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# Phytochemical screening, antiproliferative evaluation, and molecular docking studies of *Acacia nilotica* fruit from Nigeria

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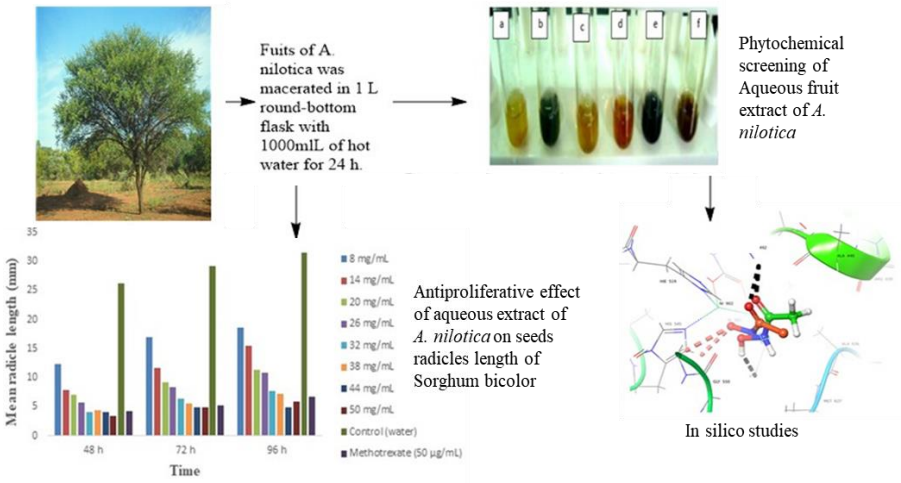
## Abstract

*Acacia nilotica*, (*Fabaceae*), is valued for its medicinal properties. We examine the antiproliferative properties of the aqueous fruit extract of *A. nilotica*. Aqueous extract from *Acacia* has been associated with potential anticancer effects in fruits and vegetables through screening, antiproliferative, and molecular docking evaluation. Phytochemical screening reveals the presence of alkaloids, saponins, tannins, flavonoids, steroids, and carbohydrates. The extracts showed significant antiproliferative effects at eight concentrations (8–50 mg $mL^{-1}$) examined in comparison to the standard (methotrexate). When compared to *Sorghum bicolor* seed radicles treated with methotrexate at 48, 72, and 96 h, 50 mg $mL^{-1}$ extract significantly inhibited the generation of seed radicals, with potent inhibitions of 87.06, 83.48, and 81.45%. Analysis of molecular docking results showed that [(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-3,4-dihydro-2*H*-chromen-7-yl]3,4,5-trihydroxybenzoate (D21), (5*R*,9*R*,10*R*,13*S*,14*S*,17*S*)-17-[(2*S*,4*R*)-4-[(2*S*)-3,3-dimethyloxiran-2-yl]-4-hydroxybutan-2-yl]-4,4,10,13,14-pentamethyl-1,2,5,6,9,11,12,15,16,17-decahydrocyclopenta[a]phenanthren-3-one (D28) and [(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-3,4-dihydro-2*H*-chromen-5-yl] 3,4,5-trihydroxybenzoate (D29) have strong tendency to inhibit dihydrofolate reductase (1VDR), capase-9 (6J15) and *Mycobacterium tuberculosis* (Mtb) (6J17) better than methotrexate and azacitidine, known antiproliferative drugs. These findings support the use of *A. nilotica* in traditional medicine for the treatment of tuberculosis and cancer.

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### Keywords

1. *Acacia nilotica*;
2. *Fabaceae* family;
3. *Sorghum bicolor* seed radicles;
4. inhibitory effects;
5. in-silico studies.

### Section Editor

**Assis Vicente Benedetti**

### Highlights

- Aqueous extract of *Acacia nilotica* fruit was obtained.
- Phytochemical screening of aqueous fruit extract of *A. nilotica.*
- Evaluation of antiproliferative properties of the aqueous fruit extract.
- Molecular docking studies of phytochemicals identified in leaves and fruits.

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# 1. Introduction

Cancer stands as one of the most terrifying diseases of the twenty-first century, emerging as a leading cause of mortality. It is a challenging inherited disorder that poses a serious public health risk, with factors such as exposure to chemicals, poor dietary habits, genetic mutations, and environmental influences contributing to its onset. Due to its high mortality rate, cancer has become a huge global public health concern (Sung *et al.,* 2021). However, recent predictions also suggested that the number of cancer-related deaths could rise to roughly 16 million by 2040 (Gopal and Sharpless, 2021), making it urgently necessary to develop the ideal anticancer treatments that would effectively treat this condition (Mukherji *et al.,* 2021). Sixty per cent of the currently used anticancer drugs were reportedly developed from medicinal plants (Enegide *et al.,* 2014; Li-Weber, 2009).

The natural diversity of medicinal plants and their diverse phytochemicals have greatly benefited the use of medicinal plants as biomodifiers. They have been successfully used as precursors in the discovery and development of novel drugs (Butler, 2008; Muschietti *et al.,* 2013). One benefit of using medicinal plants is their synergistic nature, which is superior to using individual medications to treat diseases (Yuan *et al.,* 2016). Some of the successful medications that were made from natural products include artemisinin and its analogues, which are frequently used as antimalarials, acetyldigoxin from *Digitalis lanata*, which is used as a cardiotonic, aescin from *Aesculus hippocastanum*, which is used as an anti-inflammatory, lapachol as an anticancer, and antitumor from *Tabebuia* species (Kumari *et al.,* 2019; Taylor, 2000).

The Leguminosae family includes the *Acacia nilotica* tree, which may reach heights of 15 to 18 m and a diameter of 2 to 3 m. Bagaruwa is a frequent name for it among Hausa people from northern Nigeria. In Western and Northern Nigeria, the plant is known to be used medicinally. Young delicate pods of the plant are consumed as vegetables; roasted seeds are used as a seasoning or fermented to produce alcoholic beverages; and boiling bark is used to produce a beverage that has a coffee-like flavor (Tanko *et al.,* 2014). For skin conditions, diarrhea, dysentery, cough, diabetes, eczema, wound healing, and burning feeling, as well as an astringent, demulcent, and antiasthmatic, the bark, root, gum, leaves, fruit, and flowers have all been used. Teeth are washed with delicate twigs (Roozbeh and Darvish, 2016). In Nigeria's northern region, leaves are used to cure typhoid disease. Although there are many methods to prepare it, boiling it in water and letting it cool before eating is a common method. Before consuming the herbal mixture, the patient may breathe in the steam as an alternate form of treatment (Sarkiyayi and Abdul Rasheed, 2013). Therefore, the goal of our research is to evaluate and screen the phytochemicals in *A. nilotica* fruit's aqueous extract for potential antiproliferative activity, which has historically been utilized in Nigeria to treat cancer and other ailments.

Furthermore, the main bioactive chemicals of the *A. nilotica* that had been isolated and reported (Eldeen *et al.,* 2010; Kumari *et al.,* 2020; Mohmmed and Babiker, 2019; Singh *et al.,* 2010) were downloaded from the online library, PubChem (www.https://pubchem.ncbi.nlm.nih.gov/) and screened using molecular docking method against dihydrofolate reductase (**DHFR**), X-linked inhibitor of apoptosis protein (**XIAP**) and *Mycobacterium tuberculosis* (Mtb) type VII secretion system (**T7SS**). Dihydrofolate reductase (**DHFR**, **ID: 1VDR**) is a vital enzyme in the catalysis of the NADPH-linked reduction of 7,8-dihydrofolate and subsequent production of thymidylate. DHFR and thymidylate synthase are target enzymes in cancer chemotherapy (Pieper *et al.,*1998). It has been reported that DHFR is inhibited by antineoplastic and immunosuppressive agents, such as methotrexate by the nonproliferation of malignant cells, it also serves as an antirheumatic agent (Askari and Krajinovic, 2010). Another critical form of cancer is leukemia, which affects blood-forming tissues such as the bone marrow and the lymphatic system. The causes of leukemia are X-linked inhibitor of apoptosis protein (**XIAP**, **ID: 6J15**), an apoptotic regulator protein that binds to the effector caspases-3 and -7 through its BIR2 domain, and initial caspase-9 through its BIR2 and BIR3 (Cossu *et al.,* 2009). Azacitidine is one of the medicines that work well as XIAP antagonists. The human tuberculosis pathogen Mtb infects around 33% of the global population and causes at least one million deaths annually (WHO, 2018). For the treatment of tuberculosis, several medications are available that target the type VII secretion system (**T7SS**, **ID: 6J17**). These medications, like isoniazid, must be used for an extended period (Mi *et al.,* 2022). Due to the rise of multidrug-resistant Mtb strains, these medications can be costly, have major side effects, and lose their effectiveness (Salvatore and Zhang, 2017; Wang *et al.,* 2020).

# 2. Material and methods

## 2.1 Plant

### 2.1.1. Collection and identification

Biological Science Department of Federal University Lokoja, Kogi State, Nigeria.

### 2.1.2. Plant preparation and extraction

Fifty grams (50 g) of the air-dried and pulverized fruits of *A. nilotica* were macerated in a 1 L round-bottom flask with 1,000 mL of water for 24 h. The resultant mixture was filtered. Using a rotating evaporator under reduced pressure, the clear filter was concentrated to dryness at 60–80 °C to obtain *A. nilotica* fruit aqueous extract.

## 2.2. Preliminary phytochemical screening

A powder sample of *A. nilotica* fruit was subjected to phytochemical screening using the methods outlined by (Yusuf *et al.,* 2014) to detect chemical components.

### 2.2.1. Identification of steroids and triterpenes

A test tube containing 3 g of the powdered sample and 10 mL of 50% ethanol was filled with the mixture. The tube was then submerged in a water bath and heated for 3 min. After that, it was filtered and allowed to cool to ambient temperature. After the filtrate had been dried out in an evaporating dish, 5 mL of petroleum ether was added, and the mixture was swirled for 5 min before the petroleum ether portion was decanted and disposed of. After adding 10 mL of chloroform and stirring it for around 5 min, the mixture was put into a test tube. Next, 0.5 mg of anhydrous sodium sulphate was added, gently agitated, and filtered. The filtrate was then separated into two test tubes and utilized for the subsequent experiments.

