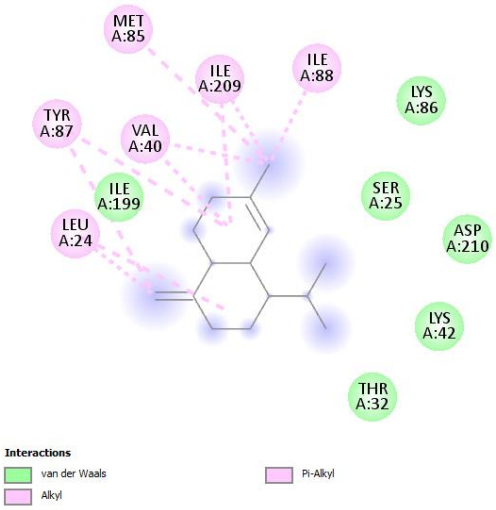

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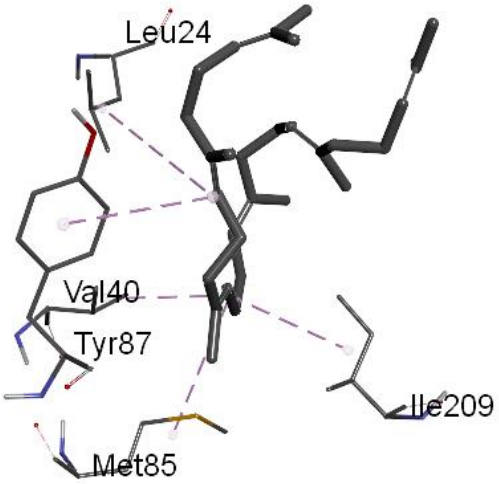

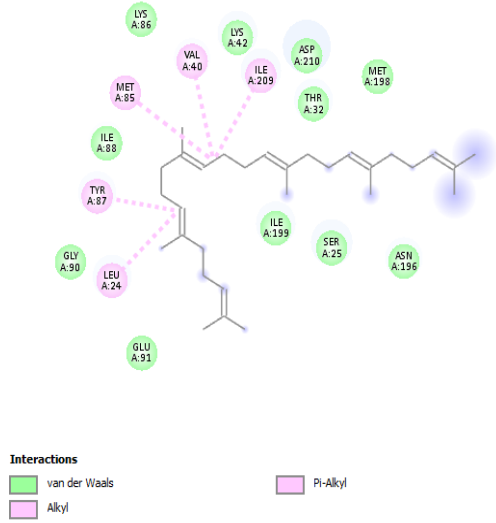

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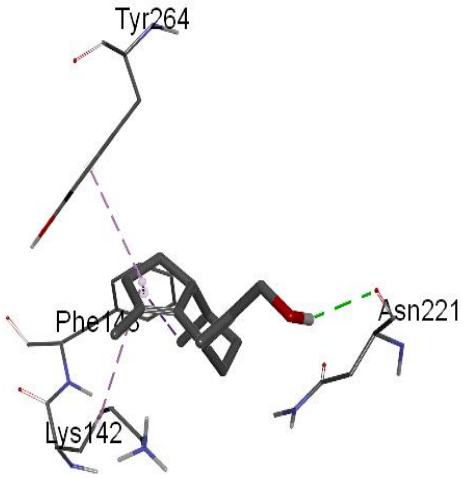

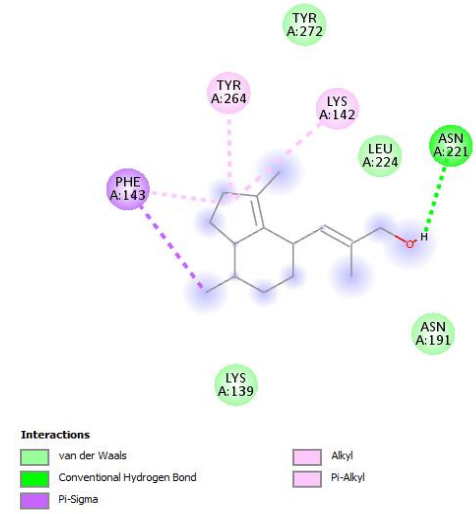

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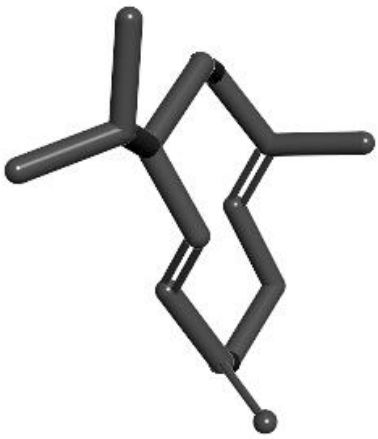

