Original article

Potential effects and relevant lead compounds of *Vigna mungo* (L.) Hepper seeds against bacterial infection, helminthiasis, thrombosis and neuropharmacological disorders

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A B S T R A C T

Multidrug-resistant bacterial infections, helminthiasis, thrombosis, anxiety and insomnia are some of the major global health concerns. *Vigna mungo* (L.) Hepper (VM) has been used traditionally to treat microbial infection, helminthic disorder, schizophrenia, memory loss, and blood circulatory problem. This research aims to discover antibacterial, anthelmintic, thrombolytic and neuropharmacological effects of the methanol extract of *Vigna mungo* seeds (MESVM), and also in-silico prediction of relevant lead compounds by molecular docking and ADME/T analysis. The crude extracts and subsequent fractions of MESVM were investigated for antibacterial activity by disc diffusion method, anthelmintic activity by paralysis and death test on earthworms, and thrombolytic activity by in vitro blood clot dissolution test. Open-field test and elevated plus maze test were performed for evaluating anxiolytic activity of the extracts. Using molecular docking, ligand poses of selected VM seeds' phytoconstituents were predicted targeting tubulin, GlcN-6-P synthase, and human tissue plasminogen activator proteins for anthelmintic, antibacterial, and thrombolytic activity, respectively. In the antibacterial activity test, the MESVM at 10000 μg/mL concentration created highest and significant (P < 0.001) zone of inhibition against *Staphylococcus aureus* (15.42 mm) and *Escherichia coli* (12 mm) compared with tetracycline. The MESVM exhibited remarkable anthelmintic activity at 50 mg/mL concentration with 35.4 min paralysis time, 75.2 min death time and were closer to the durations of standard drug albendazole. No test extract showed anxiolytic activity. In thrombolytic activity test, all concentrations of MESVM produced clot lytic activity with high significance (P < 0.001) in comparison with the blank. In docking, 2′-hydroxygenistein, cyclokievitone hydrate, and aureol displayed maximum affinity to the target proteins for anthelmintic, antibacterial, and thrombolytic activity, respectively. This research revealed that the MESVM demonstrated potential anthelmintic, antibacterial and thrombolytic effects that confirmed the folkloric uses of VM and the found relevant lead compounds might be further optimized in future drug development.
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1. Introduction

Holistic treatment using traditional medications is undoubtedly much safer than most of the conventional medicine therapy due to its negligible side-effects. Plant parts and even whole plants are great sources of traditional medicines against various diseases used by different locality people for their bioactive compounds and natural availability (Khairan et al., 2021). Bacterial diseases with resistant antibacterial agents, helminthiasis with limited anthelmintic drugs, anxiety disorders with habit-forming agents and deadly clot formation in blood vessels are serious human health-hazard issues in our recent world. Bacterial disease is a common cause of severe human sufferings for a long time and

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many antibiotics have been manufactured till date and are still in use to treat such infections. Despite abundance, antibiotics are becoming resistant due to their irrational uses. Therefore, it often requires the researchers to design and develop novel antibiotics based on natural products (Birkett et al., 2010). Helminthic disease also possesses a great threat to the people mostly from Africa, South Asia and South America (Lobo et al., 2011). In vulnerable areas with poor sanitary and extreme environmental conditions people are randomly infected by helminths (Salam and Azam, 2017). Very few anthelmintic drugs are available to treat this disease but with side effects. Some researches show that various plant species have potential anthelmintic action that is comparatively better in safety and economy than conventional treatment (Kozan et al., 2016). In modern day to day life, neurological disorders such as insomnia, anxiety, and epilepsy also affect the people badly. However, treating these disorders by synthetic psychotropic drugs creates habituation, dependence and addiction; furthermore, withdrawal symptoms arise due to the abrupt discontinuation of long-term use of these drugs. Therefore, researchers are focusing on the herbal medication with a view to gaining better CNS depressants (Thirupathy et al., 2011). A thrombus is formed by the conversion of fibrinogen into thrombin as a natural response for any injury of blood vessel or other certain conditions. However, it may be fatal due to block of internal blood circulation that eventually causes myocardial infarction, cardiac arrest, stroke and such other disorders. Synthetic fibrinolytic drugs such as tissue plasminogen activator, urokinase, streptokinase, alteplase are widely used to treat thrombosis by dissolving thrombin to reverse and improve the resulting consequences. Nevertheless, these synthetic drugs have some disadvantages such as requirement of large doses, bleeding tendency, anaphylactic shock and systemic fibrinolysis. Besides, researchers have been trying to genetically modify these drugs to eliminate their limitations, however, these are not setbacks free (Ramjan et al., 2014). Such shortcomings of the conventional synthetic drugs influence researchers to investigate more on traditional medicine for their safety, affordability, and availability.

Molecular docking, a computer-aided drug designing (CADD) technique, is widely used in the advanced computational drug design to predict whether and how a responsible protein (enzyme/receptor) interacts with its potential ligands at the atomic level. Computational biology or in silico biology simply refers to any biological investigation using computer. The purpose of performing docking simulation is to design structure-based drugs by targeting a protein that can be responsible for specific disease. After target selection, potential lead compounds for that disease can be virtually predicted by this simulation through analysis of orientation, binding affinity, and interaction of the ligands with the active site residues of the target protein. Therefore, molecular docking has been extensively used in recent phytochemical research works to explore potent, yet safer and more efficacious drugs (Niode et al., 2021).

Black gram, scientifically named *Vigna mungo* (VM), is a plant the seeds of which are one of the medicinally valued lentils used in South-Asian countries. Black gram is an annual herb and a member of papilionaceae family, the vertical length of which is almost 30 to 100 cm (Zaheer et al., 2020). The seeds are very nutritious and enriched with protein, potassium, calcium, iron, niacin, thiamine, riboflavin and folate. They also contain essential amino acids. This nutritious bean offers numerous health benefits and is used traditionally to treat heart issues by lowering cholesterol, ease internal and external inflammation, decrease oxidative stress, reduce blood sugar, soothe pain, improve blood circulation, manage nervine problems particularly hysteria, memory weakness and schizophrenia (Staughton, 2020, Boby and Leelamma, 2003). Moreover, the *Vigna* genus has anthelmintic (Shukla and Tyagi, 2017) and antimicrobial (Kingsley et al., 2014) folkloric uses.

Despite having huge traditional uses and profuse nutritional values, this pulse has received less attention to be studied as a significant candidate for extensive research. Previous research works reveal analgesic (Ahmed et al., 2015), antihyperglycemic (Mou et al., 2015), antihyperlipidemic (Solanki and Jain, 2010), antidiabetic (Yao et al., 2013), anticonvulsant (Gohel et al., 2011), antimicrobial (Kingsley et al., 2014) and cytotoxic (Nasrin et al., 2015) activities of different parts of VM. The results of antimicrobial test by Nasrin et al. (Nasrin et al., 2015) using disc diffusion method on leaves and stems, and by Kingsley et al. (Kingsley et al., 2014) using well diffusion method on germinated pulse have been found positive. However, the responsible chemical constituents of the seeds with their mechanism for antibacterial action have not been explored yet. Moreover, anxiolytic, thrombolytic and anthelmintic activities of the VM seeds have not been investigated extensively that has given scopes of this research study to assess these activities in the VM seeds. Two mice models of anxiety (open field test, and elevated plus maze test) for anxiolytic activity, in vitro blood clot lytic test for thrombolytic activity, paralysis and death test on earthworm for anthelmintic activity and disc diffusion method for antibacterial activity were used in the present research. Molecular docking simulation was performed in this study on the isolated compounds of the seeds to identify lead compounds of anthelmintic, antibacterial and thrombolytic activities interacting with tubulin protein (PDB ID: **1SA0**), GlcN-6-P synthase (PDB ID: **1XFF**) and human tissue plasminogen activator (PDB ID: **1A5H**) drug targets, respectively. Moreover, ADME/T profile analysis of the top docked compounds was done to validate the docking result.

2. Materials and methods

2.1. Crude extracts and subsequent fractions preparation

VM seeds were collected from a local seed market in Chittagong city with a voucher specimen number SU/DPS/Herb/25. Fresh seeds were shade-dried at normal environment. Then, after grinding, 650 gm ground VM seed was taken in an Erlenmeyer flask (5 L) and soaked in 3.5 L methanol for 15 days. The flask was kept stirring intermittently. After that, the liquid mixture of ground seed and methanol was strained out through a Whatman filter paper (size no. 1) to obtain a good amount of filtrate. The filtrate was run through a Heidolph rotary evaporator at 39 °C and dried in the air. Lastly, a gummy concentrate of yellow colored crude extract (15 gm) was found. The crude methanol extract of the seeds of *Vigna mungo* (MESVM) was subsequently fractionated using n-hexane (HFSVM), carbon tetra chloride (CTCFSVM) and chloroform (CFFSVM) (VanWagenen et al., 1993).

Aqueous extract of the seeds of *Vigna mungo* (AESVM) was prepared through the following process: first, 150 gm ground seed was mixed in 1 L of distilled water in an Erlenmeyer flask and soaked for 24 h with episodic shaking; second, the aqueous mixture was passed through a Whatman filter paper to get the filtrate; and finally, the filtrate was vaporized by rotovap to obtain the aqueous extract.

2.2. Phytochemical screening

To screen carbohydrates, proteins, alkaloids, glycosides, flavonoids, tannins, saponins, phenols and phytosterols in the extract, some preliminary qualitative phytochemical tests were performed on the freshly prepared MESVM (Choudhary et al., 2021, Gul et al., 2017).

2.3. Experimental animals

5–6 weeks aged and 25–30 gm weighed Swiss-albino mice (*Mus musculus*) were brought from the Animal Lab of Jahangir Nagar University, Dhaka, Bangladesh. Before executing tests, these mice had been kept at 24 ± 1 °C temperature and 55–65% relative humid environment (standard atmospheric condition) for one week providing standard laboratory feed and water *ad libitum* (Islam et al., 2019). Animals were taken care of and observed following the rules of Institutional Animal Ethics Committee of the Faculty of Biological Sciences, University of Chittagong, Bangladesh.