**Lieberman–Burchard's reaction**: An equivalent volume of acetic anhydride was added to test tube I and gently stirred. Subsequently, 1 mL of concentrated $H_2SO_4$ was poured into the tube's side. Sterols and triterpenes are indicated by the formation of a brownish-red ring at the point of contact between the two liquids and a greenish color in the separation layer.

**Salwoski's Test**: In test tube II, a lower layer was created by adding two to three drops of concentrated sulfuric acid. A steroidal ring was present when the interphase was reddish-brown.

### 2.2.2. Identification of alkaloids

Two grams of powdered leaves and 20 mL of 5% sulfuric acid in 50% ethanol were cooked together in a water bath. After cooling, the mixture was filtered. There was a reserved amount. A second part of the filtrate was added to a 100 mL separating funnel, and two drops of strong ammonia solution were added to the solution to turn it alkaline. To let the layer separate, an equal volume of chloroform was added and gently shaken. The lower layer of chloroform was drained into an additional separating funnel. The layer of ammonia was set aside. Two volumes of diluted sulfuric acid, each containing 5 mL, were used to remove the chloroform layer. Next, the different extracts were put to the following test:

**Mayer's Test**: 1 mL of Mayer's reagent was applied drop by drop to the filtrate in test tube I. Alkaloids were presented when a cream-colored or greenish-colored precipitate formed.

**Dragendorff's Test**: 1 mL of Dragendorff's reagent was applied, drop by drop, to the filtrate in test tube II. A reddish-brown precipitate's formation suggests the presence of alkaloids.

**Wagner's Test**: 1 mL of Wagner's reagent was applied, drop by drop, to the filtrate in tube III. A reddish-brown precipitate's formation suggests the presence of alkaloids.

### 2.2.3. Identification of tannins

Ten milliliters of 50% alcohol were used to extract 2 g of fruit sample. The mixture was then filtered, and the resulting filtrate was split into three sections for the subsequent tests.

**Ferric chloride test**: Three drops of a diluted $FeCl_3$ solution were applied to test tube I; the presence of tannins is indicated by the formation of a blue or greenish-black color that turns olive green when more ferric chloride is added.

**Bromine water test**: The second part of the filtrate received three drops of bromine water added to it. Condensed tannins are indicated by a buff-colored precipitate, whereas hydrolysable tannins produce none.

**Lead subacetate test**: In the third section, three drops of lead subacetate solution were added. The presence of tannins is shown by the formation of a colored precipitate.

### 2.2.4. Identification of anthraquinones

**Borntrager's test (for free anthracene derivatives)**: 5 min were spent shaking the 0.5 g of the powdered fruit of *A. nilotica* in a test tube with 5 mL of chloroform added. After the mixture was filtered, an equivalent volume of 10% ammonia solution was shaken with the filtrate. When the aqueous layer is agitated, the presence of free anthraquinone is indicated by a pink, red, or violet color.

**Modified Borntrager's test (for combined anthracene derivatives)**: 5 mL of 10% hydrochloric acid was heated with 1 g of the powdered fruit for 3 min. After cooling and filtering the heated solution in a test tube, 5 mL of benzene was carefully removed from it. After pipetting off the top layer of benzene, the test tube containing half of its volume of 10% ammonium hydroxide solution was gently shaken. The ammonia layer's color changes from rose pink to cherry red when anthraquinone is present.

### 2.2.5. Identification of saponins

**Frothing test**: A test tube containing 0.5 g of powdered fruit was filled with 10 mL of distilled water, and the mixture was forcefully shaken for 30 s. After that, it was left to stand for 30 min and examined. The presence of saponins is indicated by the formation of honeycomb foam.

**Hemolysis test**: 1 g of the fruit sample was extracted using distilled water, and 2 mL of an aqueous NaCl solution was added to a test tube along with 2 mL of the filtrate. Next, three drops of animal blood were added to the tube using a syringe, and the tube was gently mixed by inverting it (no shaking) and left to stand for 15 min. The red blood cells' tendency to settle indicates the presence of saponins.

### 2.2.6. Identification of flavonoids

Two grams of the fruit sample that had been powdered were fully detained using acetone. After letting the acetone evaporate in a water bath, the residue was removed using warm water. Following a hot filtering of the combination, the filtrate was allowed to cool before being utilized in the subsequent test:

**Shinoda's Test**: 3 mL of the aqueous solution was mixed with a small amount of magnesium chips, and two drops of diluted hydrochloric acid and heated. A pink or red color indicates the presence of flavonoids.

**Sodium Hydroxide Test**: 2 mL of a 10% NaOH solution was added to test tube II; the yellow solution shows the presence of flavonoids, which turn colorless when diluted hydrochloric acid is added.

**$FeCl_3$ Test**: After adding three drops of $FeCl_3$ solution to test tube III, a phenolic nucleus is indicated by the development of a greenish-black color.

### 2.2.7. The Molisch's test for carbohydrates

The fruit sample was extracted with 10 mL of ethanol. To extract the fruit extract, the mixture was filtered via filter paper. A dry test tube containing approximately 2 mL of the fruit sample was filled with 2-3 drops of concentrated tetraoxosulphate (VI) acid and 3 drops of 1% α-naphthol in 80% ethanol. The combination was then stirred vigorously to create an upper phase. The presence of carbohydrates is indicated by the development of a purple or brown color.

## 2.3. Experimental materials (*Sorghum bicolor*)

*Sorghum bicolor*, the experimental plant, was purchased from Lokoja's new market in Kogi State. It was put in a water-filled container to undergo a viability screening. The submerged seeds were dried for use and cleaned with alcohol while the floating seeds were thrown away (Ayinde and Agbakwuru, 2010). Commercial methotrexate was acquired from the Lokoja, Kogi State, Nigeria pharmacy.

### 2.3.1. Determination of growth inhibitory effects of *A. nilotica* fruit aqueous extract on *S. bicolor* seed radical length

*Acacia nilotica* (4,000 mg) was dissolved in 40 mL of distilled water to obtain a 100 mg $mL^{-1}$ stock solution. Various concentrations (8, 14, 20, 26, 32, 38, 44, and 50 mg $mL^{-1}$) of *A. nilotica* aqueous extract were prepared and methotrexate 0.167 mg $mL^{-1}$ was used as the positive control. Petri dishes were layered with cotton wool and filter paper (Whatman No. 1). Twenty seeds of *S. bicolor* were placed in each of the Petri dishes. The negative control samples were seeds treated with 15 mL of distilled water containing no extracts. The tested seeds were treated with 15 mL of different prepared concentrations of *A. nilotica* and methotrexate in different Petri dishes, incubated in a dark room, and observed for growth after 24 h. The mean lengths (mm) of radicles emerging from the seeds were measured after 48, 72 and 92 h respectively. The experiment was done in triplicates. The percentage inhibition was calculated as **Eq. 1**:

$$\%inb = \frac{\text{mean radicle length negative control} - \text{mean radicle length treated}}{\text{mean radicle length negative control}} \times 100 \quad (1)$$

where percentage growth = 100 – % inhibition (Ayinde *et al.,* 2011; Chinedu *et al.,*2014).

### 2.3.2. Statistical analysis

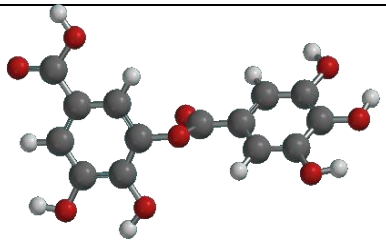
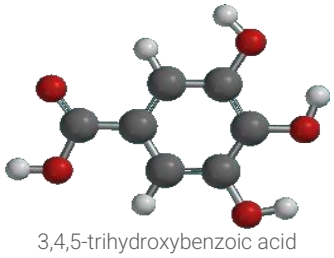
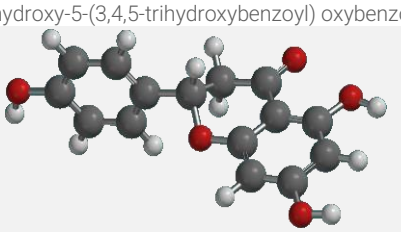
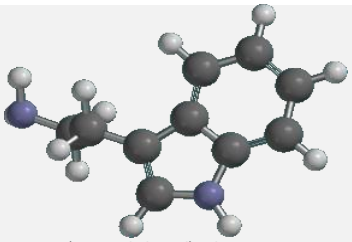
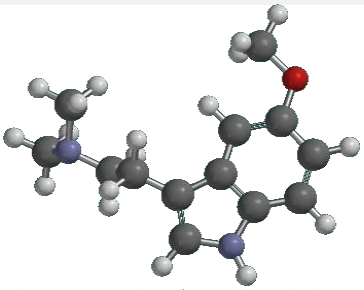
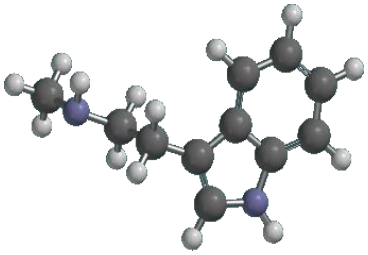
The data obtained were expressed as mean ± standard error mean. A two-way analysis of variance was used to test for significance. $P < 0.001$ was considered significant. Graph pad prism (version 6) was used for the analysis.

## 3. Procedures for molecular docking study

The biochemicals downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) as shown in **Table 1** were optimized (using Density Functional Theory (DFT-B3LYP/6-31G(d,p)) method and docked with the receptors using AutoDock Vina integrated in PyRx software (Horaira *et al.,* 2023; Krishnan *et al.,* 2022; Kirubhanand *et al.,* 2023; Shil *et al.,* 2023).

