

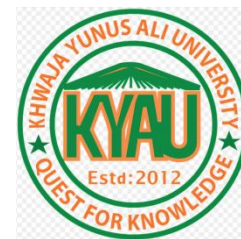
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Research Article

Evaluation of anthelmintic activities and ADME/T properties of *Ficus racemosa* Linn.

Khurshid Jahan Shammi¹, Safayet Jamil¹, Asma Akhter¹, Naznin Shahria¹, Imran Mahmud^{1*}

¹Department of Pharmacy, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh

*Correspondence to: imran.mahmud.pharm@kyau.edu.bd (Imran Mahmud, Assistant Professor, Department of Pharmacy, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh)

Abstract

The aim of this study was to determine the anthelmintic activity of ethanolic extract of *F. racemosa* against bovine parasite. After that, the isolated compounds were put through molecular docking and an ADME/T evaluation. The current investigation includes the use of ethanol extraction on dried leaves. The anthelmintic activity of the compound was determined with the help of an experimental model utilizing the worm *Paramphistomum cervi*, Schrödinger-Maestro v10.1 docking fitness was then used to conduct a molecular docking analysis to discover compounds with the highest activity against the Tubulin-Colchicine enzyme. Moreover, the Swiss ADME online-based application was used to review the ADME/T profiles. Preliminary phytochemical screening of ELFR revealed that it contains alkaloids, glycoside, flavonoids, tannins, gums, carbohydrate and quinone.

ELFR showed a dose-dependent and statistically significant anthelmintic activity on experimental worm (*Paramphistomum cervi*). ELFR having a concentration of 200 mg/ml showed lowest paralysis and death time of 8.33 and 12.84 min, respectively, which is comparable with that of standard drug albendazole. Moreover, the molecular docking study reveals that among the seven compounds isolated from the plant, β -sitosterol has the best docking score of -15.4 kcal/mol against Tubulin-Colchicine and ADME/T analysis using web-based tool ensures that the compound has not violated Lipinski's rule of five indicating its safety consumption. The findings from both in vitro and in silico studies corroborate the anthelmintic activity of *Ficus racemosa* leaves. The data support beta-sitosterol to be a potential anthelmintic agent worthy of further comprehensive clinical studies and exploring its drug-like properties.

Keywords: *F. racemosa*, Anthelmintic, Molecular docking analysis, ADME/T property

1. Introduction

Medicine is the wonder of the world and blessings for mankind. From the very ancient time people used various plant parts as therapeutic aid for alleviating ailments. It is common knowledge that herbs have a wealth of medicinal compounds that can treat a variety

of conditions. On the other hand, the ethnopharmacological applications of these medicinal plants may differ depending on the tenets of a certain individual's traditional medical system and the compositions that are made by regional ethnic communities (Alongi *et al.*, 2001).

There is also a connection between traditional medicine and medicinal plants. The term "modern medicine" refers to the numerous compositions that are established in a scientific way by trying to make use of sophisticated technology and knowledge of how they are now implemented in sophisticated pharmacopoeias for the purpose of treating diseases and improving health. These compounds are used in modern medicine to treat and prevent infection (Cragg and Newman, 2005). Scientists worked hard to discover and isolate bioactive components with therapeutic applications, which are now used in the manufacturing of modern medicine. They did this by first isolating various components of plants, then introducing those components into a biological process and conducting pharmacological tests on the substances. Some manufacturing companies were involved in the manufacture of synthetic drugs; however, after prolonged use, synthetic drugs began exhibiting severe adverse effects. As a result, even in developed countries, there is a renewed interest in natural drugs and natural foods; therefore, we may pay close attention to our herbs and medicinal plants (Berg, 1989; Husain, 1992).

An active ingredient is referred to as a "drug candidate" during the drug discovery process if it has a pharmacological or biological action that is likely to be beneficial for therapeutic purposes but still has a suboptimal framework that needs to be modified in order to fit effectively to the target. Its chemical structure is utilized as a starting point for chemical alterations that are carried out in order to increase the efficacy, specificity, or pharmacokinetic properties of the compound (Oprea *et al.*, 2001). Natural products, combinatorial chemistry, or molecular modeling are used to find lead compounds. High-throughput screenings (where active compounds are called "hits") test lead compounds to see if they can block (antagonist) or turn on (agonist) a specific receptor and figure out how selective they are (Shoibe *et al.*, 2017).

Because of their low bioactivity or high toxicity, large and intricate structures that are difficult to synthesize commercially, or poor solubility, natural products from plants provide model molecules for the design and synthesis or semi-synthesis of novel drugs (Wang *et al.*,

2019). Intestinal helminths (roundworms, tapeworms, and flukes) are parasitic nematodes transmitted through soil that infect one-third of the world's population and cause substantial economic losses due to the death of animals and crop failures (Hotez *et al.*, 2008). Unfortunately, many parasites are becoming resistant to the few medications used to treat human helminthiasis infections that have been developed during the past fifty years of research. For instance, this is becoming an urgent issue in the agricultural and environmental fields; for instance, helminthic diseases can be treated with a wide range of drugs including macrocyclic lactones, benzimidazoles, praziquantel, and imidathiazoles, but one study found that the organisms quickly developed resistance to these drugs. So, plants can help in the search for new targets for anthelmintic medicines (Oprea *et al.*, 2001).