2.4. Acute toxicity test

This test was performed following the OECD guideline to find out the toxicity of the extracts and fractions of VM seeds (OECD Guidelines, 2002). Several groups have been formed each of which contained five mice. Each mouse was kept fasted overnight before the experimental day. The groups were then treated with different doses of the MESVM, HFSVM, CTCFSVM, CFFSVM and AESVM in an increasing order up to the highest dose 2000 mg/kg and kept unfed for the following 3–4 h. All of the treated and control mice were carefully observed for any behavioral changes or mortality. The observation was continued for next 14 days as well.

2.5. Antibacterial activity test

To screen antibacterial activity of MESVM at different concentrations, the disc diffusion method was employed using three pathogenic bacteria - *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 14574) and *Escherichia coli* (ATCC 25922) (Zahan et al., 2020). The bacterial strains were provided by the Biochemistry lab of University of Chittagong, Bangladesh. Subcultures of these strains were prepared by inoculating the strains in different agar slants and then incubating them for one day at 37 °C to have fresh pure cultures. 5 mL sterilized liquid agar medium was taken in different test tubes each where the test bacteria from the pure cultures were added separately in aseptic manner. After shaking, these bacterial suspensions were subsequently poured into different marked sterilized petri dishes. Several sterile filter paper discs were impregnated separately with 30 μg/mL tetracycline (positive control), distilled water (negative control), and 10 μg/mL, 100 μg/mL, 1000 μg/mL and 10000 μg/mL of MESVM (test extracts). These impregnated discs were put on the specified zones of all the pre-loaded bacterial petri dishes. Then, zone of inhibition was noticed on each dish after one day incubation at 37 °C.

2.6. Assay of anthelmintic activity

In this study, the anthelmintic activity of MESVM at different concentrations was tested by in vitro paralysis and death test on adult earthworms (*Pheretima posthuma*) (Choudhary et al., 2021). The earthworms of 3–4 in. were divided into seven different petri dishes of five each. Among them, five petri dishes had 10, 20, 30, 40 and 50 mg/mL concentrations of MESVM, one had reference drug (albendazole at 15 mg/mL concentration) and another had negative control (distilled water at 10 mL/petri dish). After treating with the samples, any physical changes in the earthworms were continuously observed. Two parameters of the earthworms were recorded: *i)* the time for paralysis when the worms became motionless unless shaken forcefully and *ii)* the time required for death that was ascertained by no movement of the worms even after forceful shaking or dipping those in 50 °C warm water.

2.7. Neuropharmacological (anxiolytic)activity test

2.7.1. Study design

Forty mice were distributed equally into eight groups. In the negative control group (Group I), mice received only vehicle (normal saline) at 0.5 mL/mice; in the standard group (Group II), mice received the reference anxiolytic drug (diazepam) at 1 mg/kg; in the extract groups (Group III, IV, V, VI, VII and VIII), mice were respectively treated with the doses of MESVM at 200 mg/kg, MESVM at 400 mg/kg, CFFSVM at 400 mg/kg, HFSVM at 400 mg/kg, CTCFSVM at 400 mg/kg and AESVM at 400 mg/kg per oral. The study of neuropharmacological activity was performed to find out whether the seed extracts and fractions had anxiolytic effect and to evaluate exploratory behavior of mice after introducing the test samples.

2.7.2. Open-field test

To examine anxiolytic activity based on exploratory behavior, this assay was performed on mice model by the open-field test (Rakib et al., 2020). The open-field apparatus has a 0.5 m^2 floor that is marked as a series of black and white alternative squares, and is surrounded by a 40 cm high wall. At the beginning of the assay, the test samples, negative control and positive control were administered orally to all groups. Then, the number of passed squares by each mouse of all groups were recorded for 5 min at 0, 30, 60, 90, 120 min.

2.7.3. Elevated plus maze test

To explore anxiolytic activity, the elevated plus maze (EPM) test was executed on mice model. A plus-sign EPM device, positioned 50 cm above floor, with two open arms (30 × 5 cm^2) and two closed arms (30 × 5 × 15 cm^3), was used in this assay. In a calm and quiet environment, first, the grouped mice were treated with the test samples, positive control and negative control. Each treated mouse was instantly put at the maze-center fronting one of the closed arms. Then, the number of entries in open arm and the number of entries in closed arm by all mice as well as their spending time on the arms were recorded for 5 min at 0, 30, 60, 90, 120 min (Rakib et al., 2020).

2.8. Thrombolytic assay

MESVM was tested for thrombolytic effect by in vitro clot lytic test using human blood samples (Ibrar et al., 2019). First, Twenty one empty Eppendorf tubes (w_1) were taken and weighed. Second, 5 mL blood was collected from each of three healthy individuals who had not received either anticoagulant drug or contraceptive therapy. Third, collected blood from each individual was distributed into seven weighed Eppendrof tubes, each containing 0.5 mL of blood. Fourth, to form thrombus, all of these tubes were incubated at 37 °C for 45 min. After thrombus formation, serum was carefully drained out from each tube. Then, each clot containing tube (w_2) was re-weighed to determine clot weight in each tube as follows:

$$\text{Weight of clot} = w_2 - w_1$$

Fifth, in these clot containing tubes, MESVM at 2, 4, 6, 8 and 10 mg/mL concentrations (test samples), streptokinase (SK, the standard) and distilled water (negative control) were added separately at the amount of 100 μl each. Sixth, these treated tubes were re-incubated at 37 °C for 90 min. After incubation, fluid released in the clot dissolved tubes. The released fluid was drained out and the weight of clot lytic tubes (w_3) were measured to calculate the thrombolytic effect as a percentage of thrombolysis as follows:

$$\% \text{ of thrombolysis} = (w_2 - w_3)/(w_2 - w_1) \times 100\%.$$

2.9. Statistical analysis

The experimental data of neuropharmacological activity and thrombolytic activity were compared statistically with their respective negative control groups through one-way analysis of variance ANOVA, followed by post hoc Dunnett's test using the software SPSS (version 26.0). The p value < 0.05 was used as an indication of the statistical significance. Obtained data of antibacterial ZOI of test sample were also compared statistically with the positive control by the same analysis.

2.10. Molecular docking analysis

2.10.1. Ligand retrieval and preparation

A total of 29 biological compounds from VM seeds were selected for molecular docking analysis through literature review (Onyilagha et al., 2009, Zia-Ul-Haq et al., 2014, Adesanya et al., 1984, Girish et al., 2012). All of these compounds were obtained from the PubChem database (CID No.: 5280863, 92729, 92094, 14985, 173183, 12795736, 5280794, 5280934, 445639, 5280961, 5282074, 5280520, 119269, 181994, 156777, 442770, 440047, 156743, 5491648, 585939, 188458, 44257389, 131752226, 44257380, 700, 445858, 3469, 370, 72; Supplementary Table 1) in .sdf format. Ligands were then prepared by minimizing their energy form and converting their .sdf format into .pdbqt format using OpenBabel module of PyRx 0.8.

2.10.2. Protein preparation

Three-dimensional crystallographic structures of GlcN-6-P synthase (PDB ID: **1XFF**), tubulin (PDB ID: **1SA0**), and human tissue plasminogen activator (PDB ID: **1A5H**) were collected from the protein data bank (PDB) as antibacterial, anthelmintic and thrombolytic target protein, respectively (Berman et al., 2002). Initially, these protein structures were prepared in the Discovery Studio (DS) version 4.5 where the water molecules and heteroatoms were removed. Energy of each retrieved protein was minimized using Swiss-PDB Viewer (Guex and Peitsch, 1997). After that, the structures were saved in .pdb format in the Discovery Studio (Inc, 2012).

2.10.3. Molecular docking

PDBsum database was used to predict the active side residues of the selected protein structures (Laskowski 2001). In AutoDock Vina software, molecular docking was run keeping the docking parameters as default. In this software, grid box size towards X, Y, and Z-axes was set for the tubulin, GlcN-6-P synthase, and human tis-

sue plasminogen activator at 66.02 × 56.65 × 53.07 Å, 40.22 × 44. 77 × 46.51 Å, and 51.06 × 47.65 × 59.13 Å, respectively. Docking analysis results were represented as docking score in kcal/mol that indicated released energy during protein–ligand binding. It has been established that the more negative the score, the stronger the binding affinity (Trott and Olson, 2010).

2.11. Adme profile analysis

The prediction of ADME profile of the top docked compounds was carried out to further validate the molecular docking simulation. Lipinski's rule of five (RO5) was employed for this purpose (Lipinski et al., 2001). According to this rule, a compound must conform to five criteria namely hydrogen bond acceptors ($\leq$10), hydrogen bond donors ($\leq$5), molecular weight ($\leq$500 g/mol), molar refractivity (40–130), and lipophilicity (<5). Compounds passing Lipinski's filter can be deliberated as suitable drug candidates. SwissADME server (http://www.swissadme.ch/index.php) for predicting pharmacokinetics properties of molecules was used to analyze the ADME profile of the top docked compounds (Daina et al., 2017).

3. Results

Results of phytochemical screening, acute toxicity test, antibacterial activity, anthelmintic activity, neuropharmacological activity, thrombolytic activity, molecular docking analysis of antibacterial, anthelmintic and thrombolytic properties of the extracts and fractions of the VM seeds, and prediction of ADME profile of the top docked compounds are presented in this section that might help to discover the potential pharmacological effects and possible lead compounds of the VM seeds.

3.1. Phytochemical screening

From this study, we had observed that carbohydrates, proteins, alkaloids, glycosides, phenols, flavonoids, phytosterols, tannins and saponins were found in the MESVM (Table 1).

3.2. Acute toxicity test

In the result of acute toxicity test, it was found that each of the MESVM, HFSVM, CTCFSVM, CFFSVM and AESVM expressed no abnormal changes or mortality in the treated mice groups up to 2000 mg/kg. As a result, for the in-vivo tests, we chose 200 mg/kg body weight and 400 mg/kg body weight as safe doses.

3.3. Antibacterial assay

The antibacterial activity result of the MESVM, in Table 2, shows that different concentrations of the extract produce variable zone of inhibition (ZOI) against the tested bacterial cultures.