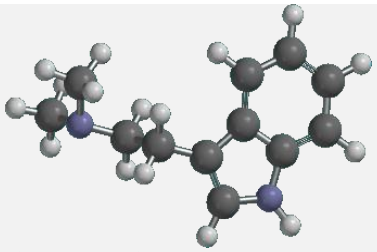
The receptor proteins (dihydrofolate reductase, (**DHFR**, **ID: 1VDR**); X-linked inhibitor of apoptosis protein (**XIAP**, **ID: 6J15**), and the type VII secretion system (**T7SS**, **ID: 6J17**)) were downloaded from protein data bank website (https://www.rcsb.org/) and were cleaned up with Edupymol version 1.7.4.4 and BIOVIA Discovery Studio 2019 as reported (Adegbola *et al.,* 2021; Adepoju *et al.,* 2022; Oyebamiji *et al.,* 2023). The BIOVIA Discovery Studio 2019 was used to view the 2-D structure of the receptor complexed with co-ligand to determine the active gorge of each receptor, and a grid box was set around the active site. The center and size of the grid box are (x = 16.3147, y = -0.4604, z = 27.9177) and (x = 28.1977, y = 25.0013, z = 25.0231) for 1VDR receptor; (x = 62.4438, y = 14.1519, z = 32.9565) and (x = 33.5779, y = 32.4740, z = 38.0195) for 6J15 receptor; and (x = 17.4933, y = 0.213, z = 14.43267) and x = 37.1836, y = 35.6242, z = 40.9731) for 6J17 receptor, respectively.

**Table 1**. Bioactive phytochemicals from *A. nilotica* leaf and fruit plant.

| Code | 3D Structure with IUPAC name | Code | 3D Structure with IUPAC name |
|---|---|---|---|
| D1 |  3,4-dihydroxy-5-(3,4,5-trihydroxybenzoyl) oxybenzoic acid | D2 |  3,4,5-trihydroxybenzoic acid |
| D3 |  5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one | D4 |  2-(1H-indol-3-yl)ethanamine |
| D5 |  2-(5-methoxy-1H-indol-3-yl)-N,N-dimethylethanamine | D6 |  2-(1H-indol-3-yl)-N-ethylethanamine |

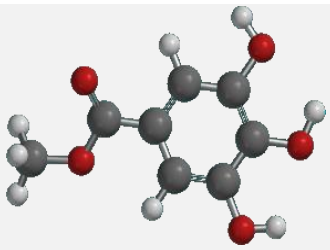
https://doi.org/10.26850/1678-4618.eq.v49.2024.e1512

D7



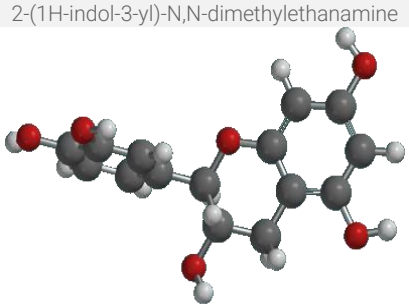
2-(1H-indol-3-yl)-N,N-dimethylethanamine

D8



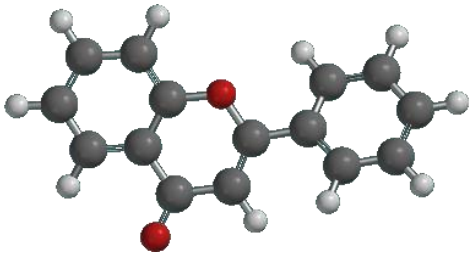
Methyl 3,4,5-trihydroxybenzoate

D9



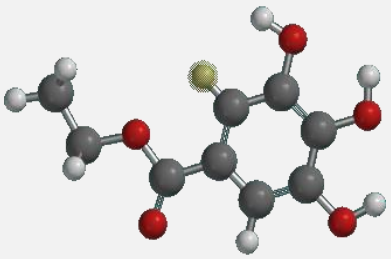
(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol

D10



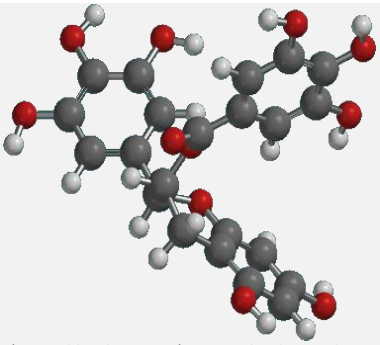
2-phenylchromen-4-one

D11



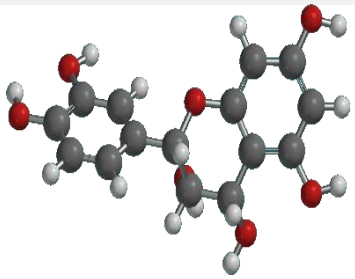
ethyl 3,4,5-trihydroxybenzoate

D12



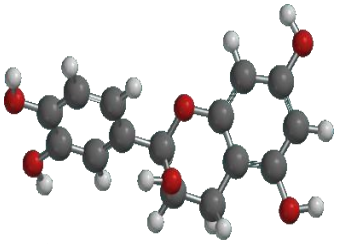
[(2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromen-3-yl] 3,4,5-trihydroxybenzoate

D13



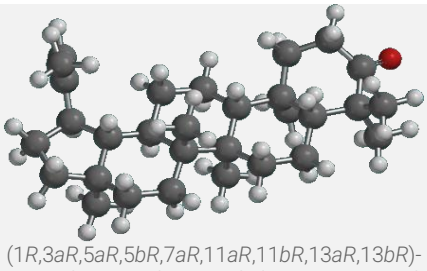
2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,4,5,7-tetrol

D14



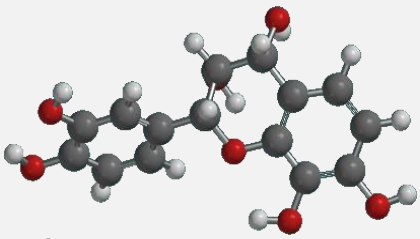
(2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol

D15



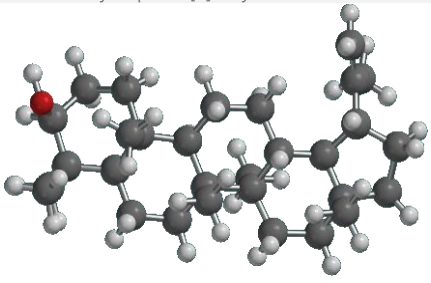
(1*R*,3a*R*,5a*R*,5b*R*,7a*R*,11a*R*,11b*R*,13a*R*,13b*R*)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl-2,3,4,5,6,7,7a,10,11,11b,12,13,13a,13b-tetradecahydro-1*H*-cyclopenta[a]chrysen-9-one

D16

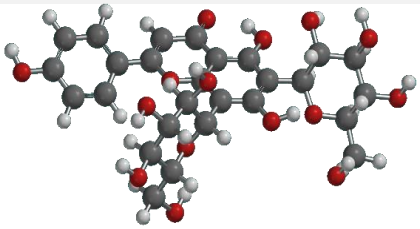


[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3,4,5-trihydroxybenzoate

D17



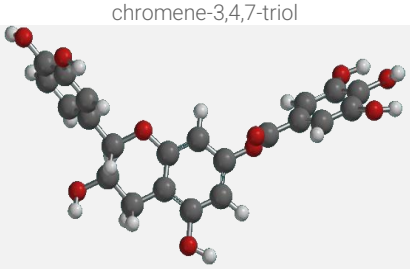
D18



(2*R*,3*R*,4*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,4,7,8-tetrol

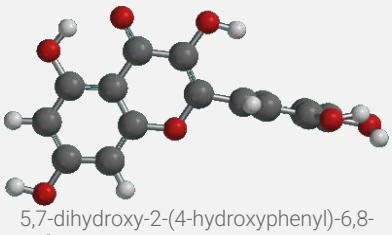
2*R*,3*S*,4*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,4,7-triol

**D19**



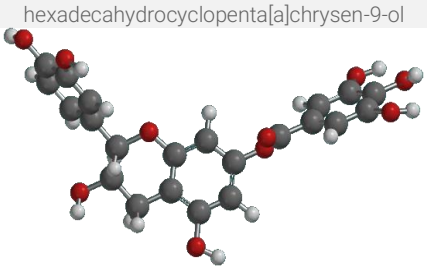
(1*R*,3a*R*,5a*R*,5b*R*,7a*R*,9*S*,11a*R*,11b*R*,13a*R*,13b*R*)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol

**D20**



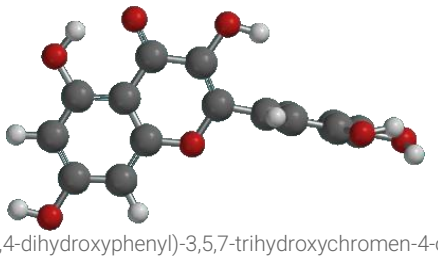
5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-bis[(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one

**D21**



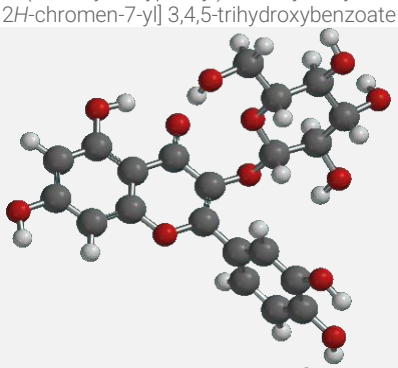
[(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-3,4-dihydro-2*H*-chromen-7-yl] 3,4,5-trihydroxybenzoate

**D22**



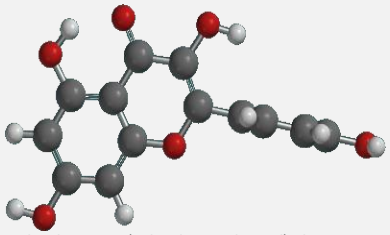
2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one