The green, modest, sprawling, lactiferous, woody *F. racemosa* Linn. (Moraceae) tree grows to a height of 15-18 m and has no noticeable aerial roots (Berg, 1989). *F. racemosa* can reach heights of more than 40 feet and widths of 20 to 40 feet. The size of this tree, classified as deciduous, is somewhere in the middle. The thick, verdant greenery provide for pleasant cooling. The length of these dark green leaves is between 7.5 and 10 centimeters. The spherical fruits of the *F. racemosa* range in size from about an inch and a half to two inches in diameter, and develop immediately on the trunk. Tiny, many, and grain-like in appearance, the seeds are in plenty. Bark thickness ranges from 0.5 to 1.8 cm and is reddish-grey or grayish-green in color with a smooth, pliable surface that is prone to cracking. The exterior rubs off in papery white flakes, while the interior is light brown, fibrous, and tastes mucilaginous without any distinctive odor. *F. racemosa* has long, dark brown roots. It smells and tastes distinctively, with a touch of bitterness. The form of a root tends to be atypical (Deep *et al.*, 2013). Seeds are another useful tool for plant reproduction. Several conditions are treated using the plant in the traditional medical system. Bark, roots, leaves, fruits, and latex are just some of the plant parts that have been put to use as astringents, carminatives, vermifuges, and anti-dysentery remedies. It helps control hunger pangs effectively. Diabetes, leucoderma, and menorrhagia are all treated with the fruit extract. Wounds, lymphadenitis, sprains, and

fibrositis all benefit from its local use to reduce inflammation (Shah *et al.*, 2016).

There are sterols, triterpenoids (such as lanosterol), alkaloids, tannins, and flavonoids found in the plant's leaves. Stem bark contains gluanol acetate, β -sitosterol (Shrivastava *et al.*, 1977), leucocyanidin-3-O-Dglucopyranoside, leucopelargonidin-3-O-Lrhamnopyranoside, lupeol, ceryl behenate, and α -amyrin acetate. Stem bark supplied lupenol, β -sitosterol, and stigmasterol. In addition to higher hydrocarbons (Hentriacontane), fruit contains gluanol acetate, glucose, tiglic acid, taraxasterol esters, lupeol, friedelin, and phytosterols. Gluanol acetate, which is 13 α , 14 β , 17 β H, 20 α H-lanosta-8, 22-diene-3-acetate, was extracted from the leaves, together with racemosic

2. Methodology

2.1 Collection of plant material

The leaves of *F. racemosa* were collected in the month of January, 2022 from Enayetpur, Sirajganj.

2.2 Extraction

The powdered leaves of *F. racemosa* were put through a cold extraction process with 95% ethanol for seven days, during which time they were stirred and shaken on a regular basis. Following the process of maceration, the mixture was separated in order to eliminate any undesired plant elements and produce a mixture that was transparent. To obtain the crude extract, the filtrate was collected and then continuously concentrated in a rotary evaporator at 40°C.

2.3 Preliminary phytochemical screening

Standard chromogenic reagents were used to identify the major phytochemical groups of secondary bioactive metabolites present in ELFR (ethanolic leaf extract of *F. racemosa*) (Sofowora, 1982).

In vitro Anthelmintic activity test

The method that had already been explained was used to figure out how well ELFR worked as an antihelmintic (Sofowora, 1982).

2.4 Experimental worm

Parasites that can reproduce asexually researchers detected *Paramphistomum cervi* (Trematoda) parasites in the blood of recently killed cattle. The parasites were scrubbed in phosphate-buffered saline (PBS) with a concentration of 0.9% and a pH of 7.4. This solution

acid. Gluanol acetate is a tetracyclic triterpene. The thermostable aspartic protease that was synthesized by plant latex was really remarkable. There was evidence of gluanol acetate present in the fruit, as well as the stem and the bark (Shrivastava *et al.*, 1977).

Despite the fact that there is not yet a paper that demonstrates in vitro and in silico that the leaves of *F. racemosa* have anthelmintic action, In light of this, the primary purpose of this research is to conduct an investigation into the anthelmintic activity of ELFR (ethanolic leaf extract of *F. racemosa*) using an experimental model, which will then be followed by an in silico molecular docking investigation and an assessment of ADME/T properties.

was prepared by dissolving 8.01 grams of sodium chloride, 0.20 grams of potassium chloride, 1.78 grams of sodium phosphate, and 0.27 grams of potassium phosphate in one liter of distilled water and then stashing the solution at 37 \pm 1°C.

2.5 Study design

The extract was tested for its anthelmintic efficacy on live bovine parasites, namely *Paramphistomum cervi*. Each batch of parasites consisted of six parasites to facilitate study. Petri plates were constructed and inoculated with extract at concentrations of 25, 50, 100, and 200 mg/mL, and with the reference standard albendazole (obtained from Square Pharmaceuticals Ltd., Bangladesh) at concentrations of 15 mg/mL in 10 mL of PBS. The placebo group received 0.1% Tween 80 in phosphate-buffered saline. In each Petri plate, six parasites were inserted for study. When no motion could be seen at all, even when violently shaken, the onset of paralysis was seen. After determining that the parasites did not react to being shaken forcefully, submerged in heated water (50°C), or otherwise stimulated, the time of death was recorded.

2.6 Preparation of sample

Triturating extracts of 0.25, 0.5, 1, and 2 gm with 0.2% v/v of Tween 80 as a suspending agent and raising the total volume to 10 ml with PBS yielded 25, 50, 100, and 200 mg/ml concentrations of ethanolic extracts of *F. racemosa*, respectively. A standard concentration of 15 mg/ml was made by mixing 150 mg of albendazole

powder with 0.2% v/v Tween 80 as a suspending agent and then filling up to 10 ml with PBS.

2.7 In silico molecular docking study for anthelmintic activity

With a view to performing molecular docking analysis, seven compounds derived from the plant were selected based on their anthelmintic potentiality reported on literature (Vezquez *et al.*, 2012; Sravani *et al.*, 2014; Bouras *et al.*, 2008; Namboodiripad *et al.*, 1968; Okoye *et al.*, 2014; Oliveira *et al.*, 2021).