The result revealed that both Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* were susceptible to the MESVM at 1000 µg/mL and 10000 µg/mL concentrations, where no ZOI was found at 10 µg/mL and 100 µg/mL. However, Gram positive *Bacillus subtilis* was resistant to all of the concentrations of the extract. Moreover, at the highest concentration (10000 µg/mL), the MESVM provided maximum and significant (P < 0.001) ZOI against *S. aureus* and *E. coli*.

3.4. Anthelmintic activity test

In Table 3, the anthelmintic activity test result of the MESVM is recorded where it is exhibited that the activity is proportionate

Table 1
Screening result of phytoconstituents in MESVM.

No.	Phytochemicals	Test	MESVM
1	Alkaloids	a) Mayer's test	+
		b) Wagner's test	+
		c) Hager's test	−
		d) Dragendroff's test	−
2	Carbohydrates	a) Molisch's test	+
		b) Benedict's test	+
		c) Fehling's test	+
3	Glycosides	a) Legal's test	+
		b) Modified Borntrager's test	+
4	Saponins	a) Froth test	+
		b) Foam test	+
5	Phytosterols	a) Salkowski's test	+
		b) Libermann-Burchard's test	+
6	Phenols	a) Ferric Chloride test	+
7	Tannins	a) Ferric Chloride test	+
8	Flavonoids	a) Alkaline reagent test	+
		b) Lead acetate test	+
9	Proteins and amino acids	a) Xanthoproteic test	+

Table 2
Zone of inhibition produced by the MESVM at different concentrations.

Test Bacteria	Zone of Inhibition Diameter (mm)				
	MESVM				Standard (Tetracycline)
	10000 μg/mL	1000 μg/mL	100 μg/mL	10 μg/mL	30 μg/mL/disc
Gram Positive Bacteria					
Bacillus subtilis	–	–	–	–	26 ± 0.21
Staphylococcus aureus	15.42 ± 0.13***	6 ± 0.67***	0.0 ± 0.0	0.0 ± 0.0	30 ± 0.67
Gram Negative Bacteria					
Escherichia coli	12 ± 0.14***	5.3 ± 0.12***	0.0 ± 0.0	0.0 ± 0.0	29 ± 0.15

Values are shown as means of triplicates (n = 3) ± SEM. *** indicates statistically high significance (P < 0.001) compared to the standard.

Table 3
Anthelmintic activity of MESVM.

Test sample	Concentration (mg/mL)	Time for paralysis (minute)	Time for death (minute)
Control (Distilled water)	–	–	–
Standard (Albendazole)	10	43.6 ± 0.68	62 ± 0.55
Methanolic extract (MESVM)	10	64 ± 0.84	140.4 ± 0.51
	20	59.8 ± 0.37	121 ± 0.71
	30	55.6 ± 0.98	101 ± 0.71
	40	44.2 ± 0.73	92 ± 0.84
	50	35.4 ± 0.81	75.2 ± 1.77

Values are shown as means of five replicates (n = 5) ± SEM.

directly to the concentration of the seed extract. It is clear from the Fig. 1 that both of the paralysis time and death time of the worms reduce with increasing order of concentrations of the seed extract. Time required for paralysis and death of the worms were found in the range of 35.4–64 min and 75.2–140 min, respectively for different concentrations of the extract and 43.6 and 62 min, respectively for the standard drug albendazole. The highest activity was found for 50 mg/mL concentration that took the lowest paralysis and death time (35.4 and 75.2 min, respectively) of the worms.

3.5. Neuropharmacological (anxiolytic) activity tests

3.5.1. Open-field test

Table 4 shows the result of the open-field test to evaluate any change of exploratory behavior of mice after introducing the crude extracts and fractions of the VM seeds. Fig. 2 presents the behavior of movement of mice for different extracts. Both 200 mg/kg and 400 mg/kg doses of the MESVM increased the number of move-
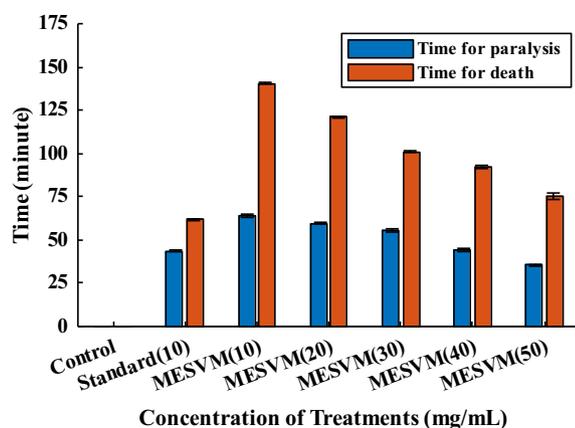
ments of mice from every initial value recorded at 0, 30, 60, 90 min. Treating different mice groups with AESVM at 400 mg/kg, CFFSVM at 400 mg/kg, CTCFSVM at 400 mg/kg and HFSVM at 400 mg/kg increased the number of their movements slightly for each case in the range from 277 to 297, 277 to 296, 275 to 294, and 278 to 297, respectively, at the same time lapse. On the other hand, the number of movements of mice treated with the standard drug diazepam decreased from 130 to 119 indicating CNS depressive property of the drug. The number of locomotion of each mouse treated with test samples increased with increasing of the doses. It indicated that no test sample had CNS depressive or anxiolytic action.

3.5.2. Elevated plus maze test

The result of anxiolytic activity of the test samples using elevated plus maze (EPM) test on mice model is shown in Table 5. The result showed that all of the test samples at all doses reduced the percentage of entries and the percentage of time spent of mice in the open arms of the EPM in comparison with the control, where the standard drug increased both values. It explicated that no seed extract and fraction had anxiolytic property. The entry and time spent percentages of all test extracts are approximately close to the control values that are pictorially presented in Fig. 3.

3.6. Thrombolytic activity test

The thrombolytic activity test result of the MESVM are set out in Table 6 and diagrammed in Fig. 4. The result revealed that the MESVM at each concentration showed concentration dependent clot lytic activity with statistically significance (where P value was<0.001) comparing with the blank. The maximum thrombolytic activity (31.52%) of the MESVM was recorded at its highest concentration of 10 mg/mL, where the positive control yielded 74.52% clot lytic activity.

3.7. Molecular docking analysis

In the case of tubulin (PDB ID: **1SA0**), 16 active site residues (Ser178, Thr179, Ala180, Val181, Val238, Cys241, Leu242, Ala250, Lys254, Leu255, Asn258, Met259, Val315, Ala316, Lys352, and Ile378) were found in the PDBsum database. For GlcN-6-P synthase (PDB ID: **1XFF**), there were 10 interactive amino acid residues (Cys1, Arg73, Trp74, Thr76, His77, His86, His97, Asn98, Gly99, and Asp123) in the active site. The active site for human tissue plasminogen activator (PDB ID: **1A5H**) included 14 amino acid residues namely Asp97, Thr98, Tyr99, Arg174, Asp189, Ala190, Gln192, Ser195, Ile213, Trp215, Gly216, Gly219, Cys220, Gly226. Docking scores of the VM seeds' selected phytoconstituents towards tubulin (PDB ID: **1SA0**), GlcN-6-P synthase (PDB ID: **1XFF**) and human tissue plasminogen activator (PDB ID: **1A5H**) are represented in Table 7.

Fig. 1. Paralysis and death time changes of *Pheretima posthuma* for different concentrations of MESVM in anthelmintic activity test.

Table 4
Open field test activity in different extracts of the VM seeds.

Groups	Dose (p. o.) (mg/kg)	Number of movements			
		0 min	30 min	60 min	90 min
Control	0.5 mL/mice	278.33 ± 0.33	284 ± 0.58	290 ± 4.20	298.56 ± 5.60
Diazepam	1	130.45 ± 5.62	125.65 ± 1.23***	119.68 ± 3.45***	115.56 ± 4.21***
MESVM	200	270.33 ± 0.88	281 ± 0.58	285 ± 0.00	294.67 ± 2.91
MESVM	400	275 ± 0.00	283 ± 1.15	287 ± 0.58	296 ± 3.06
CFFSVM	400	277.67 ± 1.45	280.33 ± 0.88	289 ± 1.53	295.7 ± 0.67
HFSVM	400	278.7 ± 0.67	283.56 ± 1.76	289 ± 0.58	296.33 ± 1.86
CTCFSVM	400	275 ± 2.65	282.67 ± 1.86	286 ± 0.58	293.67 ± 1.45
AESVM	400	277 ± 0.58	284 ± 1.15	288.33 ± 3.84	296.67 ± 0.33

Values are shown as means of five replicates (n = 5) ± SEM. *** and ** indicate statistically high significance (P < 0.001) and moderate significance (P < 0.01), respectively comparing with the negative control.
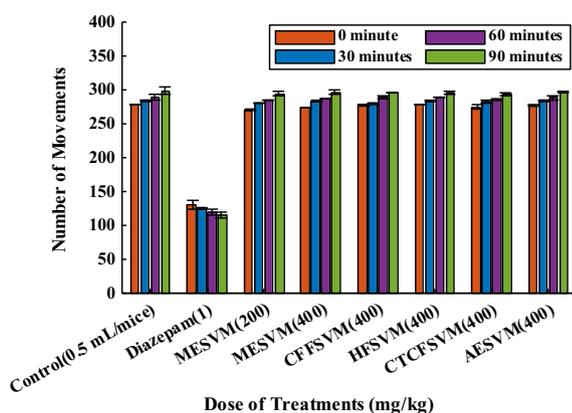

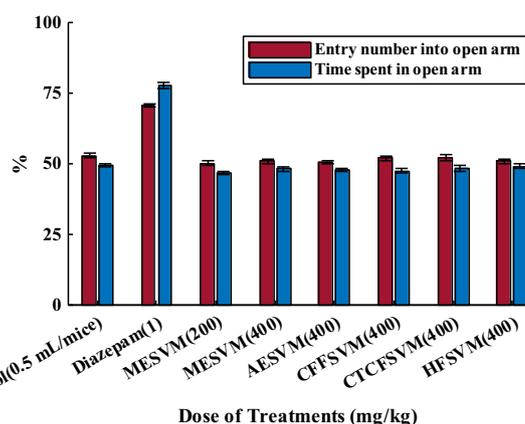**Fig. 2.** Behavior of movement of mice in open field test.