**D23**



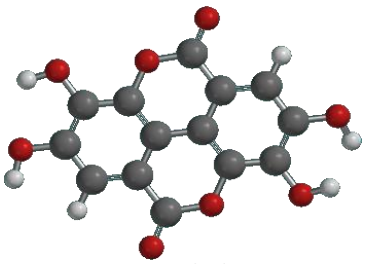
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one

**D24**



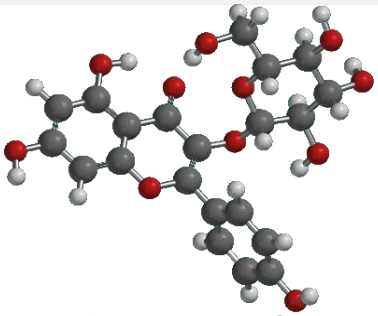
3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one

**D25**



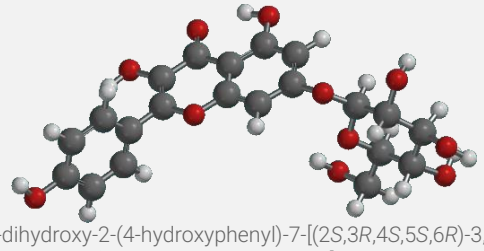
6,7,13,14-tetrahydroxy-2,9-dioxatetracyclo[6.6.2.0$^{4,16}$.0$^{11,15}$]hexadeca-1(15),4,6,8(16),11,13-hexaene-3,10-dione

**D26**



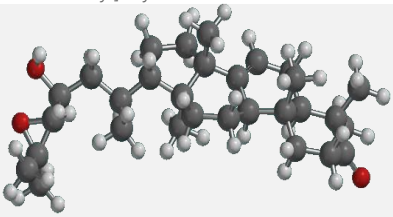
5,7-dihydroxy-2-(4-hydroxyphenyl)-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one

**D27**



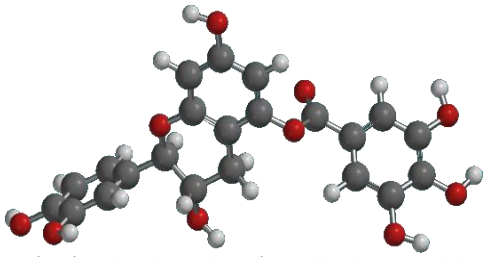
3,5-dihydroxy-2-(4-hydroxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one

**D28**



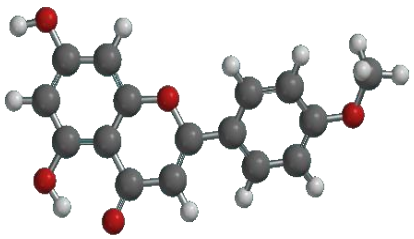
(5*R*,9*R*,10*R*,13*S*,14*S*,17*S*)-17-[(2*S*,4*R*)-4-[(2*S*)-3,3-dimethyloxiran-2-yl]-4-hydroxybutan-2-yl]-4,4,10,13,14-pentamethyl-1,2,5,6,9,11,12,15,16,17-decahydrocyclopenta[a]phenanthren-3-one

D29



[(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-3,4-dihydro-2*H*-chromen-5-yl] 3,4,5-trihydroxybenzoate

D30



5,7-dihydroxy-2-(4-methoxyphenyl)chromen-4-one

# 4. Results and discussion

## 4.1. Phytochemical screening

Five grams of the dried aqueous extract of *A. nilotica* fruit was obtained from a sample of 50 g of powdered *A. nilotica* fruits by water infusion. The yield is 10% (w/w%). **Table 2** displays the findings of the phytochemical analysis of the fruit aqueous extract of *A. nilotica.*

**Table 2.** Preliminary Phytochemical screening of aqueous fruit extract of *A. nilotica.*

| S/No. | Test | Water |
|---|---|---|
| **1** | Carbohydrates | + |
| **2** | Flavonoids | + |
| **3** | Saponins | +++ |
| **4** | Tannins | + |
| **5** | Anthraquinones | - |
| **6** | Alkaloids | + |
| **7** | Terpenoids | + |
| **8** | Steroids | + |

*Note:* + ve indicates presence, whereas; - ve indicates the absence.

According to the results of the phytochemical analysis, *A. nilotica* fruit contains tannins, alkaloids, saponins, flavonoids, terpenes, steroids, and carbohydrates. Due to the compelling experimental data supporting their capacity to alter how the body responds to allergens, viruses, and cancer-causing substances, flavonoids have also been referred to as *natural biological response modifiers.* According to Bello *et al.* (2011), most identified alkaloids and flavonoids exhibit antiallergic, anti-inflammatory, antibacterial, and anticancer properties. Tannins have antibacterial, antiviral, and antitumor properties, while saponins have antioxidant, anticancer, and anti-inflammatory properties (Kunle and Egbarevba, 2009).

## 4.2 Inhibitory effects of A. nilotica fruit on the growth of S. bicolor seed radicle

**Table 3** and **Fig. 1** show the mean radicle length of *S. bicolor* seeds from treated and untreated *A. nilotica* fruit at 48, 72, and 92 h after incubation. As well as the negative control and the positive control, **Table 4** shows the percentage growth inhibition and percentage growth at 48, 72, and 92 h for seeds treated with various doses (8–50 mg $mL^{-1}$) of aqueous fruit extract of *A. nilotica* fruit.

This study reveals that *A. nilotica* fruit has good growth-inhibitory effects on *S. bicolor* seed radicals as compared to the negative control seeds. In the lack of nutrients (the negative control), the *S. bicolor* seed sprouts quickly, mimicking the development of cancer cells in people. Like seeds treated with methotrexate (the reference standard), the length of the radicles of seeds exposed to various concentrations of the extract dramatically shrank. *Acacia nilotica* fruit antiproliferative actions are concentration-dependent because the maximum percentage of inhibitions was seen at 50 mg $mL^{-1}$ after 48, 72, and 96 h of incubation, respectively (**Table 4**). Thiagarajan *et al.* (2020) revealed *A. nilotica* leaf extract's antiproliferative properties against the human cancer cell line KB. *A. nilotica* fruit species have reportedly been shown to possess analgesic, antiplasmodial, antimicrobial, antihyperglycemic, and antimicrobial activities (Abd-Ulgadir and El-Kamali, 2017; Raji *et al.,* 2002;). According to Ogbadoyi *et al.* (2011), the methanol extract of *A. nilotica* pods has antihypertensive properties, while the aqueous extract of the seeds has spasmogenic and vasoconstrictor properties.

**Table 3.** Antiproliferative effect of aqueous extract of *A. nilotica* fruit on seeds radicle length of *S. bicolor*.

| S No. | Concentration (mg $ml^{-1}$) | Percentage inhibition | | | Percentage growth | | |
|---|---|---|---|---|---|---|---|
| | | 48 h | 72 h | 96 h | 48 h | 72 h | 96 h |
| **1** | $H_2O$ (negative control) | 0 | 0 | 0 | 100 | 100 | 100 |
| **2** | MTX (positive control) | 83.92 | 82.19 | 78.76 | 16.08 | 17.80 | 21.24 |
| **3** | 8 | 52.99 | 42.05 | 41.03 | 47.01 | 57.95 | 58.97 |
| **4** | 14 | 69.74 | 59.86 | 50.86 | 30.26 | 40.14 | 49.14 |
| **5** | 20 | 73.45 | 68.59 | 64.09 | 26.55 | 31.41 | 35.90 |
| **6** | 26 | 78.02 | 71.16 | 65.68 | 21.98 | 28.84 | 34.32 |
| **7** | 32 | 84.20 | 78.17 | 75.51 | 15.79 | 21.83 | 24.49 |
| **8** | 38 | 84.77 | 80.91 | 76.94 | 15.23 | 19.09 | 23.06 |
| **9** | 44 | 85.14 | 82.98 | 80.05 | 14.86 | 17.02 | 19.95 |
| **10** | 50 | 87.06 | 83.48 | 81.45 | 12.94 | 16.52 | 18.55 |

*Note:* MTX = methotrexate.

**Table 4.** Mean radical length growth of *S. bicolor* seeds treated with aqueous extract of *A. nilotica* fruit and reference standards.

| S No. | Concentration (mg ml$^{-1}$) | Mean radical length (mm) | | |
|---|---|---|---|---|
| | | 48 h | 72 h | 96 h |
| 1 | Water (-ve control) | 26 ± 2 | 29 ± 2 | 31 ± 2 |
| 2 | Methotrexate (+ve control) | 4.2 ± 0.2 | 5.2 ± 0.4 | 6.7 ± 0.8 |
| 3 | 8 | 12.4 ± 0. 9 | 17 ± 1 | 19 ± 1 |
| 4 | 14 | 8.0 ± 0.4 | 11.7 ± 0.7 | 16 ± 1 |
| 5 | 20 | 7.0 ± 0.6 | 9.2 ± 0.7 | 11.3 ± 0.8 |
| 6 | 26 | 5.8 ± 0.4 | 8.4 ± 0. 7 | 10.8 ± 0.6 |
| 7 | 32 | 4.2 ± 0.4 | 6.4 ± 0.8 | 8 ± 1 |
| 8 | 38 | 4.0 ± 0.5 | 5.6 ± 0.8 | 7 ± 1 |
| 9 | 44 | 3.8 ± 0.3 | 5.0 ± 0.5 | 6.3 ± 0.9 |
| 10 | 50 | 3.4 ± 0.5 | 4.8 ± 0.7 | 6 ± 1 |

*Note:* Values measured as mean ±standard deviation.