2.8 Ligand and protein preparation

To name a few of the chemicals found in *F. racemosa*, we have taraxasterol (PubChem CID: 115250), tiglic acid (PubChem CID: 125468), β -sitosterol (PubChem CID: 521199), lupeol (PubChem CID: 259846), and kaempferol (PubChem CID: 259847). (PubChem CID: 52801). The Protein Data Bank was consulted in order to ascertain the three-dimensional structure of the TUBULIN-COLCHICINE receptor, which was then used in the protein synthesis process (ID: 1SAO). After that, the structure was built and refined with the help of the protein order to prepare wizard (Schrodinger-Maestro v. 10.1), which included doing things like attributing charges and bond ordering, trying to add hydrogens to the heavy atoms, changing selenomethionines to methionine, and removing all the water portions. However, the amide groups of asparagines, glutamines, and the imidazole ring of histidines were tweaked at a neutral pH, and the thiol and hydroxyl groups of certain amino acids were rotated. Minimization was performed using the OPLS 2005 force field, and the maximum RMSD for heavy atoms was set at 0.30 Å (Friesner *et al.*, 2004).

2.9 Receptor grid generation

Grids were produced in Glide with the default values of the van der Waals scaling factor set to 1.00 and the charge cut-off set to 0.25. These grids were then exposed to the OPLS 2001 force field. For the receptor, a three-dimensional cube with well-defined dimensions and a center point that corresponds to the centroid of the active site residues was created. It is necessary to locate the active binding site in the target protein, and the

bounding box was set to have dimensions of 16 squares by 16 squares by 16 squares.

2.10 Glide standard precision (SP) ligand docking

Adjustable ligand docking was performed using the Schrodinger-Maestro software Glide, version 10.1. Within this system, non-cis/trans amide bond interactions were punished (Friesner *et al.*, 2004). These molecules were docked with the TUBULIN-COLCHICINE enzyme in Glide's XP mode using the default values for all docking parameters, and a score of above 4 kcal/mol was found, indicating a hit. There were no restrictions placed on bonding during the docking calculations. The ligand poses for all of the input molecules were generated using the Monte Carlo random search method. Every molecule's expected binding affinity to the TUBULIN-COLCHICINE enzyme was calculated using its Glide docking score. For docked molecular energy estimations, the empirical E model score function was also applied. The OPLS 2005 force field was used for the post-docking minimization, and a single posture was captured for each ligand. When calculating the docking score, a penalty equal to one-fourth of the strain difference in energy was imposed on matches when the difference in energy between the bound and free forms of the ligand was more than 4 kcal/mol.

2.11 ADME/T Properties Analysis

Lipinski's Rule of Five (RO5) was followed in this investigation; it states that if a material satisfies no more than two of the following conditions, it will have high resistivity and passive absorption (Lipinski, 2004). The molecular weight should be between 40 and 130, the hydrogen bond donor should be between 5 and 10, the high lipophilicity should be given as a log Po/w value of 5, and the hydrogen bond acceptor should be between 5 and 10. The canonical SMILES for each compound is obtained from PubChem and then transferred into the Swiss ADME online tools (Lipinski *et al.*, 1997) so that the physicochemical properties, lipophilicity, pharmacokinetics, and drug similarity of each molecule may be analyzed. A successful drug candidate development process will proceed with the constituent that best meets the aforementioned criteria.

3.0 Results

Preliminary phytochemical screening

Phytochemical screening of ELFR (ethanolic leaf extract of *F. racemosa*) revealed the presence of

alkaloids, glycoside, flavonoids, tannins, gums, carbohydrate and quinone.

Table 1: Chemical groups present in the Ethanolic leaf extract of *F. racemosa*

Name of the group	Ethanolic leaf extract of <i>F. racemosa</i>
Alkaloids	+
Glycoside	+
Flavonoids	+
Saponins	-
Tannins	+
Proteins	-
Terpenoids	-
Gums	+
Steroids	-
Carbohydrate	+
Quinone	+

Here, (+) indicates Presence, (-) indicates Absence

In vitro anthelmintic activity test

The anthelmintic properties of ELFR were investigated in *Paramphistomum cervi*. The extract showed concentration-dependent anthelmintic activity, which was evaluated in relation to the gold standard anthelmintic medication albendazole. At greater doses, the extract caused paralysis in *P. cervi* more rapidly. Paralysis times of 23.50, 16.66, 11.16, and 8.33 minutes

and death times of 28.66, 21.66, 14.83, and 12.84 minutes were observed for ELFR at concentrations of 25, 50, 100, and 200 mg/ml, whereas these times for the reference medicine albendazole were 6.5 and 9.83 minutes, respectively (Table 2). The study's findings suggest that an extract at 200 mg/ml exhibited anthelmintic effects that were on par with those of the gold standard medication albendazole (Figure 4 & 5).

Table 2: Anthelmintic activity of *F. racemosa*

Groups	Time taken for Paralysis (min) \pm SEM	Time taken for Death (min) \pm SEM
Control (Water)	0.00	0.00
Standard 15 mg/ml (Albendazole)	6.5 \pm 0.56	9.83 \pm 0.83
Extract 25 mg/ml	23.50 \pm 0.43	28.66 \pm 0.80
Extract 50 mg/ml	16.66 \pm 0.61	21.66 \pm 0.81
Extract 100 mg/ml	11.16 \pm 0.75	14.83 \pm 0.74
Extract 200 mg/ml	8.33 \pm 0.80	12.84 \pm 0.47