Fig. 3. Percentages of entry and time spent of mice in the open arm in EPM test.

Table 5
EPM test activity in different extracts of the VM seeds.

Groups	Dose (mg/kg)	% of entry number into the open arm	% of time spent in the open arm
Control	0.5 mL/mice	53 ± 1.05	49.60 ± 0.51
Diazepam	1	70.80 ± 0.66***	77.60 ± 1.03***
MESVM	200	50.20 ± 0.86	46.80 ± 0.66
MESVM	400	51 ± 0.84	48.20 ± 0.66
AESVM	400	50.40 ± 0.51	47.80 ± 0.73
CFFSVM	400	52 ± 0.71	47.40 ± 0.81
CTCFSVM	400	52.40 ± 1.03	48.40 ± 1.03
HFSVM	400	51 ± 0.71	49.20 ± 0.66

Values are shown as means of five replicates (n = 5) ± SEM. *** and ** indicate statistically high significance (P < 0.001) and moderate significance (P < 0.01), respectively comparing with the negative control.

3.8. Molecular docking associated with antibacterial activity

The interaction between selected biological compounds of VM seeds and GlcN-6-P synthase (PDB ID: **1XFF**) were determined for antibacterial activity. Here, all the compounds interacted with our target enzyme. The docking score ranges from −8.0 to −3.5 kcal/mol. The docking results of the top five compounds and the standard compound glutamic acid that docked with the GlcN-6-P synthase are presented in Table 8. Cyclokievitone hydrate (−8.0 kcal/mol) displayed the utmost binding affinity towards our target enzyme followed by 2′-hydroxygenistein (−7.7 kcal/mol), 5-deoxykievitone hydrate (−7.6 kcal/mol), kaempferol (−7.5 kcal/mol), and protocatechuic acid (−7.5 kcal/mol). Importantly, all the top five selected compounds exhibited better docking score than the standard compound glutamic acid (−6.6 kcal/mol). Cyclokievitone hydrate bound with the target protein through

three H-bonds with Cys1, Gly99, Asp123 residues, and four hydrophobic interactions with Cys1, Arg73, His97, Gly99 residues. The standard compound glutamic acid formed hydrogen bonds with Cys1, Arg73, Thr76, His77, His86, Gly99, Asp123 residues of the active site and hydrophobic interactions with Asn98 residue. All the residues of cyclokievitone hydrate formed interactions at the active site of the enzyme. The superimposed representation of cyclokievitone hydrate and docked co-crystallized ligand with the native co-crystallized ligand glutamic acid, two-dimensional and three-dimensional interactions of cyclokievitone hydrate and glutamic acid with the enzymatic pockets of GlcN-6-P synthase are shown in Fig. 5 and Fig. 6.

3.9. Molecular docking associated with anthelmintic activity

Interaction between the selected biological compounds of *Vigna mungo* and the target protein tubulin (PDB ID: **1SA0**) were determined for anthelmintic activity. Surprisingly, all of the compounds interacted with our target protein. The docking score ranges from −8.3 to −3.1 kcal/mol. The docking results of the top five compounds and the standard compound DAMA-colchicine that docked with the tubulin are presented in Table 9. 2′-hydroxygenistein (−8.3 kcal/mol) displayed the utmost binding affinity towards our target followed by 2′-hydroxydihydrodaidzein (−8.2 kcal/mol), stigmasterol (−7.8 kcal/mol), dalbergioidin (−7.6 kcal/mol), and aureol (−7.6 kcal/mol). Importantly, all of the selected compounds exhibited better docking score than the standard compound DAMA-colchicine (−6.6 kcal/mol). The best scored compound 2′-hydroxygenistein bound with the target tubulin through two H-bonds with Ala250, Val315 active site residues, and five hydrophobic interactions with Leu248, Lys254, Met259, Ala316, Lys352 residues. All the residues except Leu248 formed interactions at the

Table 6
Thrombolytic activity of the MESVM at different concentrations.

Sample no.	Concentration (mg/mL)	Initial weight (w_1 mg)	Weight after serum removed (w_2 mg)	Weight after clot lysis (w_3 mg)	% Thrombolysis	Mean ± SEM
MESVM 1	2	803	1050	1017	13.36	13.67 ± 0.61
		802	1005	979	12.81	
		795	1024	990	14.85	
MESVM 2	4	815	1106	1049	19.50	18.96 ± 0.59
		804	1059	1009	19.60	
		795	1093	1040	17.79	
MESVM 3	6	806	1048	990	23.97	23.63 ± 0.84
		799	1085	1022	22.03	
		807	1048	988	24.89	
MESVM 4	8	810	1055	984	28.98	29.09 ± 0.44
		792	1036	963	29.90	
		780	1044	969	28.40	
MESVM 5	10	802	1035	959	32.60	31.52 ± 0.56
		810	1050	975	31.25	
		798	1036	963	30.70	

Values are shown as means of triplicates (n = 3) ± SEM. For all concentrations, in comparison with control $P < 0.001$.
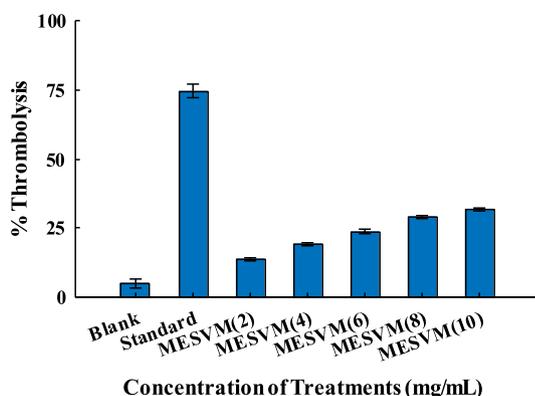

Fig. 4. Clot lytic activity by blank, standard drug and different concentrations of the MESVM.

active site of the target protein. The standard compound DAMA-colchicine formed interactions with Cys241, Ala250, Ala316, Lys352, Ala354, Leu248, Thr353 residues. Importantly, the best docked compound 2′-Hydroxygenistein displayed better interactions at the active site of the tubulin compared to the native co-crystallized ligand DAMA-colchicine. The superimposed representation of 2′-hydroxygenistein and docked co-crystallized ligand with the native co-crystallized ligand DAMA-colchicine, two-dimensional and three-dimensional interactions of 2′-hydroxygenistein and DAMA-colchicine with the binding pockets of tubulin are shown in Fig. 7 and Fig. 8.

3.10. Molecular docking associated with thrombolytic activity

The interaction between selected biological compounds of *Vigna mungo* and human tissue plasminogen activator (PDB ID: **1A5H**) were determined for thrombolytic activity. Surprisingly, all of the compounds interacted with our target enzyme. The docking score ranges from −8.4 to −3.3 kcal/mol. The docking results of the top five compounds and the standard compound bis-benzamidine that docked with the human tissue plasminogen activator are presented in Table 10. Aureol (−8.4 kcal/mol) displayed the utmost binding affinity towards our target enzyme followed by cyclokievitone hydrate (−8.3 kcal/mol), cyclokievitone (−8.3 kcal/mol), 2′-Hydroxygenistein (−8.0 kcal/mol), and 2′-Hydroxydaidzein (−8.0 kcal/mol). The standard compound bis-benzamidine (−9.0 kcal/mol) displayed the highest docking score. Aureol formed

Table 7
Docked results of the selected phytoconstituents towards the individual target proteins.

Compound	Docking score (kcal/mol)		
	1SA0	1XFF	1A5H
Kaempferol	−7.0	−7.5	−7.5
γ-Tocopherol	−5.7	−5.8	−6.6
δ-Tocopherol	−6.3	−5.9	−7.3
α-Tocopherol	−5.8	−5.8	−7.1
Campesterol	−7.5	−7.0	−7.9
Avenasterol	−7.2	−6.6	−7.8
Stigmasterol	−7.8	−7.5	−7.9
α-Linolenic Acid	−5.1	−5.8	−6.1
Oleic Acid	−4.8	−5.4	−4.9
2′-Hydroxygenistein	**−8.3**	−7.7	−8.0
2′-Hydroxydaidzein	−6.7	−6.8	−8.0
Kievitone	−7.1	−7.1	−7.9
Dalbergioidin	−7.6	−7.0	−7.5
Cyclokievitone	−7.2	−7.4	−8.3
5-Deoxykievitone	−6.5	−6.9	−8.0
2′-Hydroxydihydrodaidzein	−8.2	−6.6	−7.3
Isoferreirin	−6.8	−6.8	−7.3
Aureol	−7.6	−7.5	**−8.4**
Demethylvestitol	−6.8	−6.8	−7.2
Kievitone Hydrate	−6.8	−6.8	−7.5
4′-O-Methylkievitone	−6.8	−7.4	−7.6
Cyclokievitone Hydrate	−7.4	**−8.0**	−8.3
5-Deoxykievitone Hydrate	−6.8	−7.6	−7.3
Glycinol	−3.1	−3.5	−3.3
Ferulic Acid	−5.7	−5.7	−5.9
Gentisic Acid	−6.0	−5.7	−6.4
Gallic Acid	−5.5	−5.7	−6.4
Protocatechuic Acid	−5.9	−7.5	−6.3
Standard (co-crystallized ligand)	**−6.6**	**−6.6**	**−9.0**

Bold text indicates the best docking score.

complex with the target enzyme through four H-bonds with Asp189, Ala190, Gln192, Ser195 active site residues and three hydrophobic interactions with Ala190, Cys191, Trp215 residues. The standard compound bis-benzamidine formed hydrogen bonds with Asp97, Thr98, Thr175, Ser214 residues and hydrophobic interactions with Arg174, Tyr99, Cys191, Trp215 residues. Importantly, the best docked compound aureol displayed better interactions at the active site of the human tissue plasminogen activator compared to the native co-crystallized ligand bis-benzamidine. The superimposed representation of aureol and docked co-crystallized ligand with the native co-crystallized ligand bis-benzamidine, two-dimensional and three-dimensional interac-

Table 8

Molecular docking interaction analysis of the top five selected compounds of VM with GlcN-6-P synthase.