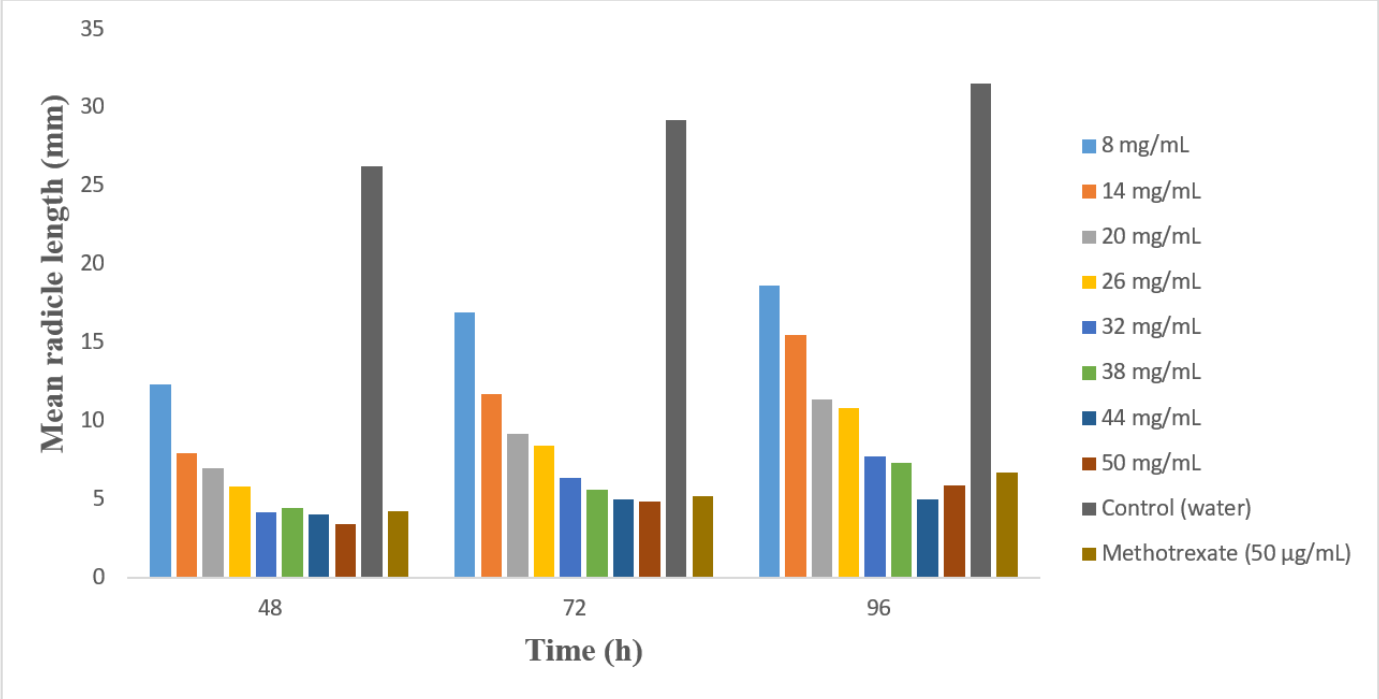



**Figure 1.** The growth inhibitory effects of the aqueous extract of *A. nilotica* fruit on *S. bicolor* seeds radical length and reference standards.

## 4.3 Molecular docking analysis

The binding affinities calculated from docking simulation of the bioactive compounds or phytochemicals in the leaf and fruit of the *A. nilotica* plant with the cell proliferating enzymes are presented in **Table 5**. The phytochemicals are docked against dihydrofolate reductase (**DHFR**) for the proliferation of cancer cells (**ID: 1VDR**), X-linked inhibitor of apoptosis protein (**XIAP**) that initials another enzyme called capase-9 for accelerating spreading of cancer (**ID: 6J15**) and enzyme type VII secretion system (**T7SS**) that aids the multiplications of Mtb in human body (**ID: 6J17**), and the interacting modes (hydrogen bonding, hydrophobic and Van der Waal interactions) of the phytochemicals with promising affinities are displayed in **Figs. 2–4**.

Molecular docking results of dihydrofolate reductase (DHFR; 1VDR) docked with the phytochemicals from *A. nilotica* showed that most of the ligands have higher binding affinities than methotrexate, a chemotherapeutic drug for cancer patients. The calculated binding affinities for the most outstanding compounds are **D12** (–36.0 kJ mol$^{-1}$), **D15** (–39.7 kJ mol$^{-1}$), **D21** (–38.5 kJ mol$^{-1}$), **D19** (–38.9 kJ mol$^{-1}$), **D27** (–35.6 kJ mol$^{-1}$), **D28** (–37.7 kJ mol$^{-1}$) and **D29** (39.7 kJ mol$^{-1}$) compare to methotrexate with binding affinity of –35.1 kJ mol$^{-1}$ (**Tables 1 and 5**), indicating that these compounds can inhibit dihydrofolate reductase better than methotrexate. The drug-likeness of a compound increases with decreasing binding affinity, thus, increasing the ability of the compound to inhibit well (Oyewole *et al.,* 2020; Omotayo *et al.,* 2022). In **1VDR**–ligand complex, **D12** is hydrogen bonded with Tyr 108, also interacted through π–π stacking and π–alkyl with Tyr 33 and Ile 15, respectively; **D15** is interacted with Ala 103 through hydrogen bond, whereas **D19** formed hydrogen with Gly 19; **D21** is hydrogen bonded to Gly 19, Gly 20, Thr 48, Val 6 and Ala 8; π–alkyl with Leu 21, Tyr 33 and Ala 8; **D27** formed two hydrogen bonds with Gly 19 and Ala 8; **D28** interacted via hydrogen bond with Asp 29 and Lys 30 while **D29** is hydrogen bonded to Tyr 108 and Ile 26 (**Fig. 2**).

Similarly, results from docking of **XIAP (ID: 6J15)** with the ligands showed that six phytochemicals have higher inhibitory activities than azacitidine (Table 5) vis-à-vis **D12** (–31.8 kJ mol$^{-1}$), **D15** (–31.4 kJ mol$^{-1}$), **D21** (–33.9kJ mol$^{-1}$), **D27** (–32. 2 kJ mol$^{-1}$), **D28** (31.8 kJ mol$^{-1}$) and

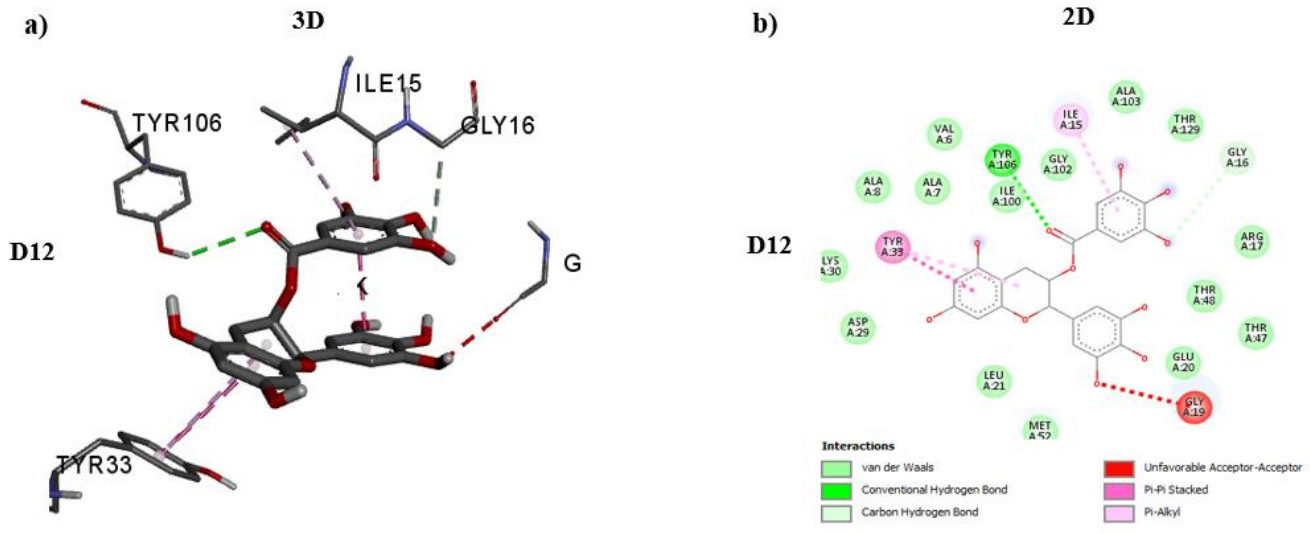
D29 (–33.9 kJ mol$^{-1}$). Thus, these phytochemicals can be better drug candidates than azacitidine (–23.8 kJ mol$^{-1}$) for the treatment of leukemia. The 6J15-ligand complex revealed that D15, D21, D27 and D28 were involved in nonbonding interactions with 6J15. However, D12 formed a hydrogen bond with Trp 99 and Val 48, while D29 interacted through a hydrogen bond with Gly 97 (Fig. 3).

Furthermore, for Mtb (ID: 6J17), the docking results presented in Table 5 revealed that D9 (–32.6 kJ mol$^{-1}$), D21 (–33.1 kJ mol$^{-1}$), D22 (–32.6 kJ mol$^{-1}$), D26 (–32.2 kJ mol$^{-1}$), D27 (–33.5 kJ mol$^{-1}$) and D29 (–38.1 kJ mol$^{-1}$) displayed outstanding binding affinities against Mtb than isoniazid (–22.2 kJ mol$^{-1}$), a chemotherapeutic drug for the treatment of tuberculosis. In 6J17–ligand complex showed that D9 has four hydrogen bonds, D21 and D29 have five hydrogen bonds, D22 has one hydrogen bond and D27 has six hydrogen bonds with amino acid residues in the active site of the 6J17.