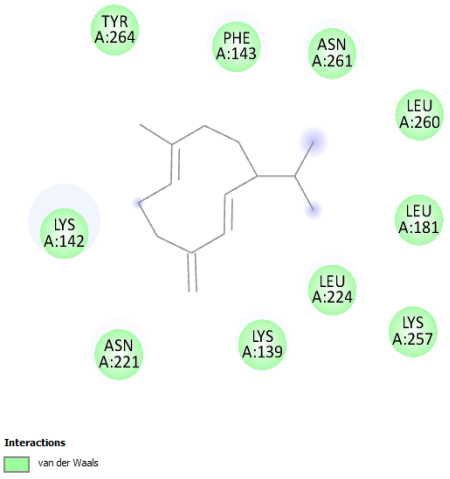

Table 7. Docking ligand-receptor complexes of selected ligands with the binding affinity (PDB ID: 5V5Z).

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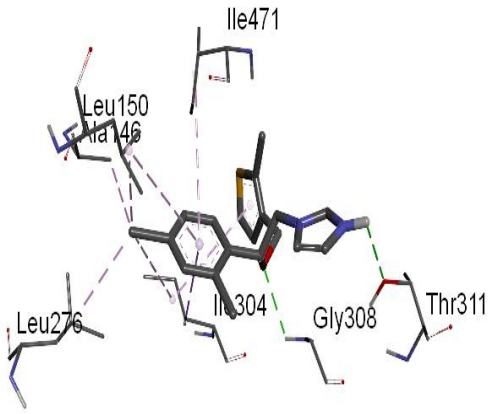
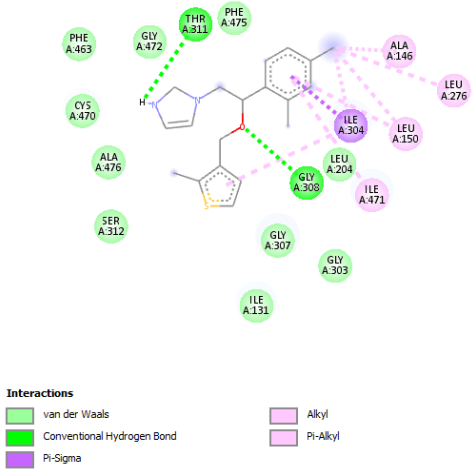
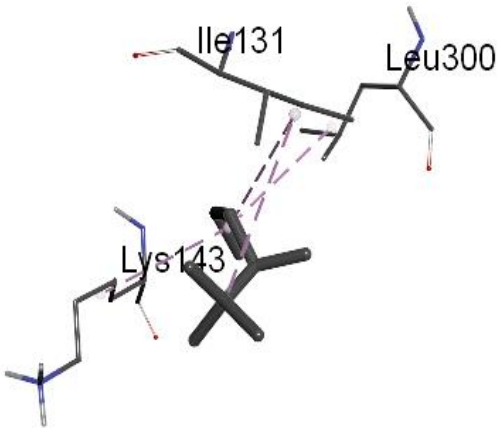
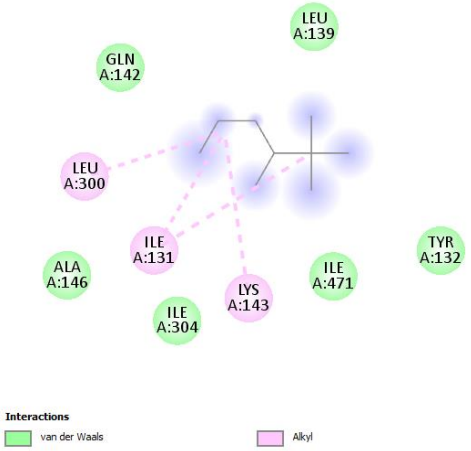
5482		
28021		

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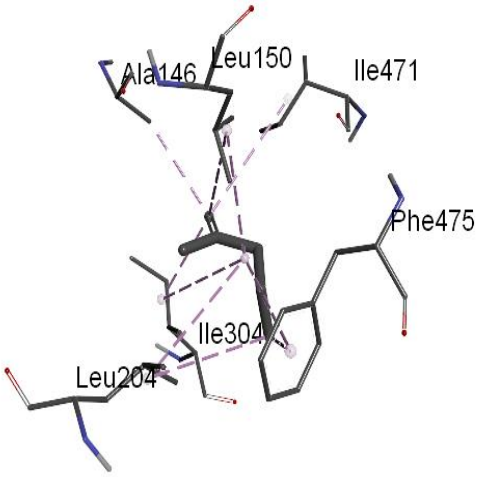