***Each value in the table is represented as mean \pm SEM (n = 6)

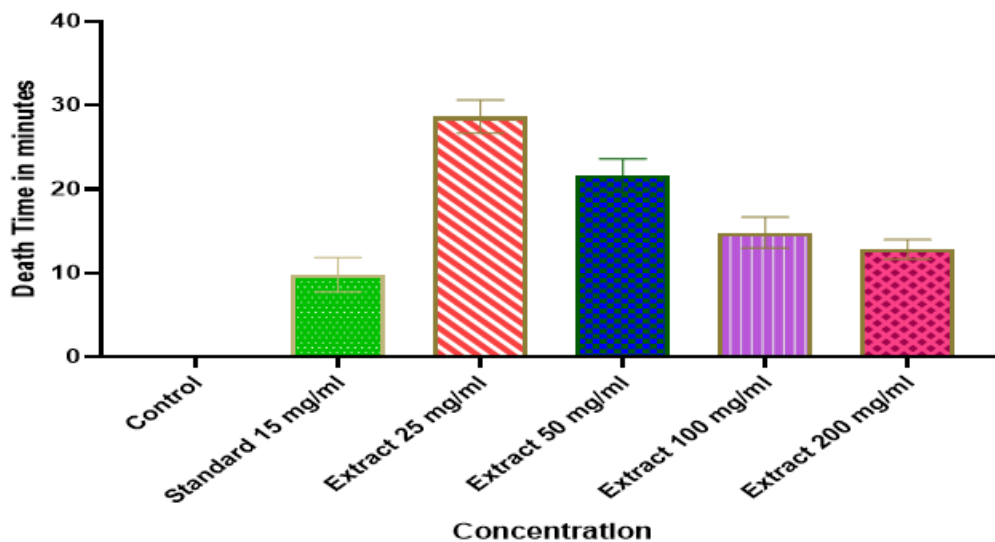


Figure 4: Effect of different doses of ethanolic leaf extract of *F. racemosa* (Paralysis time)

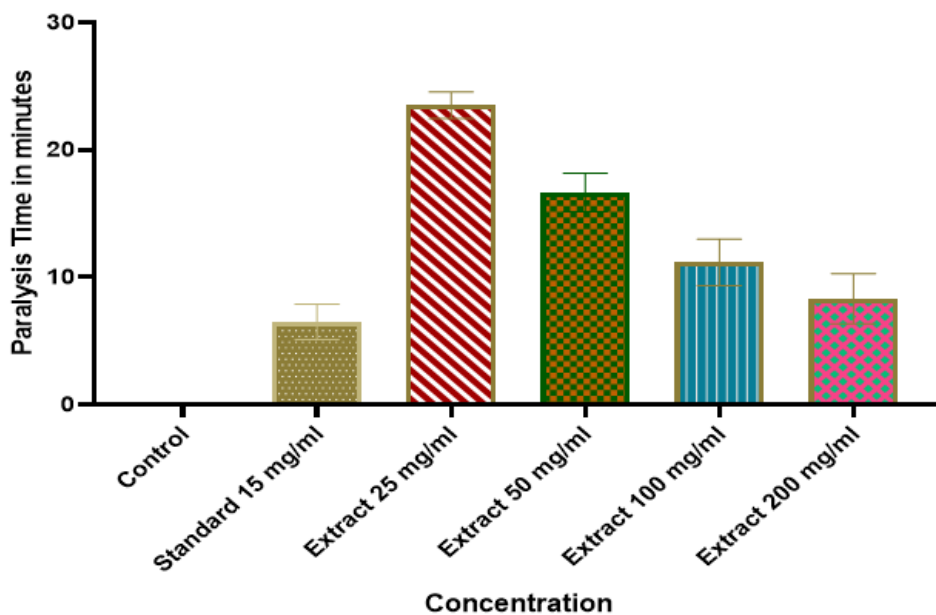


Figure 5: Effect of different doses of ethanolic leaf extract of *F. racemosa* (Death Time)

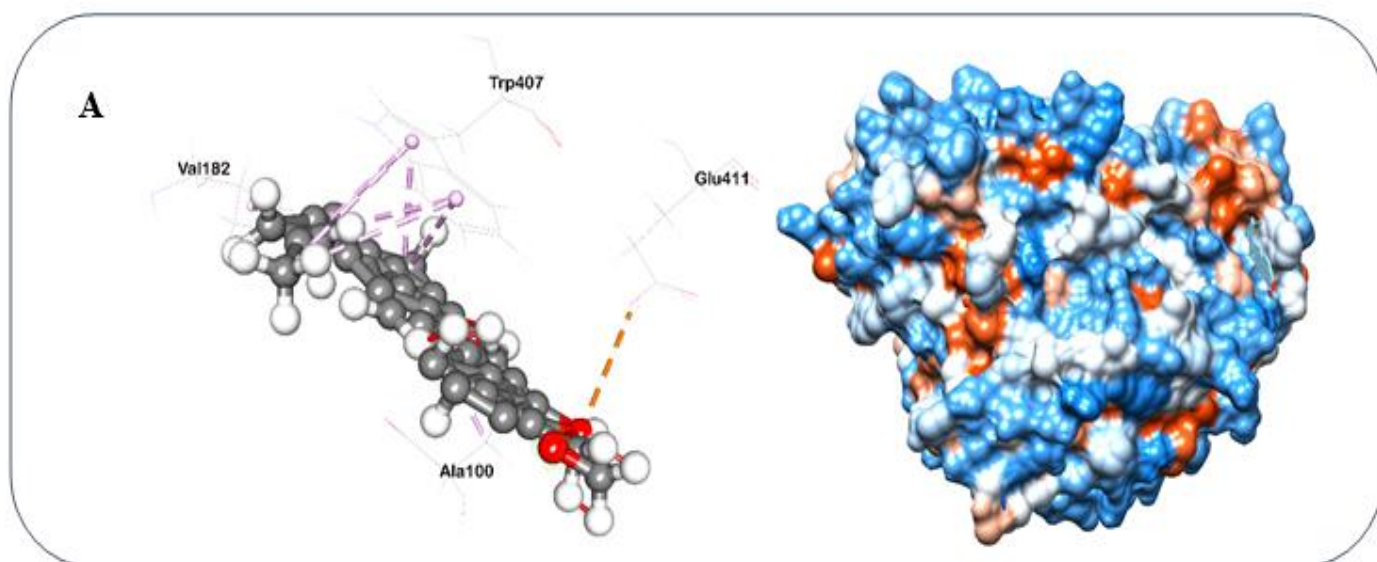
Compound Name	Compound ID	Docking Score (Kcal/mol)
β -sitosterol	521199	-15.4
Lupeol	259846	-13.7
Taraxasterol	115250	-13.5
α -amyrin	345510	-13.5
α -amyrin acetate	345510	-13.4
Kaempferol	5280863	-10.4
Tiglic acid	125468	-5.1
Albendazole	2082	-10.5