1XFF Compounds	Docking Score (kcal/mol)	Hydrogen bond interactions	Hydrophobic Bonds (Pi-alkyl/Alkyl interaction)	Hydrophobic Bonds (Pi-Pi/Pi-sigma/Pi-cation/Pi-anion/Amide-Pi interaction)	Hydrophobic Bonds (Pi-sulfur/carbon-hydrogen interaction)
Cyclokievitone Hydrate	**−8.0**	**Cys1, Gly99, Asp123**	**Arg73**	**His97**	**Cys1, Gly99**
2′-Hydroxygenistein	−7.7	Cys1, His86, Gly99	Arg73	Trp74	Cys1, Trp74, Gly99
5-Deoxykievitone Hydrate	−7.6	Cys1	Pro177	Arg26	Ser191
Kaempferol	−7.5	Cys1, Arg73, Trp74	Arg73	–	–
Protocatechuic Acid	−7.5	Thr76, His77	Arg73	–	Asn98
Standard (co-crystallized ligand)	**−6.6**	**Cys1, Arg73, Thr76, His77, His86, Gly99, Asp123**	**–**	**–**	**Asn98**

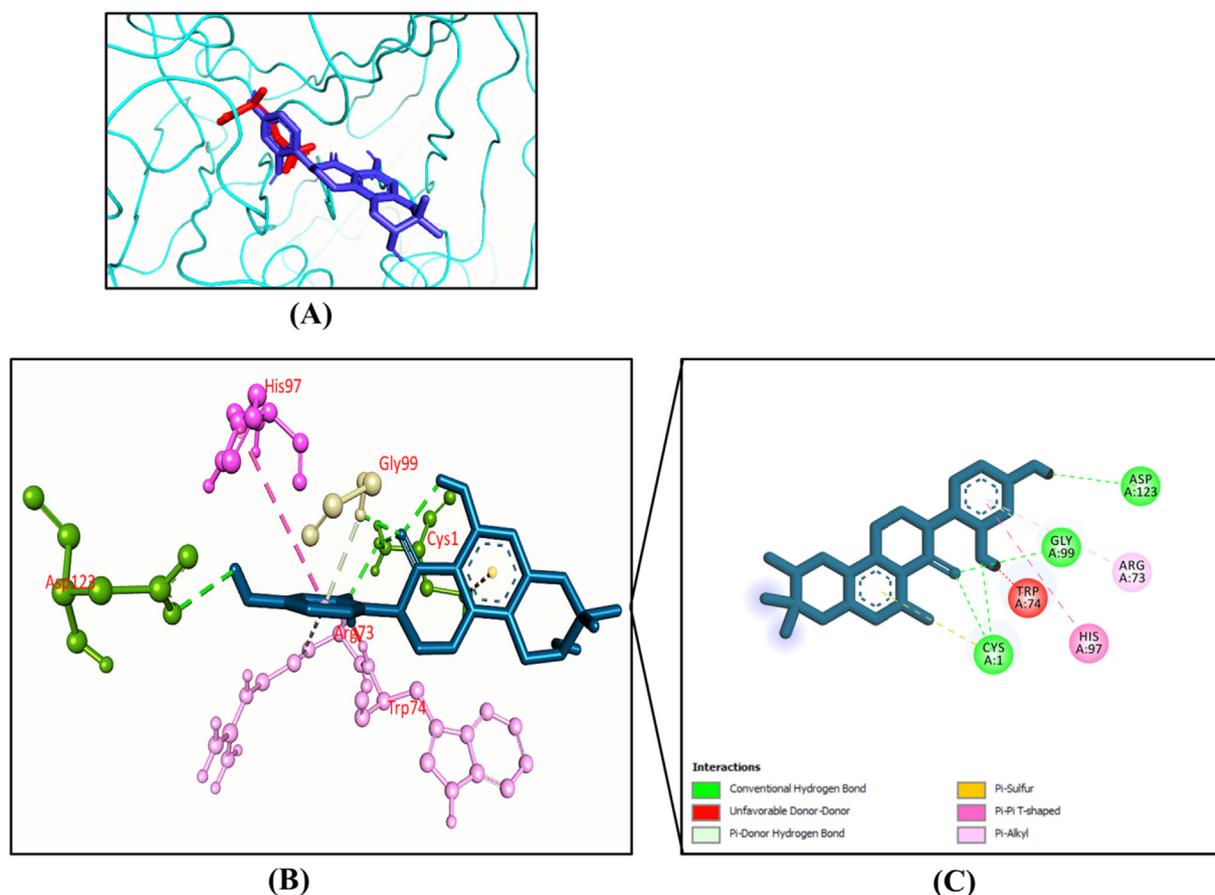Bold text indicates the best docking score.

Fig. 5. Best-ranked poses of Cyclokievitone Hydrate. Here, (A) superimposed representation of Cyclokievitone Hydrate (blue stick) with native co-crystallized ligand Glutamic Acid (red stick) of the enzyme; (B) 3D representation, and (C) 2D representation. Green for hydrogen bonds, deep pink for hydrophobic bonds (Pi–Pi/Pi–sigma interactions), light pink for hydrophobic bonds (Pi–alkyl/alkyl interactions stacking), and white for Pi donor–hydrogen bonds.

tions of aureol and bis-benzamidine with the binding pockets of human tissue plasminogen activator are shown in Fig. 9 and Fig. 10.

3.11. Prediction of ADME profile

The prediction of ADME profile of the top docked compounds was conducted based on Lipinski's rule of five (RO5) to further validate the molecular docking results. Importantly, all the top docked compounds except stigmasterol successfully passed the Lipinski's

filter as these compounds did not contravene more than one parameter. The result of the ADME/T analysis is shown in Table 11.

4. Discussion

Phytomedicine is revived for its safer use and long-lasting effectiveness, yet it becomes almost extinct in the 21st century. Phytochemicals present in the plants are responsible for various pharmacological activities (Choudhary et al., 2021). From the phytochemical analysis it was seen that the studied sample contained
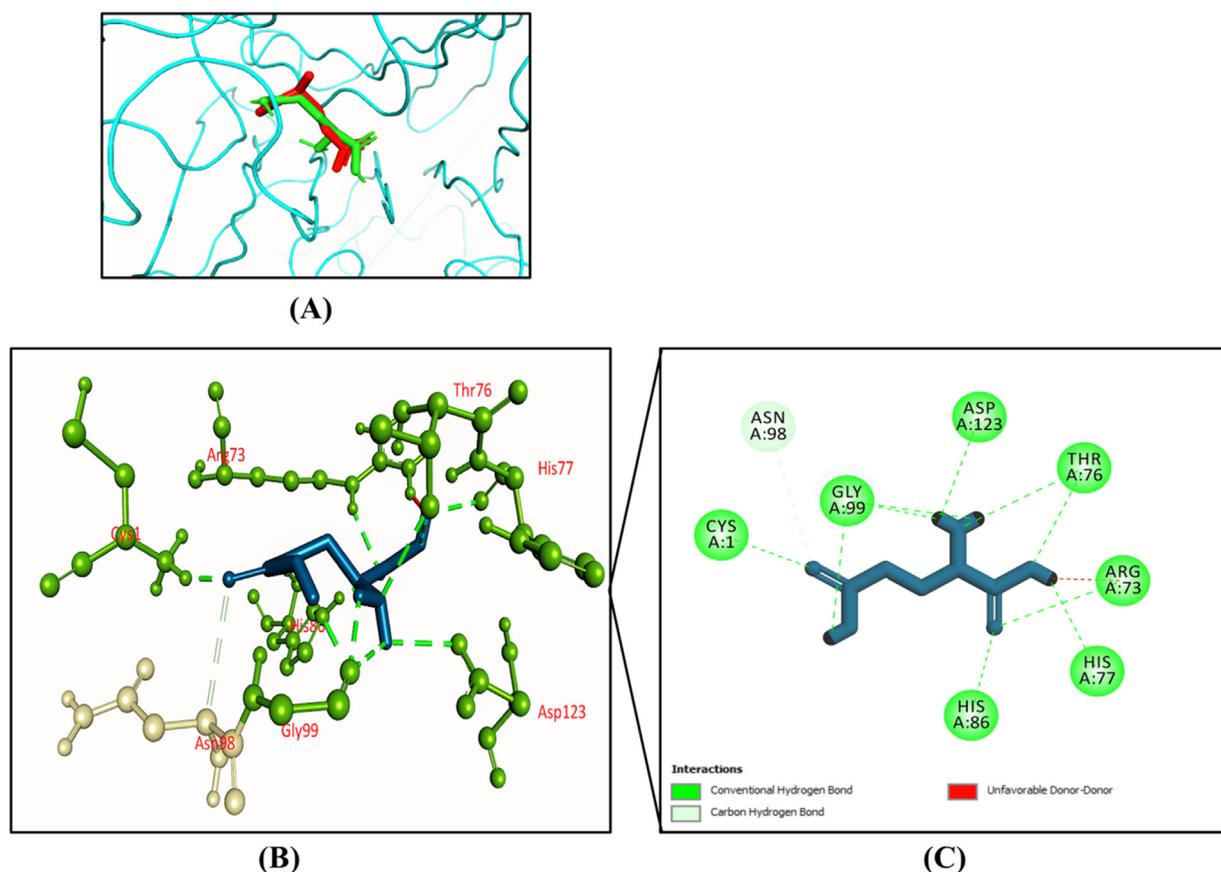
Fig. 6. Best-ranked poses of Glutamic Acid (co-crystallized ligand). Here, (A) superimposed representation of docked co-crystallized ligand (green stick) with native co-crystallized ligand Glutamic Acid (red stick) of the enzyme; (B) 3D representation, and (C) 2D representation. Green for hydrogen bonds, and white for carbon–hydrogen bonds.

Table 9
Molecular docking interaction analysis of the top five selected compounds of VM with tubulin.