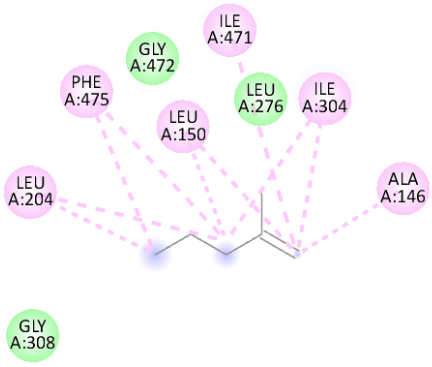

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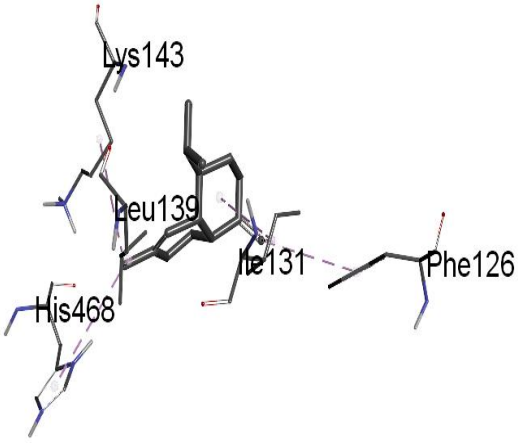

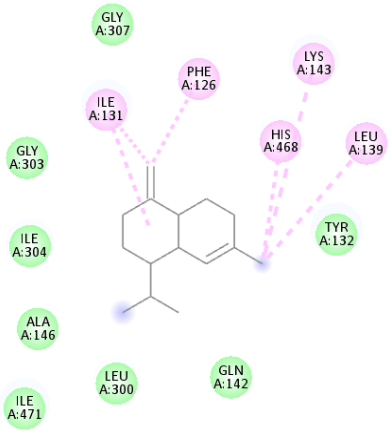

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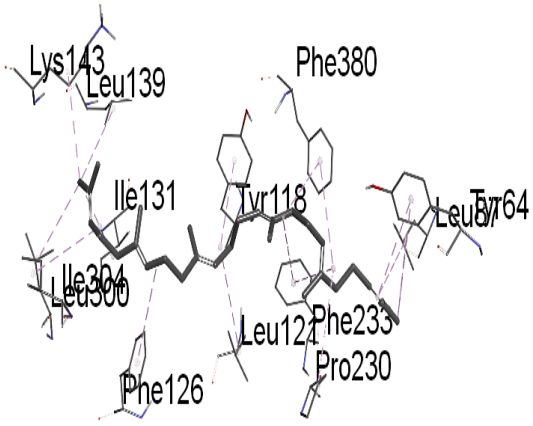