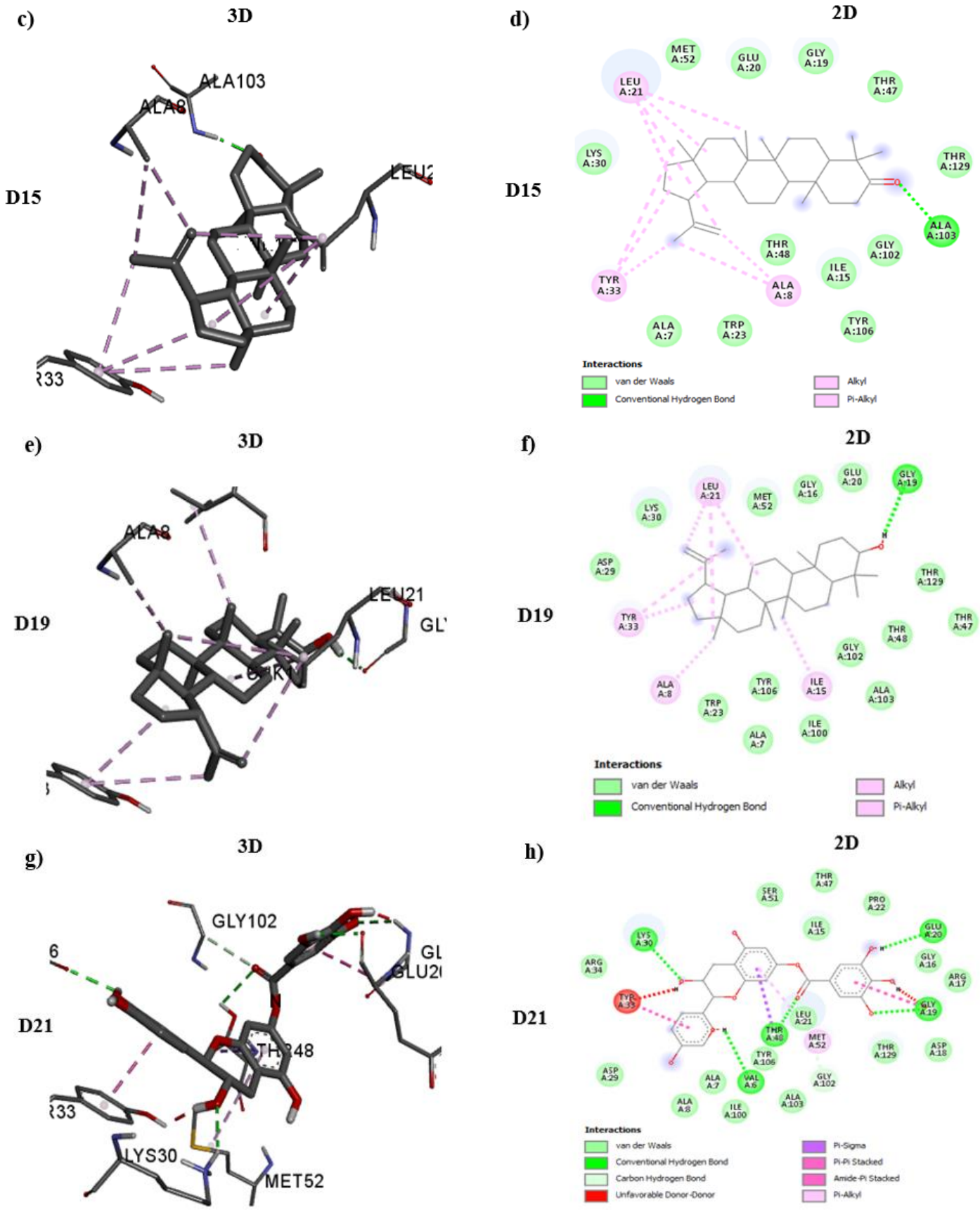
Therefore, both experimental and *in silico* results supported the use of *A. nilotica* for the treatment of cancer and related diseases including tuberculosis, and that hydrogen bonding, hydrophobic and Van der Waal interactions (Figs. 2–4) play crucial roles in inhibitory activities of these phytochemicals.

**Table 5.** Binding affinities of active phytochemicals with DHFR (ID: 1VDR), XIAP (ID: 6J15), and T7SS (ID: 6J17).

| S/N | Ligand | PDB ID: 1VDR | | PDB ID: 6J15 | | PDB ID: 6J17 | |
|---|---|---|---|---|---|---|---|
| | | Binding affinity (kJ mol$^{-1}$) | Inhibitory constant (Ki, µmol/L) | Binding affinity (kJ mol$^{-1}$) | Inhibitory constant (Ki, µmol/L) | Binding affinity (kJ mol$^{-1}$) | Inhibitory constant (Ki, µmol/L) |
| 1 | D1 | −32.6 | 1.90 | −29.3 | 7.35 | −30.1 | 5.24 |
| 2 | D2 | −23.4 | 78.14 | −20.9 | 215.24 | −23.8 | 66.00 |
| 3 | D3 | −33.9 | 1.15 | −28.5 | 10.30 | −32.2 | 2.25 |
| 4 | D4 | −23.8 | 66.00 | −23.8 | 66.00 | −23.4 | 78.14 |
| 5 | D5 | −26.4 | 23.96 | −21.8 | 153.55 | −22.6 | 109.54 |
| 6 | D6 | −25.5 | 33.59 | −23.4 | 78.14 | −24.3 | 55.74 |
| 7 | D7 | −24.3 | 55.74 | −22.2 | 129.69 | −22.2 | 129.69 |
| 8 | D8 | −22.6 | 109.54 | −20.9 | 215.24 | −25.1 | 39.77 |
| 9 | D9 | −32.6 | 1.90 | −28.9 | 8.70 | −32.6 | 1.90 |
| 10 | D10 | −33.9 | 1.15 | −30.1 | 5.24 | −31.0 | 3.74 |
| 11 | D11 | −23.0 | 92.52 | −21.3 | 181.80 | −23.8 | 60.00 |
| 12 | D12 | −36.0 | 0.49 | −31.8 | 2.67 | −32.2 | 2.25 |
| 13 | D13 | −33.1 | 1.61 | −26.8 | 20.24 | −30.1 | 5.24 |
| 14 | D13 | −32.6 | 1.90 | −28.5 | 10.30 | −31.4 | 3.16 |
| 15 | D15 | −39.7 | 0.11 | −31.4 | 3.16 | −31.8 | 2.67 |
| 16 | D16 | −30.1 | 5.24 | −26.8 | 20.24 | −29.3 | 7.35 |
| 17 | D17 | −32.2 | 2.25 | −29.7 | 6.21 | −31.4 | 3.16 |
| 18 | D18 | −33.1 | 1.61 | −28.9 | 8.70 | −31.4 | 3.16 |
| 19 | D19 | −38.9 | 0.15 | −31.0 | 3.74 | −31.0 | 3.74 |
| 20 | D20 | −33.5 | 1.34 | −31.0 | 3.74 | −31.0 | 3.74 |
| 21 | D21 | −38.5 | 0.18 | −33.9 | 1.15 | −33.1 | 1.61 |
| 22 | D22 | −33.1 | 1.61 | −28.0 | 12.19 | −32.6 | 1.90 |
| 23 | D23 | −33.9 | 1.15 | −27.6 | 14.44 | −30.5 | 4.43 |
| 24 | D24 | −32.6 | 1.90 | −27.2 | 17.09 | −31.4 | 3.16 |
| 25 | D25 | −33.5 | 1.36 | −28.0 | 12.19 | −31.8 | 2.67 |
| 26 | D26 | −34.3 | 0.97 | −30.1 | 5.24 | −32.2 | 2.25 |
| 27 | D27 | −35.6 | 0.58 | −32.2 | 2.25 | −33.5 | 1.36 |
| 28 | D28 | −37.7 | 0.25 | −31.8 | 2.67 | −30.5 | 4.43 |
| 29 | D29 | −39.7 | 0.11 | −33.9 | 1.15 | −38.1 | 0.21 |
| 30 | D30 | −31.0 | 3.74 | −28.0 | 12.19 | −31.0 | 3.74 |
| Methotrexate | | −35.1 | 0.69 | | | | |
| Azacitidine | | | | −23.8 | 47.08 | | |
| Isoniazid | | | | | | −22.2 | 129.69 |