1SA0 Compounds	Docking Score (kcal/mol)	Hydrogen bond interactions	Hydrophobic Bonds (Pi-alkyl/Alkyl interaction)	Hydrophobic Bonds (Pi-Pi/Pi-sigma/Pi-cation/ Pi-anion/Amide-Pi interaction)	Hydrophobic Bonds (Pi-sulfur/carbon-hydrogen interaction)
Dalbergioidin	−7.6	Asn258	Cys241, Leu248, Lys254, Ala316, Ala354	–	–
Stigmasterol	−7.8	–	Cys241, Leu248, Ala250, Lys254, Leu255, Ala316, Ala354	–	–
2′-Hydroxydihydrodaidzein	−8.2	Asn258	Cys241, Leu248, Lys254, Ala316, Ala354	–	–
2′-Hydroxygenistein	**−8.3**	**Ala250, Val315**	**Leu248, Lys254, Met259, Ala316, Lys352**	**–**	**–**
Aureol	−7.6	Asn249, Asn258	Leu248, Ala250, Lys254, Met259, Ala316, Lys352	–	–
Standard (co-crystallized ligand)	**−6.6**	**Cys241, Ala250**	**Ala316, Lys352, Ala354**	**Leu248**	**Thr353**

Bold text indicates the best docking score.

different phytochemical compounds such as carbohydrates, alkaloids, glycosides, proteins, phytosterols, flavonoids, phenols, tannins and saponins.

Antibacterial activity was tested in the extract in a view to getting new sources of antimicrobial lead compounds because almost all conventional antibiotics are becoming multi-resistant and show more side effects than the traditional medicines (Parekh et al., 2005). As the considerable and significant zone of inhibition is discovered in the MESVM against *Staphylococcus aureus* and *Escheri-

chia coli* but not against *Bacillus subtilis*, the seed extract is thought to have antibacterial potentiality against gram (+) ve and gram (−) ve bacteria. The underlying reason behind the antibacterial activity of the MESVM may be the presence of flavonoids, terpenoids and saponins in the extract (Koche et al., 2016). The extract could provide antibacterial action by inhibiting bacterial cell wall, inhibiting bacterial protein biosynthesis, DNA replication inhibition or folic acid metabolism inhibition (Kapoor et al., 2017). The reason behind appearance of no zone of inhibition against *B.*
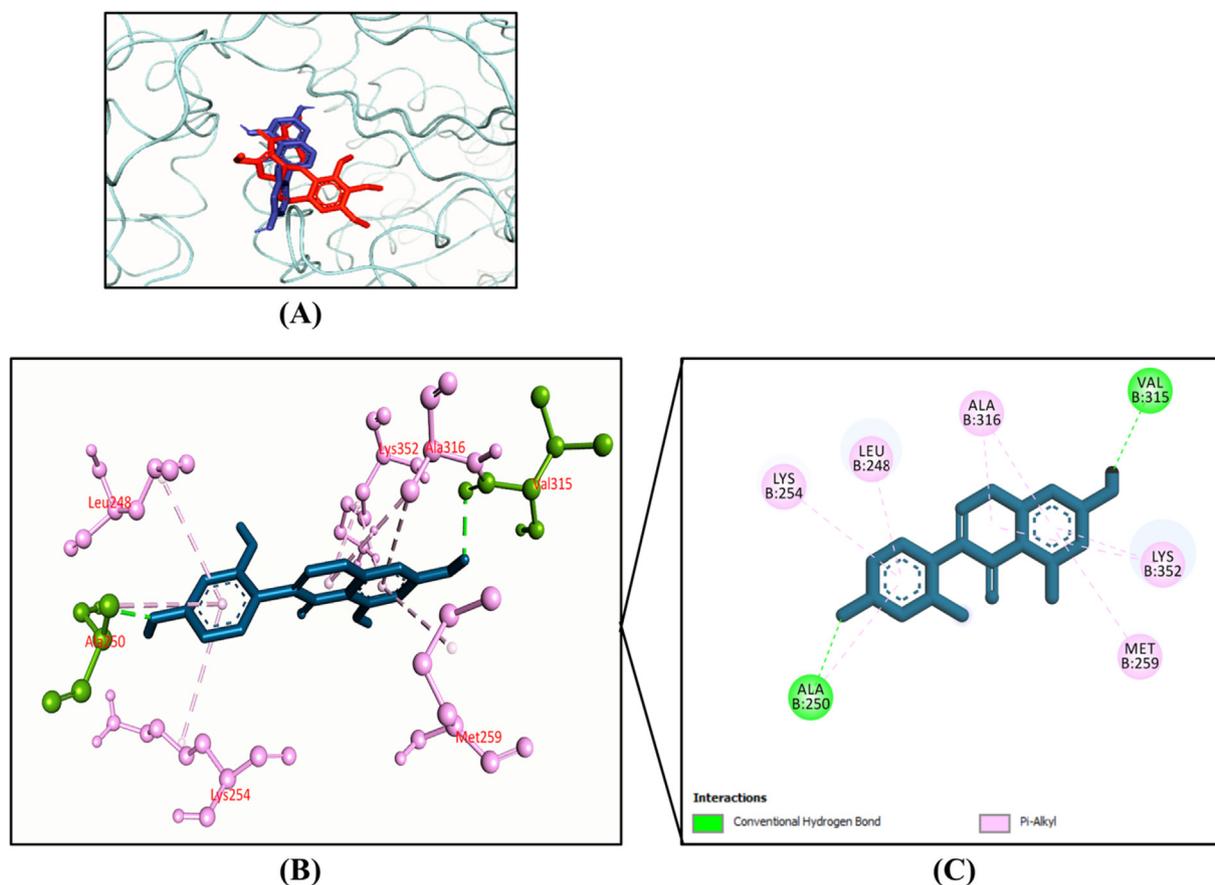
Fig. 7. Best-ranked poses of 2′-Hydroxygenistein. Here, (A) superimposed representation of 2′-Hydroxygenistein (blue stick) with native co-crystallized ligand DAMA-colchicine (red stick) of the target protein (B) 3D representation, and (C) 2D representation. Green for hydrogen bonds, and light pink for hydrophobic bonds (Pi–alkyl/alkyl interactions stacking).

subtilis is not identified, yet another research study has also discovered same result for the MESVM against this bacterium (Chanda et al., 2010). Further investigations might be needed to validate the result and to find out the underlying reasons.

Anthelmintic activity of the MESVM was evaluated in this study against earthworm (*Pheretima posthuma*) using albendazole as a standard drug. *Pheretima posthuman* is used in the initial evaluation of anthelmintic activity, because it has physiological and anatomical resemblance with intestinal helminths (Das et al., 2017). The standard drug albendazole inhibits polymerization of alpha-tubulin and beta-tubulin by binding to the beta-tubulin at its colchicine sensitive site that ultimately causes loss of cytoplasmic microtubules at the worms' intestinal cells. Moreover, this drug provides its activity by inhibiting glucose uptake by helminthic cells. Our study exhibited that increasing concentration of the MESVM gradually decreased paralysis and death time of the earthworms. It indicates that the extract has concentration dependent anthelmintic activity. The metabolites responsible for anthelmintic activity are alkaloids, terpenoids and polyphenolic compounds such as tannins (Das et al., 2017). All of these phyto-constituents are found in the test extract that may influence the positive response of helminth destruction of the MESVM by the mechanism of inhibiting tubulin polymerization.

Neuropharmacological test was performed to determine whether the extract had any CNS depressive or anxiolytic property. It is found from the results (see Table 4 and Table 5) that no extract or fraction exhibits CNS depressive or anxiolytic actions. Two mice models of anxiety were carried out to evaluate the anxiolytic neuropharmacological activity- one was open-field and another was