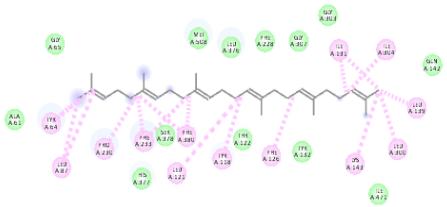

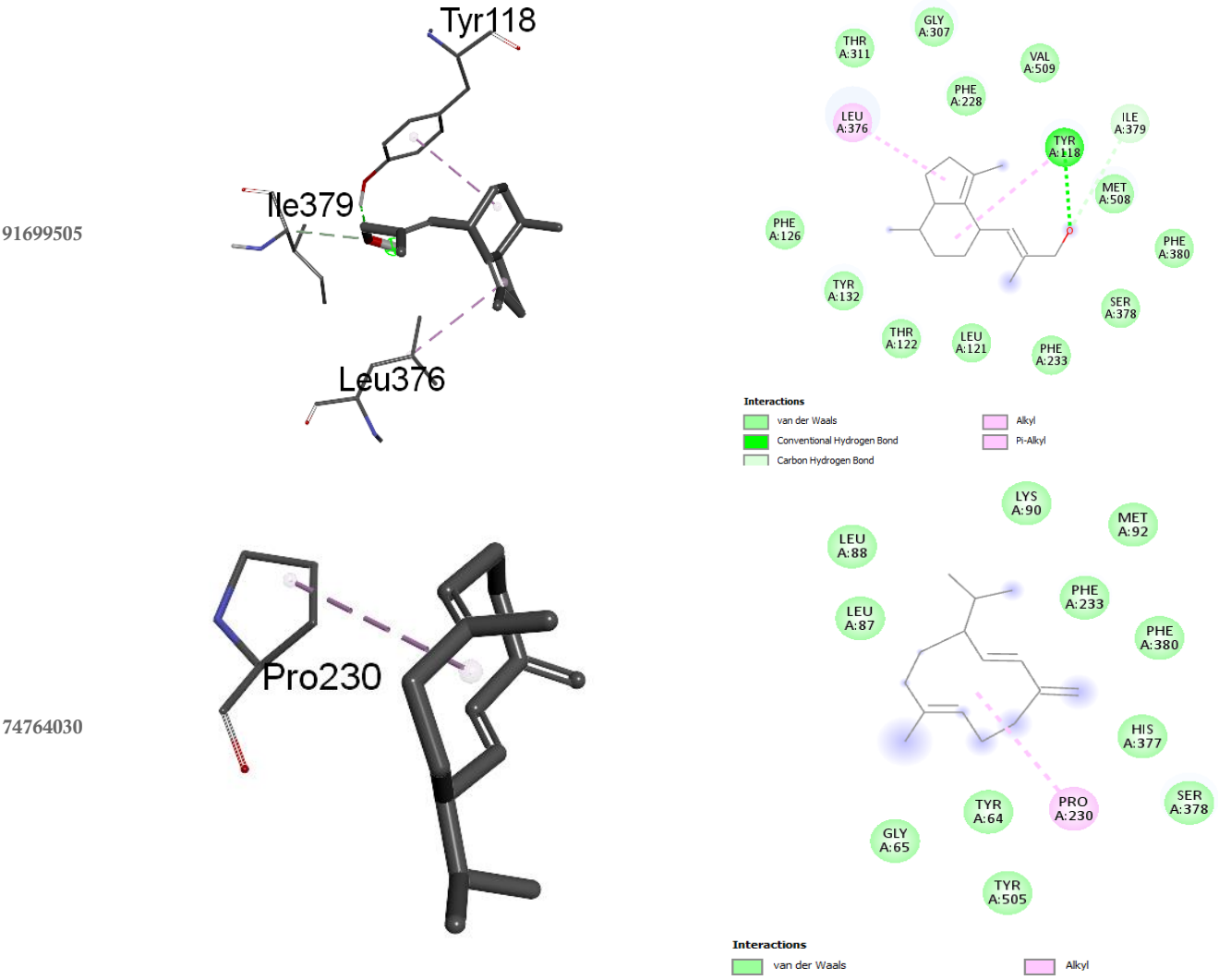

4. Conclusions

For the first time, chemical components, antibacterial and antifungal properties of the volatile oil from the roots are reported. This study is the first attempt to accurately characterize the volatile oil from the roots of *C. zenkeri* and conduct antibacterial and antifungal tests on it. The volatile oil is a potential candidate for the development of antibiotic drugs due to its antimicrobial activity against various tested strains of bacteria and fungi. Our study is the first report on the antimicrobial properties of the essential oil from *C. zenkeri*. The *in silico* molecular docking analysis of the volatile oil-identified compounds revealed good agreement with the outcomes of the *in vitro* antibacterial assay, when a compound's binding affinity decreases, its drug-likeness increases, and its inhibitory potency increases. The components of the volatile oil from *C. zenkeri* roots were also shown by the molecular docking analysis to be potential good sources for protein targeted antimicrobial compounds. This supports the use of *C. zenkeri* in folklore remedies for infections caused by microorganisms.

Authors' contributions

Conceptualization: Ejike Onwudiegwu Okpala; William Ojoniko Anthony; Godfrey Okechukwu Eneogwe; **Data curation:** Ejike Onwudiegwu Okpala; William Ojoniko Anthony; Godfrey Okechukwu Eneogwe; **Formal Analysis:** Ejike Onwudiegwu Okpala; William Ojoniko Anthony; Godfrey Okechukwu Eneogwe; **Funding acquisition:** Not Applicable; **Investigation:** Ejike Onwudiegwu Okpala; William Ojoniko Anthony; Godfrey Okechukwu Eneogwe; **Methodology:** Ejike Onwudiegwu Okpala; Oluwakayode Olubunmi Odeja; Michael Gabriel Ibok; **Project administration:** Joel Ojogbane Onoja; Samuel Akinniyi Odewo; Shedrach Ndubuisi Ike; **Resources:** Olusimbo Adesegun Onanuga; Samuel Akinniyi Odewo; Shedrach Ndubuisi Ike; **Software:** Ejike Onwudiegwu Okpala; William Ojoniko Anthony; Godfrey Okechukwu Eneogwe; **Supervision:** Banjo Semire; **Validation:** Ejike Onwudiegwu Okpala; William Ojoniko Anthony; Godfrey Okechukwu Eneogwe; **Visualization:** Ejike Onwudiegwu Okpala; William Ojoniko Anthony; Godfrey Okechukwu Eneogwe; **Writing – original draft:** Ejike Onwudiegwu Okpala; William Ojoniko Anthony; **Writing – review & editing:** Oluwakayode Olubunmi Odeja; Michael Gabriel Ibok.

Data availability statement

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

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