https://doi.org/10.26850/1678-4618.eq.v49.2024.e1512



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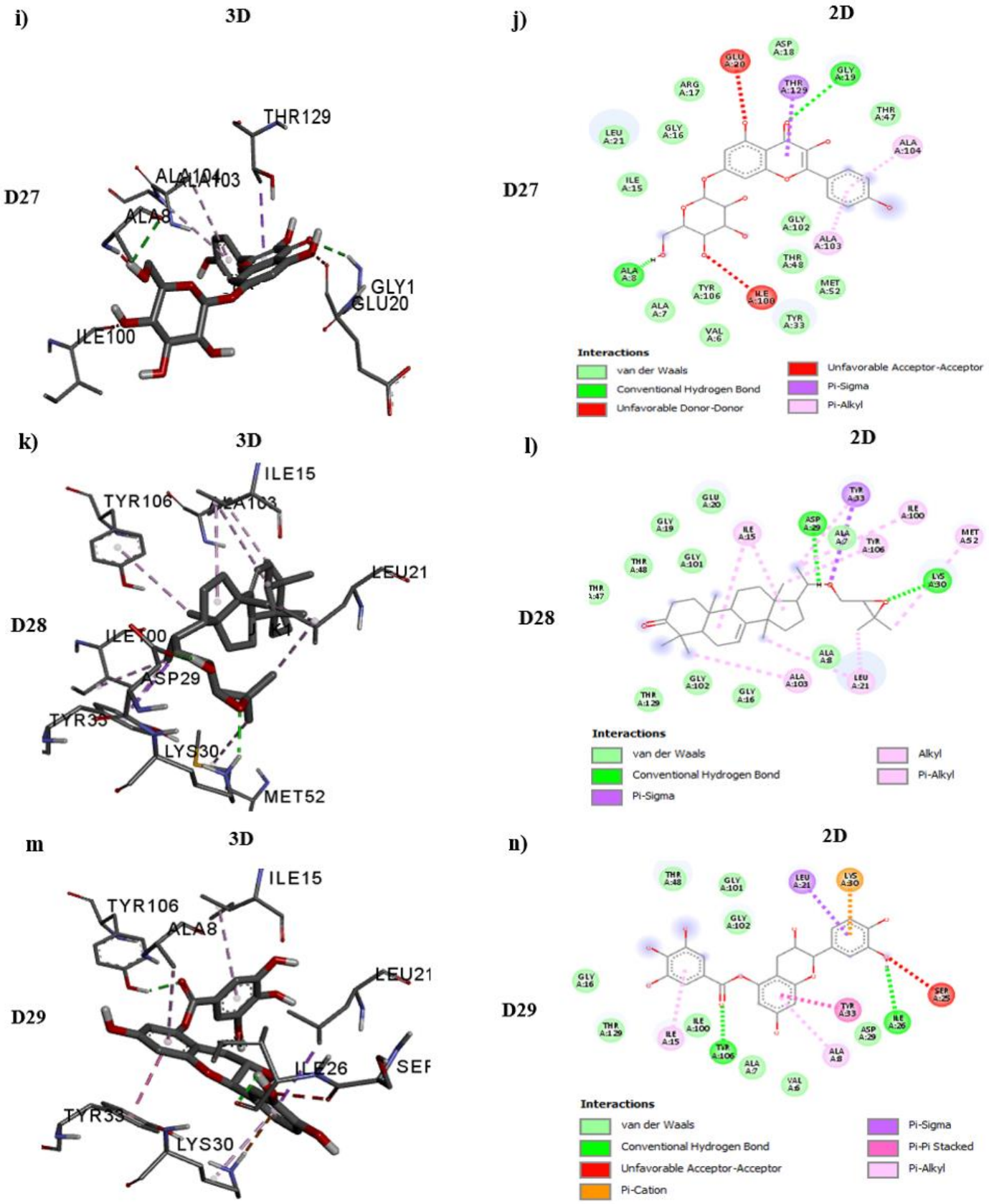
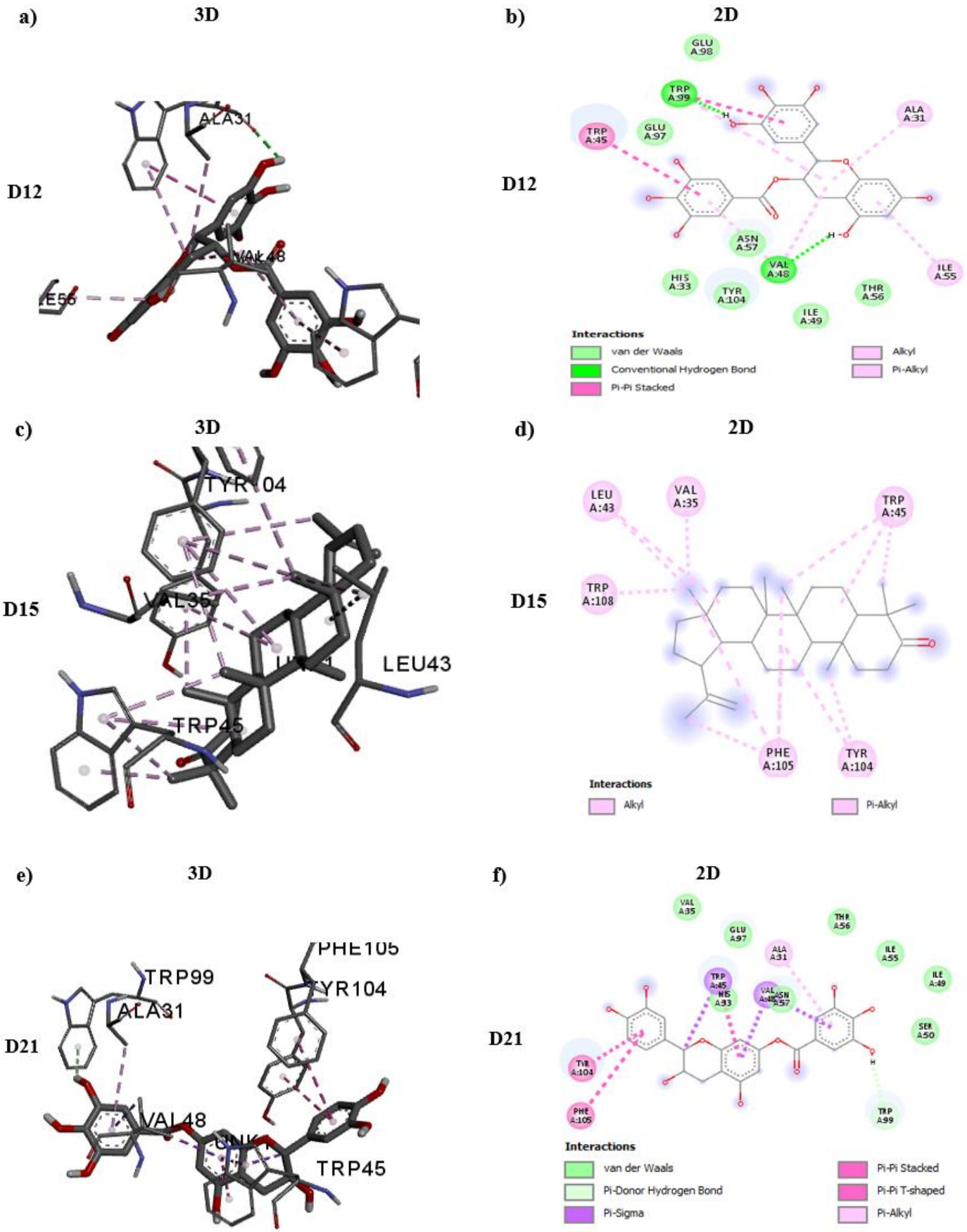



**Figure 2.** Docking ligand-receptor complexes of selected ligands with the highest binding affinity (PDB ID: 1VDR).

https://doi.org/10.26850/1678-4618.eq.v49.2024.e1512



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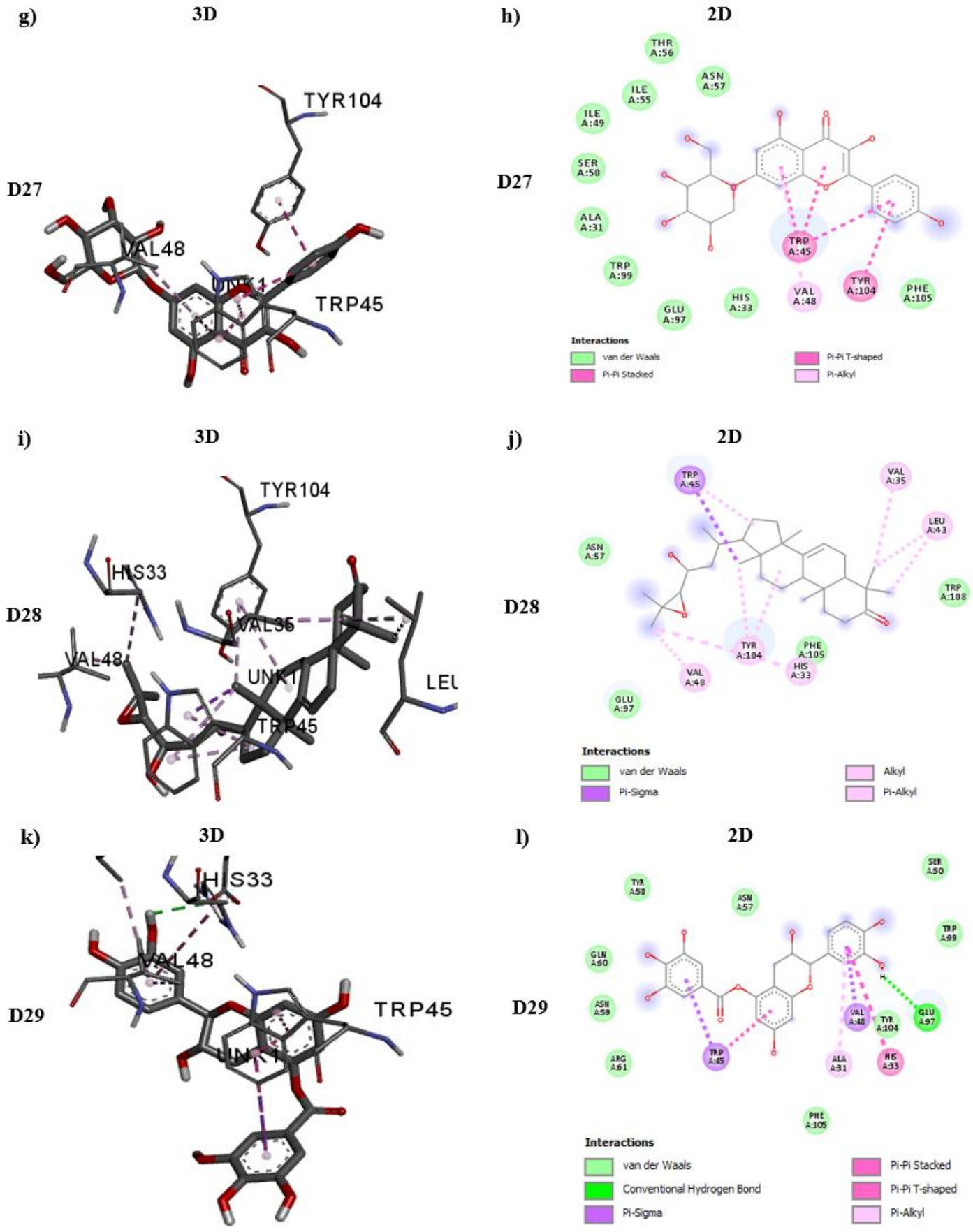
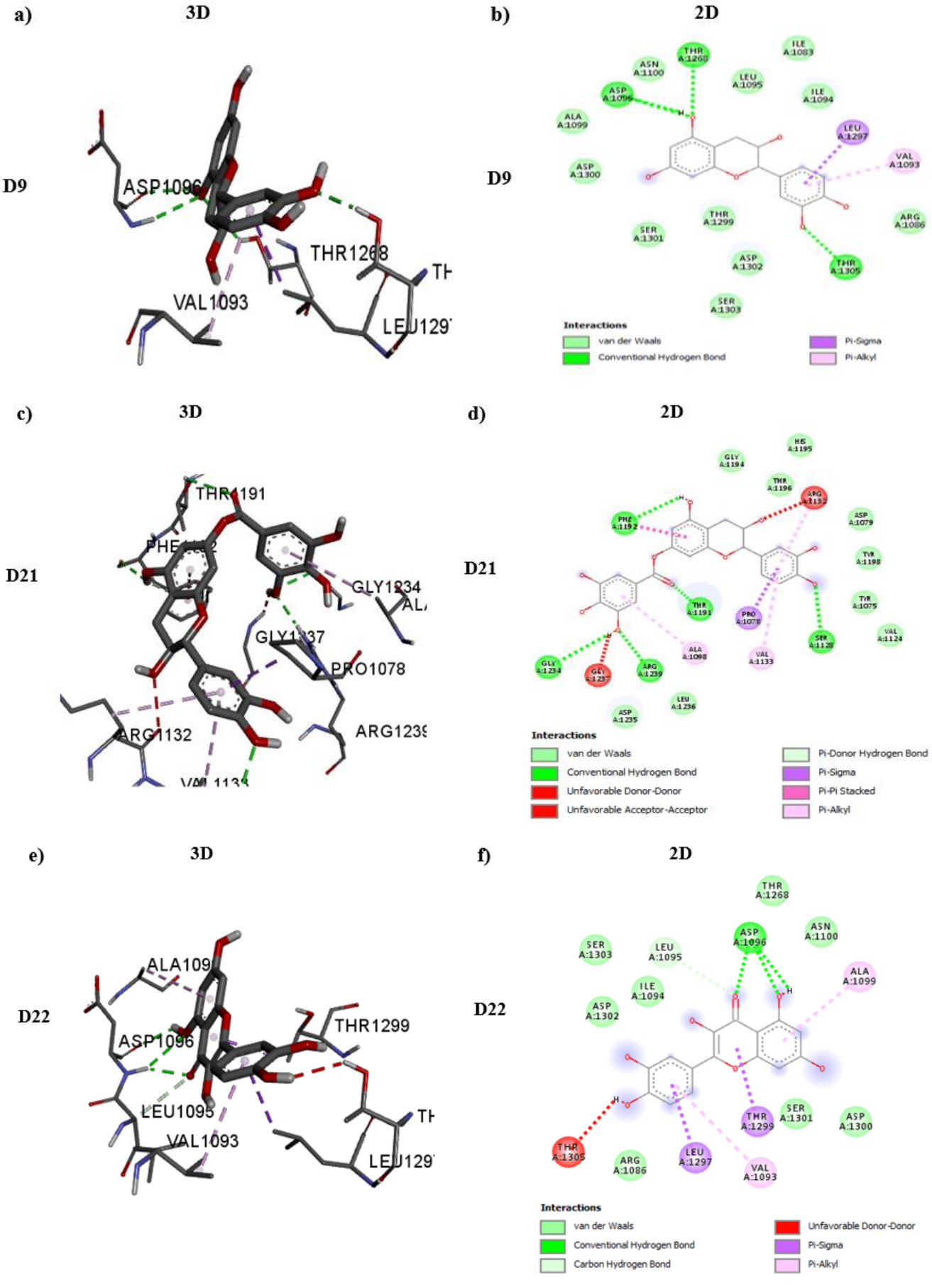