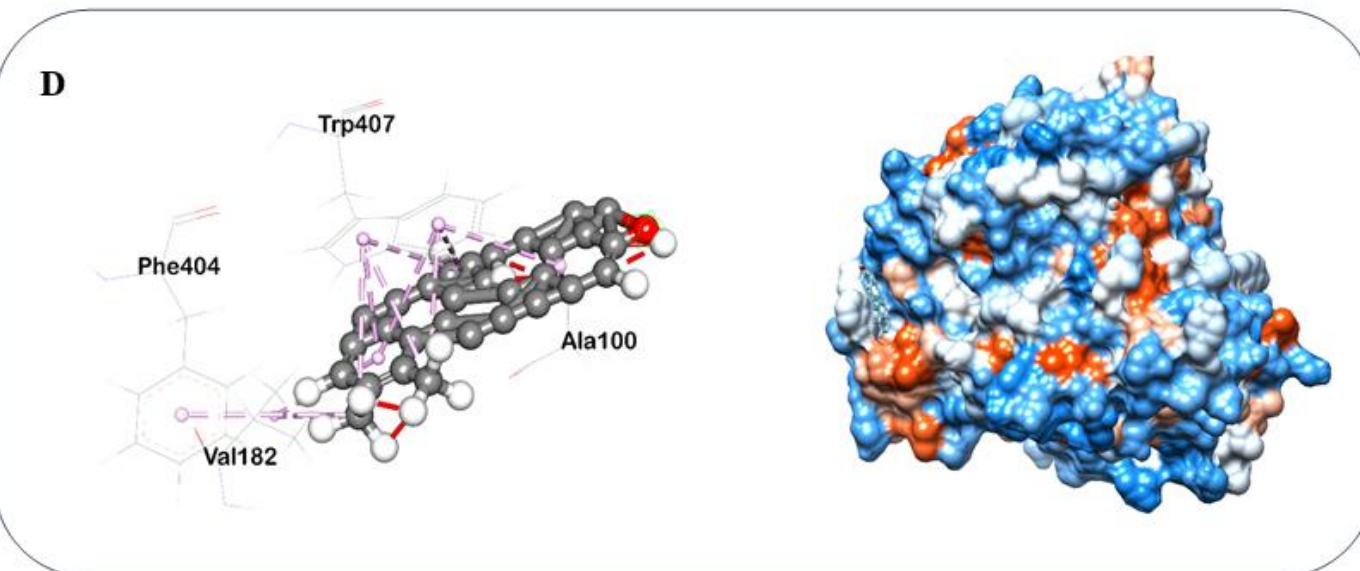
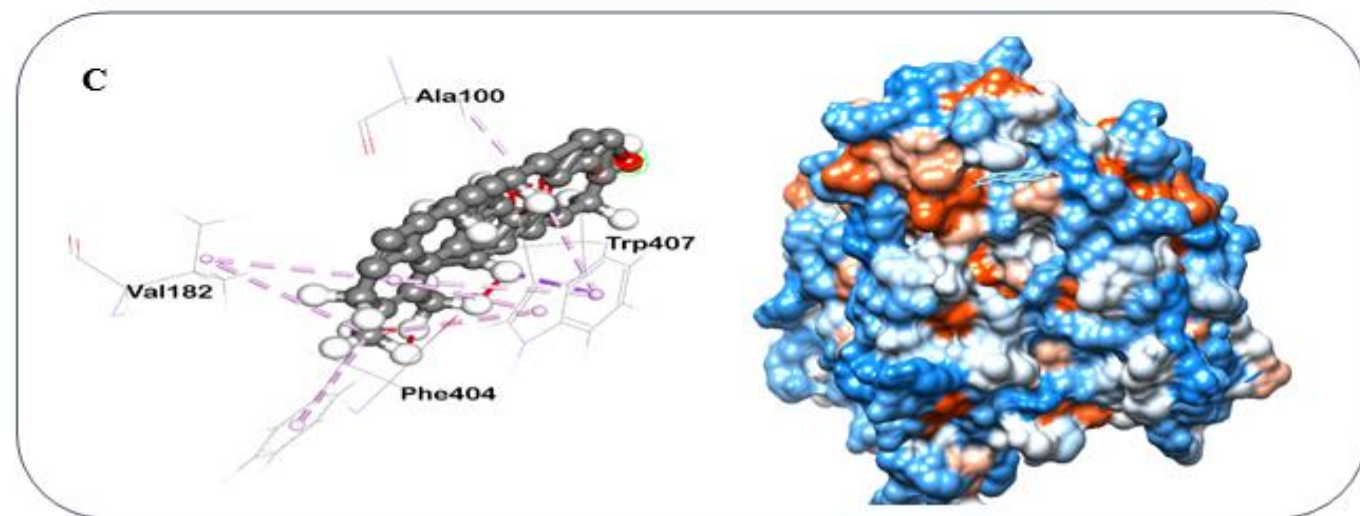
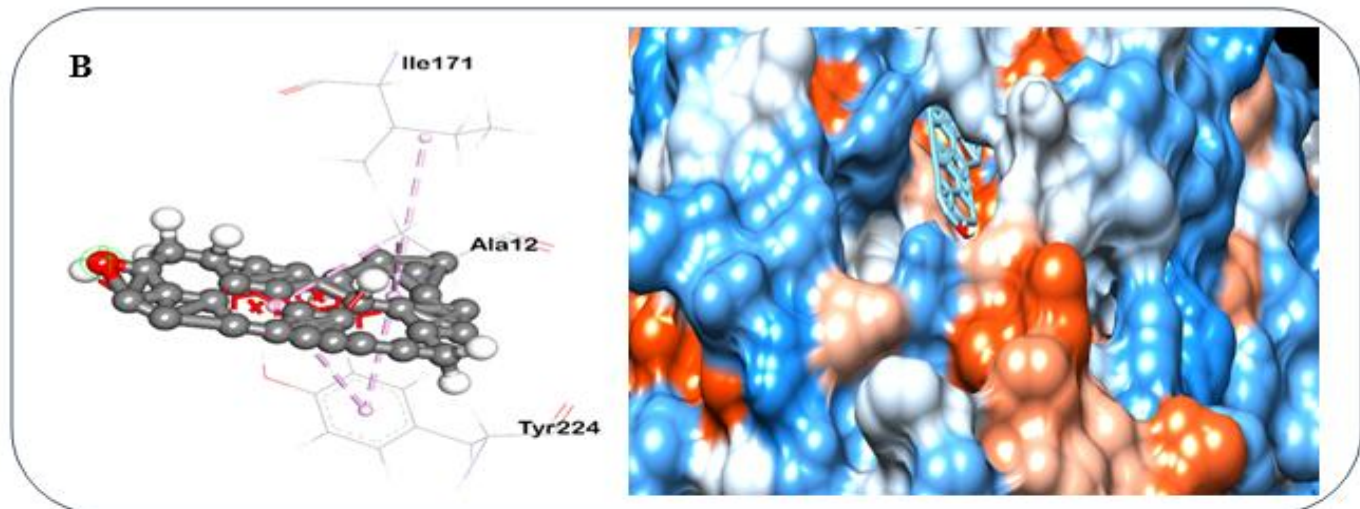
In silico molecular docking study for anthelmintic activity