EPM. The open-field test measures exploration and locomotion based anxiolytic activity in rodents. Increase of locomotion indicates alertness and decrease of locomotion shows CNS depression or sedation of rodents (Thakur and Mengi, 2005). In the open-field test, mice treated with the standard anxiolytic drug diazepam showed downward number of movements from 130 to 119 that may be the output of GABA-induced CNS depression of the drug in the test animals. No extracts or fractions notably decrease locomotion of the mice that reflects that the seed has no sedative property, thus cannot be used as anxiolytic agent. The EPM test is an useful and widely used test to screen anxiogenic and anxiolytic potentials of established drugs and test samples (Sharmen et al., 2014). Anxiolytics reduce the innate fear of mice toward height and openness that is revealed by increasing percentage of both of the entry number and the time-spent in the open arm of EPM. In the contrary, anxiogenics increase the inherent less entry number and less time spending nature of mice in the open arm (Subramanian et al., 2013). From this study, it has been seen that the entry and spent time of the standard drug-treated mice in the open arm of EPM apparatus increased with time. It may be the outcome of GABA-induced inhibitory action of CNS that makes them less afraid of staying in the open part of the apparatus. Nevertheless, no mice treated with the test samples stayed in the open arm for noteworthy time. From the previous studies, alkaloids, flavonoids and sterols are known to cause CNS depression and antipsychotic effects (Jain et al., 2019, Emran et al., 2018). Despite containing alkaloids, flavonoids and phytosterols, the seed extract did not show CNS depression or anxiolytic activity. The seeds have traditional use as nervine tonic and previous report has claimed
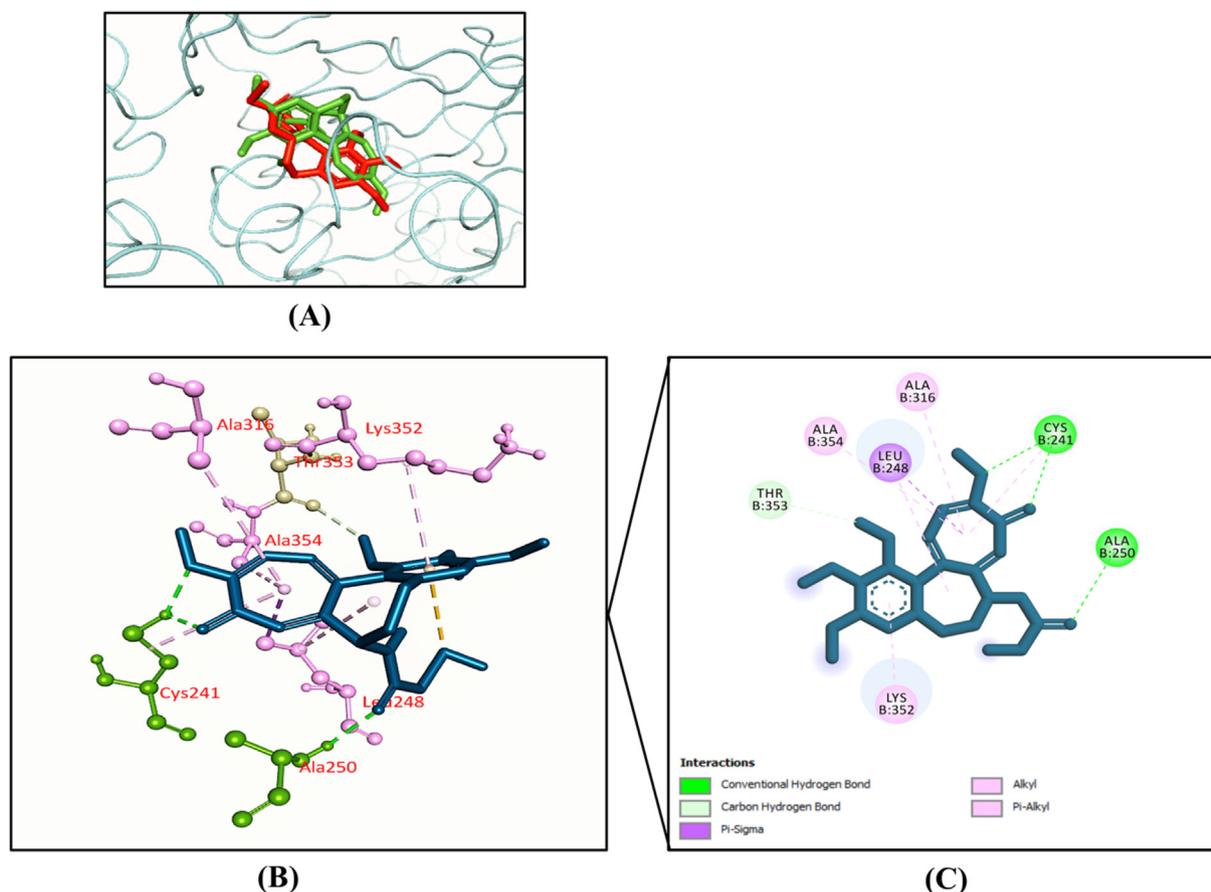
Fig. 8. Best-ranked poses of DAMA-colchicine (co-crystallized ligand). Here, (A) superimposed representation of docked co-crystallized ligand (green stick) with native co-crystallized ligand DAMA-colchicine (red stick) of the protein; (B) 3D representation, and (C) 2D representation. Green for hydrogen bonds, pink for hydrophobic bonds (Pi–alkyl/alkyl interactions stacking), and white for carbon–hydrogen bonds.

Table 10
Molecular docking interaction analysis of the top five selected compounds of VM with human tissue plasminogen activator.

1A5H Compounds	Docking Score (kcal/mol)	Hydrogen bond interactions	Hydrophobic Bonds (Pi-alkyl/Alkyl interaction)	Hydrophobic Bonds (Pi-Pi/Pi-sigma/Pi-cation/ Pi-anion/Amide-Pi interaction)	Hydrophobic Bonds (Pi-sulfur/carbon-hydrogen interaction)
Cyclokievitone	−8.3	Gly193, Gly216	Arg174	Tyr99, Trp215	Gln192
Cyclokievitone Hydrate	−8.3	Gly216	Arg174	Tyr99, Trp215	–
Aureol	**−8.4**	**Asp189, Ala190, Gln192, Ser195**	**Ala190**	**Cys191, Trp215**	**–**
2′-Hydroxygenistein	−8.0	Asp189, Gly193, Ser194	Ala190	Cys191, Trp215	–
2′-Hydroxydaidzein	−8.0	Ser195, Gly216	Ala190	Cys191, Trp215	–
Standard (co-crystallized ligand)	**−9.0**	**Asp97, Thr98, Thr175, Ser214**	**Arg174**	**Tyr99, Cys191, Trp215**	**–**

Bold text indicates the best docking score.

their anticonvulsant property (Gohel et al., 2011). As no clear reason is found behind its negative result of CNS inhibitory activity, further research should be studied to verify this test result.

A thrombus or blood clot is formed by converting fibrinogen into fibrin in the presence of activated thrombin. Damaged tissues secrete tissue plasminogen activator (t-PA) that transforms the plasma protein plasminogen into plasmin and then the plasmin dissolves the blood clot. Fibrinolytic agents are used in the patients having occlusion in the vein or artery that exert thrombolytic action by activating tissue plasminogen activator (t-PA) and thus remove vascular thrombi. The VM seed extract was evaluated to find out natural thrombolytic agent in it. In this study, streptokinase, a fibrinolytic agent was used as a standard drug that provided 74.52% clot lysis against the negative control, where the extract showd concentration-reliant clot lysis and the highest (31.52%) clot lytic activity was found at 10 mg/mL of the MESVM. Secondary metabolites like saponins, alkaloids and tannins are responsible for the thrombolytic activity (Das et al., 2013). All of these phytoconstituents were found in the MESVM that may be the significant reason for potent clot lytic effect of the sample.

In the computational drug design, molecular docking is one of the most frequently used method for predicting the orientation
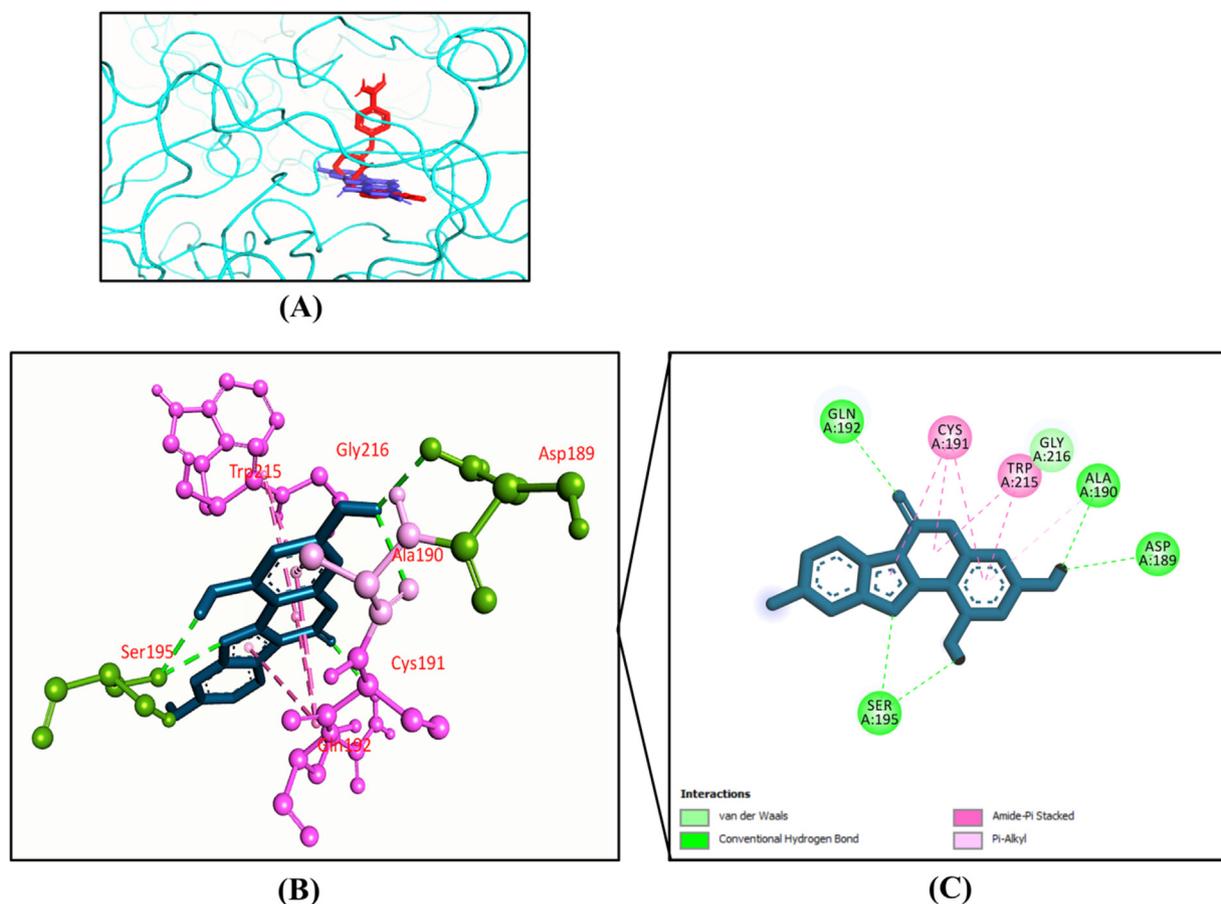
Fig. 9. Best-ranked poses of Aureol. Here, (A) superimposed representation of Aureol (blue stick) with native co-crystallized ligand Bis-Benzamidine (red stick) of the target enzyme; (B) 3D representation, and (C) 2D representation. Green for hydrogen bonds, deep pink for hydrophobic bonds (Pi–Pi/Amide–Pi interactions), and pink for hydrophobic bonds (Pi–alkyl/alkyl interactions stacking).