**Figure 3**. Ligand-receptor complexes of THE selected ligands with the highest binding affinity (PDB ID: 6J15).

https://doi.org/10.26850/1678-4618.eq.v49.2024.e1512



https://doi.org/10.26850/1678-4618.eq.v49.2024.e1512

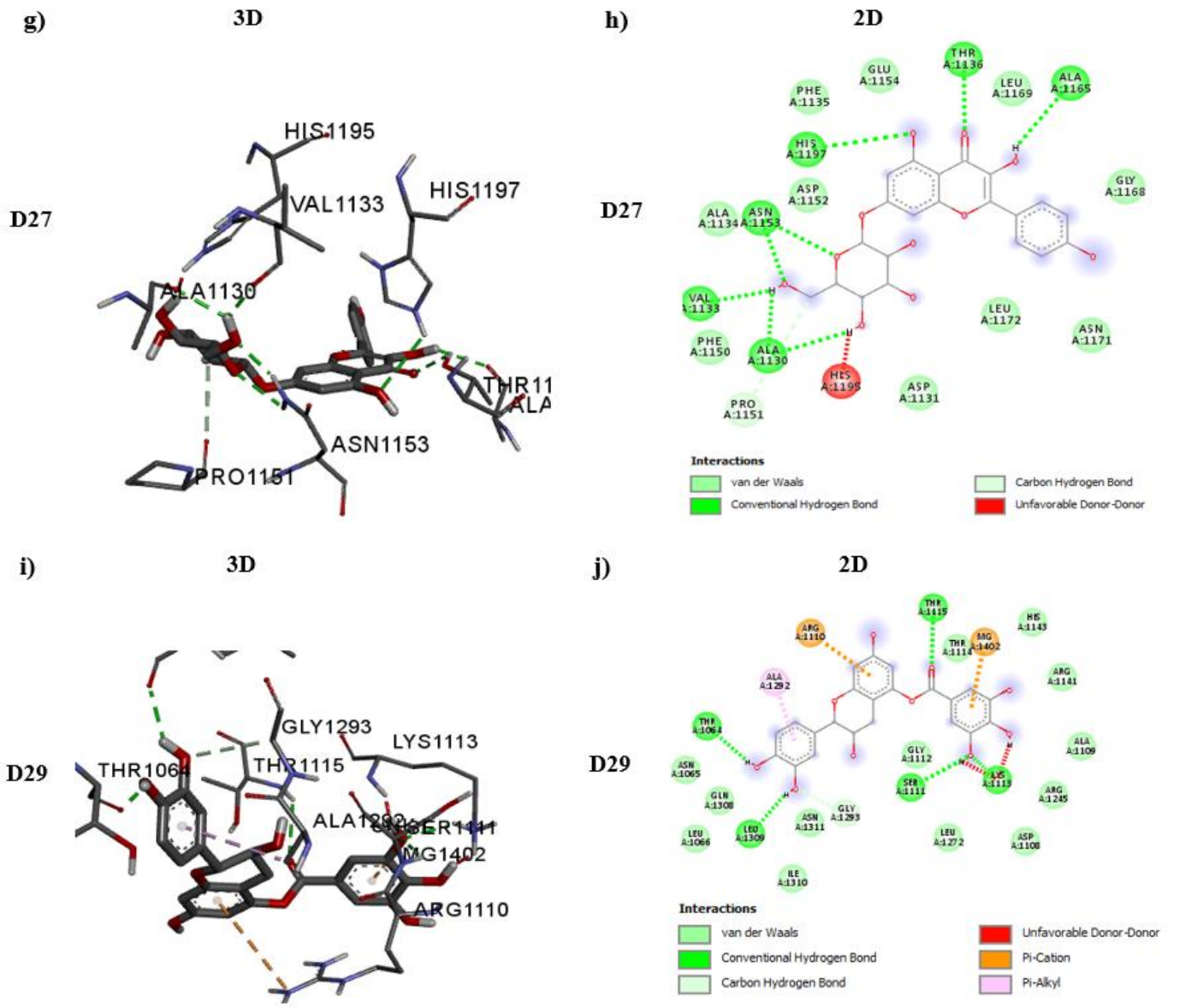



**Figure 4.** Ligand-receptor complexes of selected ligands with the highest binding affinity (PDB ID: 6J17).

## 4. Conclusions

The phytochemical screening on the *A. nilotica* fruit aqueous extract was carried out using standard procedures to identify chemical constituents and saponins are observed to be present in abundance in the aqueous extract. The aqueous extract showed maximum antiproliferative activity at 50 mg $mL^{-1}$ on seed radicles of *S. bicolor.* Molecular docking results revealed that **D21**, **D28** and **D29** displayed stronger binding affinities against **1VDR**, **6J15** and **6J17** receptors than the standard drugs used in the work. Although the compound **D29** ([(2R,3S)-2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-3,4-dihydro-2H-chromen-5-yl] 3,4,5-trihydroxybenzoate) has highest binding affinity, these compounds might play essential roles in the antiproliferation activity of *A. nilotica*. Thus, the docking results supported the antiproliferative activity of *A. nilotica*, which may rationalize the use of *A. nilotica* as a traditional medicine for the treatment of cancer and other related diseases.

## Authors' contributions

**Conceptualization**: William, O. A.; Semire, B.; **Data curation:** William, O. A.; Semire, B.; Ejike, O. O.; **Formal Analysis:** William, O. A.; Semire, B.; Godfrey, O. E.; **Funding acquisition:** Not applicable; **Investigation:** William, O. A.; Semire, B.; **Methodology:** William, O. A.; Semire, B.; **Project administration:** Semire, B.; Obiyenwa, K. G.; **Resources:** William, O. A.; Semire, B.; Ejike, O. O.; **Software:** William, O. A.; Semire, B.; Ejike, O. O.; **Supervision:** Semire, B.; Ejike, O. O.; Obiyenwa, K. G.; **Validation:** Godfrey, O. E.; Semire, B.; Obiyenwa, K. G.; **Visualization:** William, O. A.; Semire, B.; **Writing – original draft:** William, O. A.; Semire, B.; **Writing – review & editing:** William, O. A.; Semire, B.; Ejike, O. O.; Obiyenwa, K. G.; Godfrey, O. E.

## Data availability statement

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

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