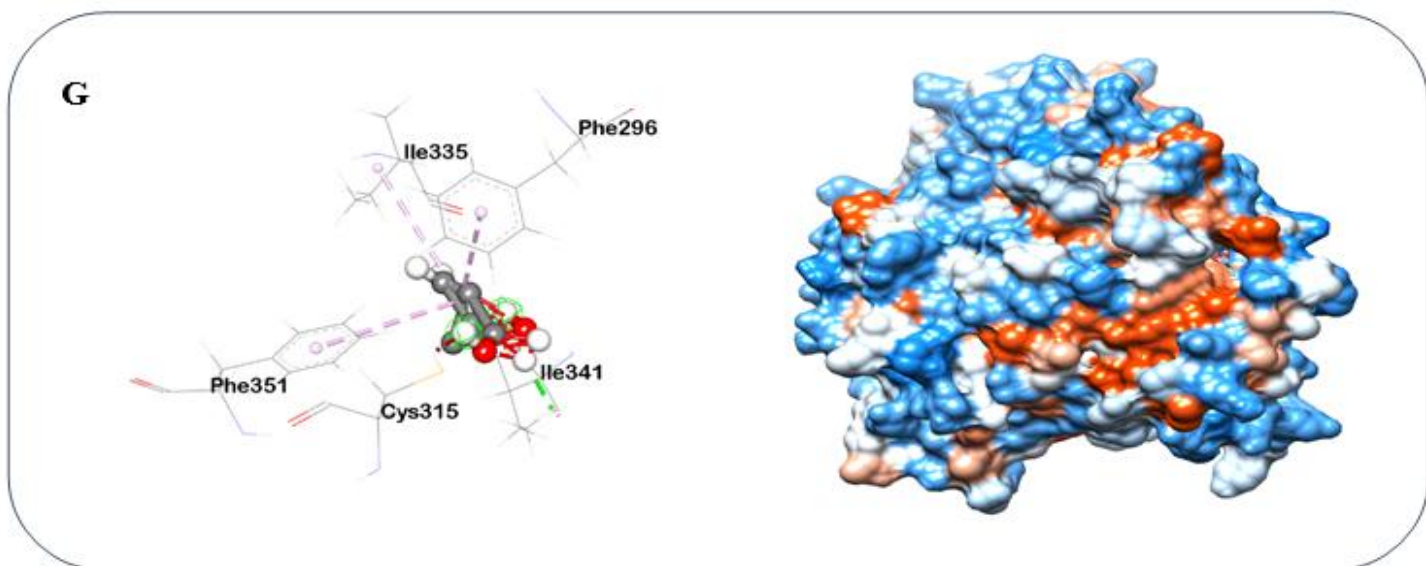
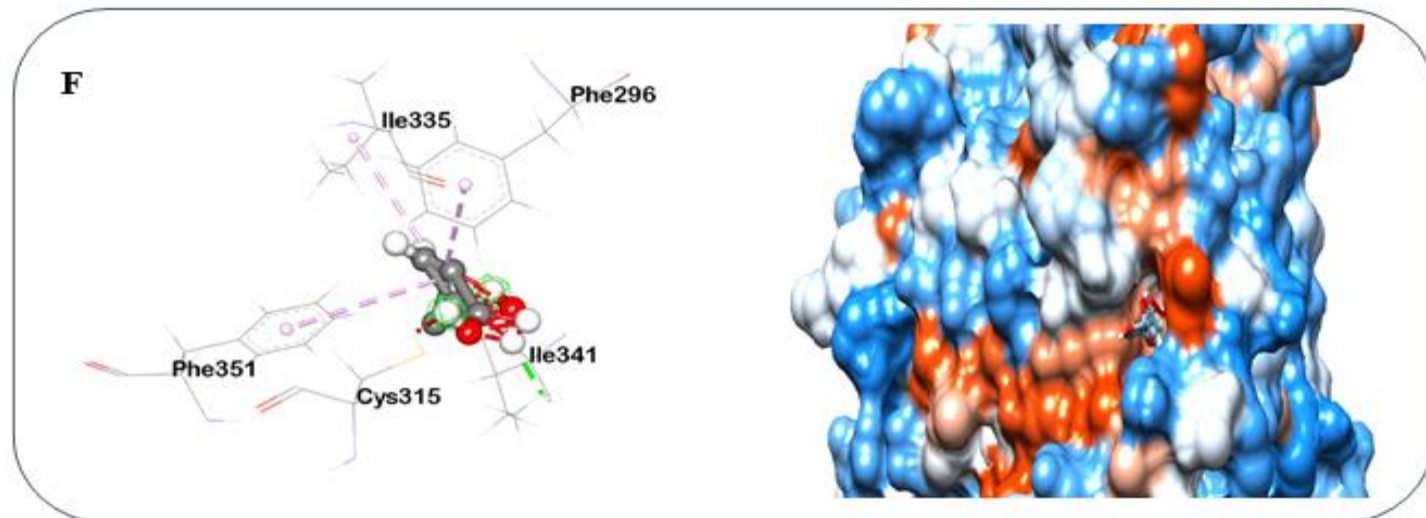
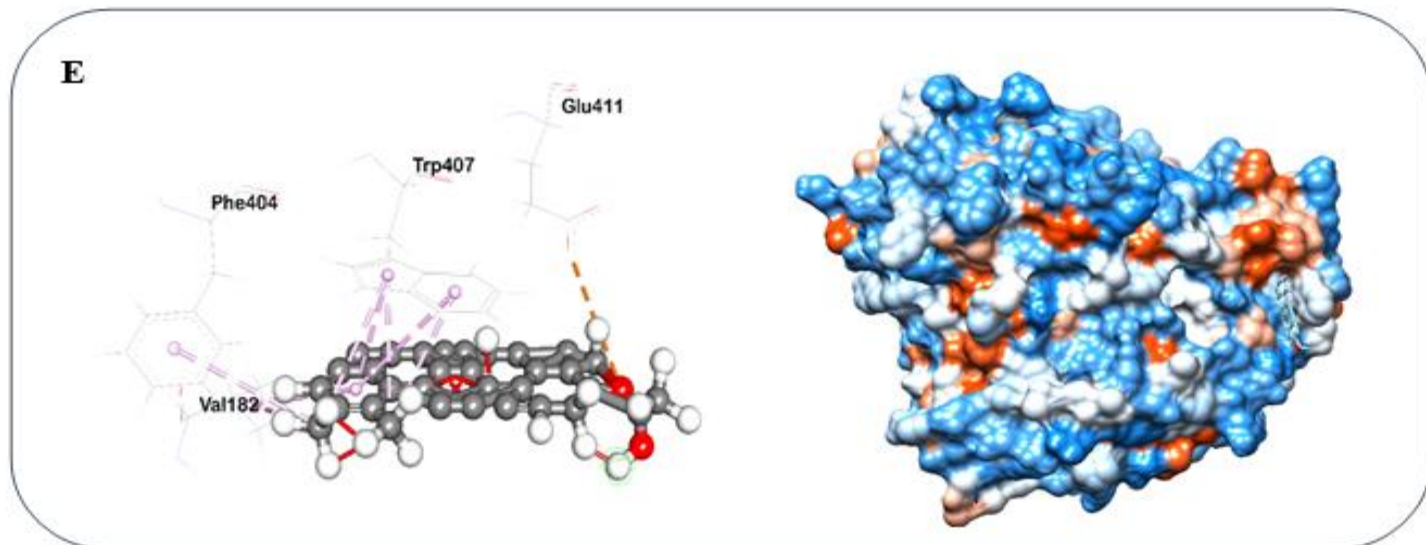
Seven plant-isolated chemicals were chosen for molecular docking analysis in this investigation. Five of these chemicals bind to the Tubulin-Colchicine enzyme more strongly than the gold-standard medication albendazole. The standard medicine albendazole has a docking score of -10.5 kcal/mol; however, the