of small molecules bound to the binding pocket of an enzyme or receptor (Hossen et al., 2021). This method is based on specific scoring algorithm which is designed to assign binding energy to the ligands fitted with target protein and expresses binding affinity. A ligand-target protein complex becomes highly stable when the interaction generates low binding energy. (Shoichet et al., 2002). In our current study, docking simulation was run for investigating binding affinities of the selected phytoconstituents from VM seeds against various target proteins. The inhibition of the polymerization of microtubule is an effective way to arrest helminth infections (Köhler and Bachmann, 1981). It is reported that, benzimidazole drugs such as thiabendazole can selectively bind to the beta-tubulin of parasites and prevents the formation of microtubule (Martin et al., 1997). Tubulin is the dimeric subunit protein of microtubules, which can be primarily targeted for the anthelmintic effects. Therefore, tubulin was targeted to predict the anthelmintic activities of the selected compounds in the molecular docking analysis. Antibacterial activity was predicted against GlcN-6-P syun

nthase. Presently, the pathogenic organisms are developing resistance against antibacterial drugs. Therefore, the development of new antibacterial drugs to combat these cases is essential. GlcN-6-P synthase enzyme works relatively as a newer antibacterial target that catalyzes the reaction between L-glutamine and fructose-6-phosphate (Padmavathi and Tajne, 2016). This reaction produces glucosamine-6-phosphate that further produces UDP-N-acetylglucosamine, a crucial compound for bacterial peptidoglycan layer. Any particular ligand inhibiting GlcN-6-P synthase enzyme

may elicit antibacterial action by suppressing the glucosamine-6-phosphate production and thus preventing bacterial cell wall formation. Balachandran et al depicted the antimicrobial purpose of GlcN-6-P synthase inhibition in a previous study (Fikrika et al., 2016). To predict the thrombolytic activities, the selected compounds were docked against human tissue plasminogen activator. This is a serine protease that dissolves fibrin by transforming plasminogen into plasmin. Tissue plasminogen activator is also beneficial in the clinical treatment of intracranial artery injuries and other heart diseases (Jin et al., 2013). Thrombolytic medications including streptokinase, urokinases are widely used. However, the previous types activators are used more effectively and safely than the later ones (Hossain et al., 2019). In anthelmintic molecular docking study, 2′-hydroxygenistein displayed the best docking score compared to the other selected compounds. It not only displayed better docking scores but also formed more interactions with the target protein at its active site compared to the standard compound DAMA-colchicine. Cyclokievitone hydrate displayed the best binding interactions compared to other selected compounds and standard compound glutamic acid in the antibacterial molecular docking study. It interacted with the GlcN-6-P synthase at its active site via Cys1, Gly99, Asp123, Arg73, and His97 residues. Arg73 and Asp123 are considered a key residue for substrate recognition of our target enzyme (Isupov et al., 1996). In thrombolytic molecular docking study, aureol displayed the utmost binding affinity towards the human tissue plasminogen activator where it formed complex with the target enzyme at its active site involving Asp189, Ala190, Gln192, Ser195, and Trp215 residues. A salt bridge
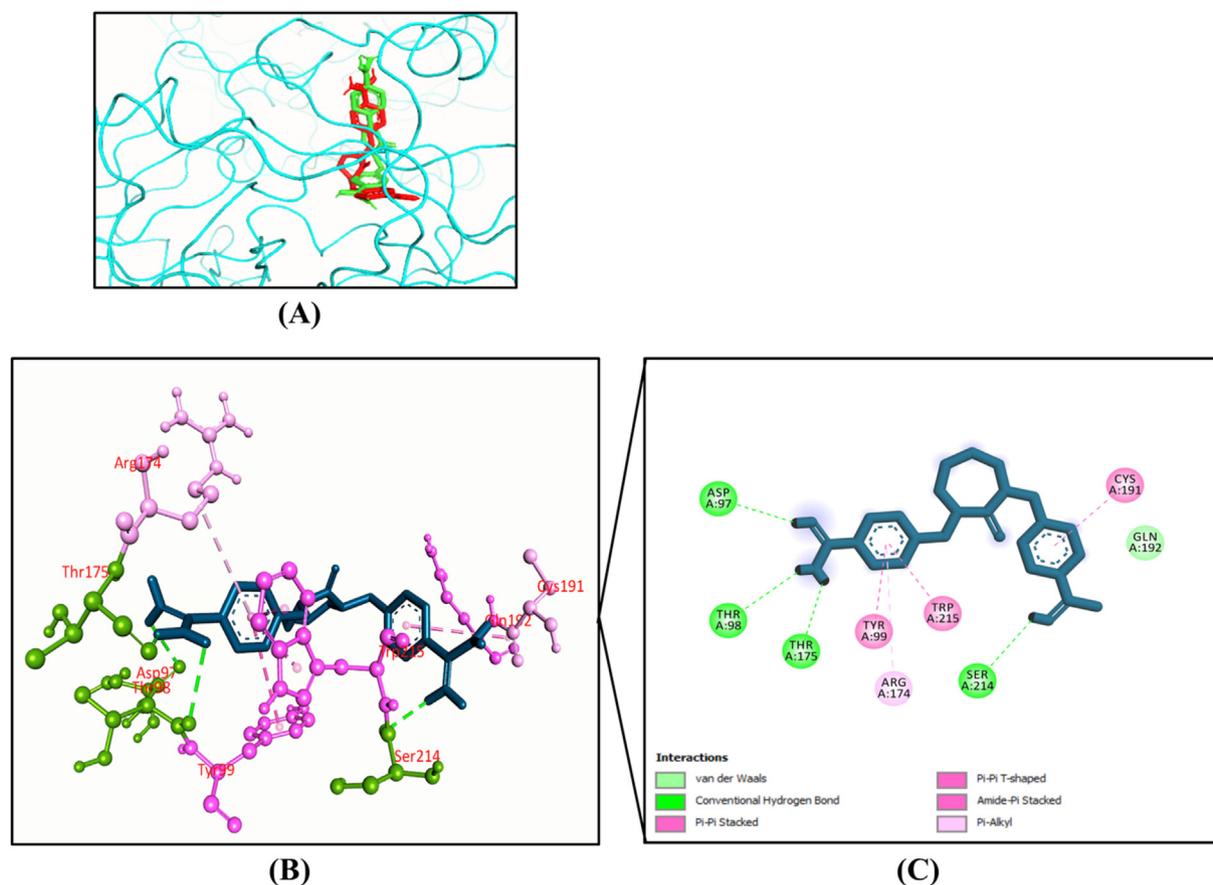
Fig. 10. Best-ranked poses of Bis-Benzamidine (co-crystallized ligand). Here, (A) superimposed representation of docked co-crystallized ligand (green stick) with native co-crystallized ligand Bis-Benzamidine (red stick) of the target enzyme; (B) 3D representation, and (C) 2D representation. Green for hydrogen bonds, deep pink for hydrophobic bonds (Pi–Pi/Pi–sigma interactions), light pink for hydrophobic bonds (Pi–alkyl/alkyl interactions stacking).

Table 11
Prediction of ADME profile of the top compounds obtained from the molecular docking analysis.

Compounds	Molecular Weight[a] (g/mol) <500	Hydrogen Bond Acceptors[b] ≤10	Hydrogen Bond Donors[c] ≤5	MlogP[d] <5	Molar Refractivity[e] 40–130	No. of Lipinski violations[f] ≤1
Cyclokievitone Hydrate	372.37	7	4	0.55	96.54	0
2′-Hydroxygenistein	286.24	6	4	−0.03	76.01	0
5-Deoxykievitone Hydrate	358.39	6	4	1.09	96.96	0
Kaempferol	286.24	6	4	−0.03	76.01	0
Protocatechuic Acid	154.12	4	3	0.40	37.45	1
Dalbergioidin	288.25	6	4	0.16	73.59	0
Stigmasterol	412.69	1	1	6.62	132.75	2
2′-Hydroxydihydrodaidzein	272.25	5	3	0.71	71.57	0
Aureol	284.22	6	3	1.21	75.83	0
Cyclokievitone	354.35	6	3	1.28	95.69	0
2′-Hydroxydaidzein	270.24	5	3	0.52	73.99	0

Note: [a] Molecular weight less than 500 g/mol, [b] Less than or equal 10 hydrogen bond acceptor, [c] Less than or equal 5 hydrogen bond donors, [d] High lipophilicity (expressed as LogP) less than 5, [e] Molar refractivity should be between 40 and 130, [f] Lipinski's rule of five violations less than or equal 1.

is formed between Asp189 and basic residues at the base of the enzyme (Renatus et al., 1997). Salt bridge formation contributes to the molecular recognition and conformational specificity. The salt bridge involving Asp with basic residues such as His, Lys represents extremely well-defined geometric preferences (Donald et al., 2011). Importantly, aureol exhibited better interactions with the target at its active site compared to the standard compound bis-benzamidine. These top docked compounds also showed significant results in the ADME prediction analysis. All of the ligands interacted with the specific targets at their active sites and their

pharmacokinetic properties indicate that these ligands may serve as potential lead compounds for the highlighted effects.

5. Conclusion

In this paper, the MESVM was evaluated for antibacterial, anthelmintic, thrombolytic and neuropharmacological activities and phytoconstituents of the seeds were analyzed by molecular docking to identify possible lead compounds. Moreover, AESVM

and subsequent fractions of MESVM were tested for investigating their anxiolytic effect. The MESVM showed ZOI against *S. aureus* and *E. coli* indicating its potential broad spectrum antibacterial activity that conformed the results of previous studies done on different parts of this plant. It displayed concentration-dependent notable anthelmintic activity. No test samples showed CNS depressive and anxiolytic effects compared with untreated sample. The result of thrombolytic activity test depicted that the MESVM had highly significant clot lytic activity. This clot lytic activity enhanced gradually with increasing concentration of the extract. In silico molecular docking analysis revealed that most of the phytoconstituents of the VM seeds exhibited antibacterial, anthelmintic and thrombolytic potentials. Among these, 2′-hydroxygenistein, cyclokievitone hydrate, and aureol showed the highest affinity towards their respective biological targets. These novel activities of the VM seeds might attract researchers to further explore the seeds for its potential lead compounds and formulate them into safer and more efficacious therapeutic compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors' contributions

TEM set up all of the necessary experimental preparations and designed the overall study. Experimental works, data collection and analysis were done by TEM, SZ and SAS. TEM performed statistical investigation. SMNU and MR supervised the work. Manuscript was prepared by TEM and SAS. All authors reviewed the manuscript. TEM edited the manuscript and SMNU approved the manuscript for submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.03.008.

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