molecular docking study revealed that β -sitosterol had the highest docking score against the Tubulin-Colchicine enzyme, at -15.4 kcal/mol. The results are shown in Table 3, and the ligand-Tubulin-Colchicine enzyme interactions are depicted in Figure 6.

Table 3: Docking score of phytoconstituents of *F. racemosa* and standard drug against Tubulin-Colchicine enzyme.







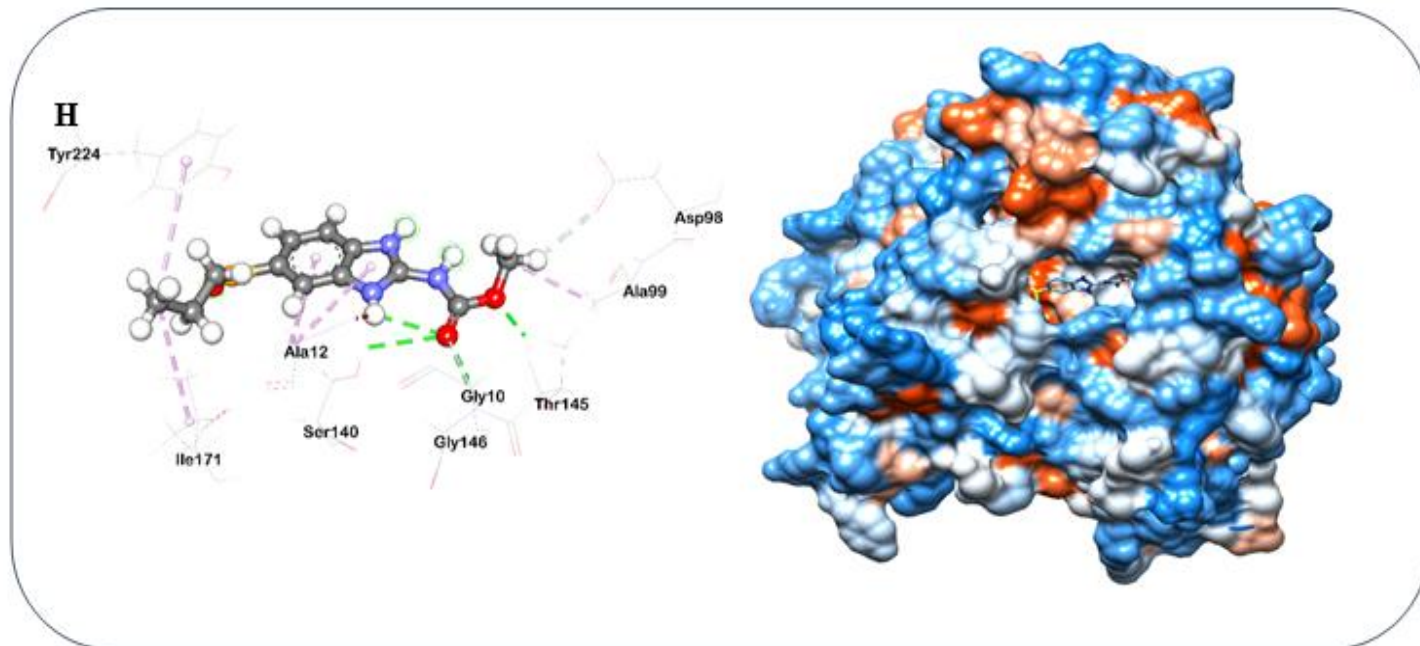


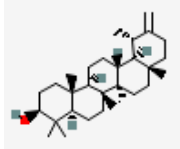
Figure 6: Pose from the docking analysis showed the binding orientation map of important amino acids for (A) β -sitosterol, (B) Lupeol, (C) Taraxasterol, (D) α -amyrin, (E) α -amyrin acetate, (F) Kaemferol, (G) Tiglic acid and (H) Albendazole (standard drug), showing hydrogen bond interaction (right) and binding mode of ligands (left) into Tubulin-Colchicine

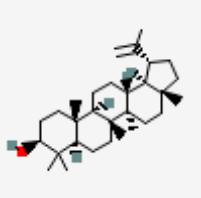
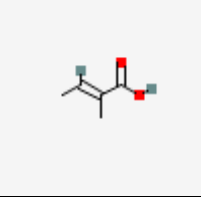
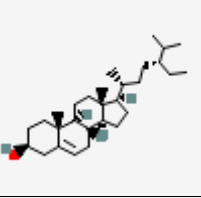
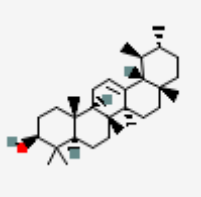
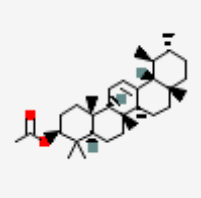
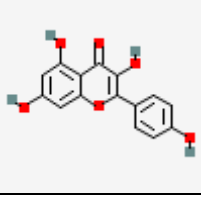
ADME/T Properties analysis

Swiss ADME web-based tools used ADME features to rank ligand compounds for their potential as drugs. Table 4 displays the results of an analysis of the ADME characteristics of seven different substances. It is common knowledge that the specified qualities affect

metabolism, cell permeability, and bioavailability. The data showed that all compounds followed Lipinski's formula of five. Since the rule allows for one infraction, the compounds taraxasterol, lupeol, beta-sitosterol, alpha-amyrin, and alpha-amyrin acetate are all safe to use.

Table 4: ADME/T properties of different compounds from *F. racemosa*

Name of Molecule	Structure	MW ^a	HB donor ^b	HB acceptor ^c	Log p ^d	Molar refractivity ^e
Taraxasterol		426.72 g/mol	1	1	4.80	135.14

Lupeol		426.72 g/mol	1	1	4.68	135.14
Tiglic acid		100.12 g/mol	1	2	1.14	27.45
β -sitosterol		414.71 g/mol	1	1	4.79	133.23
α -amyrin		426.72 g/mol	1	1	4.77	135.14
α -amyrin acetate		468.75 g/mol	0	2	4.89	144.88
kaempferol		286.24 g/mol	4	6	1.70	76.01

^aMolecular weight (acceptable range: <500).

^bHydrogen bond donor (acceptable range: ≤ 5).

^cHydrogen bond acceptor (acceptable range: ≤ 10).

^dHigh lipophilicity (expressed as LogP, acceptable range: <5).

^eMolar refractivity should be between 40-130

4. Discussion

The use of natural compounds generated from plants has been studied more closely as a possible source of novel medicinal medicines. Plants have been studied for their potential pharmacological activities, low toxicity, and commercial viability, all of which have led to an increased interest in their medicinal properties. Based on this assumption, the anthelmintic activity of the plant was assessed against the round worm after that molecular docking analysis was

performed. Helminth infections are a major health concern for both people and animals because they often cause life-threatening, long-lasting illnesses that can even lead to drug resistance (Hotez *et al.*, 2008; (Kirtikar and Basu, 1913). To stop helminth infections, scientists need to study organic ingredients like herbs that can be eaten. These ingredients provide new active molecules with no or few side effects, are easy for people in poor countries to get, and, most importantly, work better with the human body than

standard medicines (Sofowora *et al.*, 2013). By using computational approaches, solubility and permeability of drugs can be predicted efficiently (Lipinski *et al.*, 1997). By assessing their *in vitro* anthelmintic activity and conducting a molecular docking study on a subset of those compounds, we can depict the binding of molecules of those substances in the combining site of specific target proteins and demonstrate the chemical reactions of the anthelmintic activity. Table 4 shows

that β -sitosterol (-15.4), Lupeol (-13.7), Taraxasterol (-13.5), α -amyrin (-13.5), and α -amyrin acetate (-13.4) all had docking scores that were statistically significant and equivalent to the reference medicine Albendazole (-10.5). β -sitosterol had the best docking score of any chemical examined in this investigation. The results of the molecular docking analysis show that these substances, and β -sitosterol in particular, show promise as potential novel anthelmintic agents.

5. Conclusion

The *in vitro* study revealed that the plant possesses significant anthelmintic property which was validated by molecular docking study and ADME/T profile of the isolated compounds. It can be concluded that the plant possesses several bioactive compounds that are responsible for the anthelmintic activity of the plant.

6. Conflict of interest

Authors declare no conflict of interest on this research.

7. Acknowledgement

We would like to express our sincere gratitude to Department of Pharmacy, Khwaja Yunus Ali University for providing laboratory facilities and logistic support for this research.

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