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Unlocking the anthelmintic potential of *Grewia bilamellata* Gagnep.: *In-vitro* and molecular docking studies on adult Indian earthworms

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ABSTRACT

Anthelmintic resistance remains a significant challenge for the treatment of gastrointestinal parasites. The search for novel compounds is costly, but the traditional knowledge of Sashechalam hill practitioners led us to investigate *Grewia bilamellata* Gagnep. We assessed its anthelmintic activity against Indian earthworms (*Pheretima posthuma*) using various extract concentrations (10, 20, 50, and 100 mg/mL), with albendazole as the positive control and normal saline as the negative control. The duration of paralysis and death indicated anthelmintic efficacy. *G. bilamellata* ethanol extract (GBEE) demonstrated a significant concentration-dependent effect. The IC_{50} values for albendazole, *G. bilamellata* petroleum ether extract (GBPE), *G. bilamellata* ethyl acetate extract (GBEA), and GBEE were 181.947, 310.337, 270.488, and 223.468 mg/mL, respectively. GBEE exhibited potent anthelmintic activity comparable to that of albendazole, with the lowest paralysis and death rates in the model. The HR-LC-MS analysis of GBEE identified 38 phytoconstituents, of which 22 compounds obeyed Lipinski's rule. Molecular docking with β-tubulin revealed that 15 compounds exhibited superior binding energy (-8.3 to -6.3 kcal/mol) compared to albendazole (-6.1 kcal/mol). Further investigations are crucial to isolate and evaluate these compounds for the development of new anthelmintic drugs. Our findings support the traditional use of *G. bilamellata* Gagnep. as an anthelmintic, and highlight its potential for future therapeutic applications.

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1. Introduction

Helminth infections affect hundreds of millions of people annually. Worm invasions are typically observed in tropical climates and are often associated with anemia, eosinophilia, and malnutrition (Pandiyan et al., 2022). Helminthiasis is primarily caused in humans by the species of worms known as pinworms, tapeworms, and roundworms. Where sanitation is not up to standard, infected persons excrete helminth eggs in their excrement resulting in soil contamination. Typically, helminths live in the gastrointestinal tract but can also penetrate the liver and other organs (Borah et al., 2022). Currently, anthelmintic drugs are ineffective against gastrointestinal helminths. Helminth infection is the most common cause of the illness (Sreejith et al., 2013).

The total number of species of the genus *Grewia* is 323 making it the most varied genus within the family Malvaceae. *Grewia bilamellata* Gagnep. is a shrub that stands 3 to 4 m in height. Its leaves are arranged alternately and have caducous stipules 1.5 mm in length. The petioles are 3-5 mm in length, while the lanceolate blade ranges from 4 to 6 cm in length and 1.5 to 2 cm in width. The blades have an obtuse base, dentate margins, and a pointed apex. The inflorescence is a 3-flowered cyme located in the axillary region. The sepals are five (approximately 7-8 mm). The petals are five, half the length of the sepals. The fruit has a glabrous capsule that is subglobose in shape. The plant is endemic to the Western Ghats, a mountain range in southwestern India. It is found wildly in Kerala, Karnataka, and Tamil Nadu. *Grewia bilamellata* Gagnep. grows in evergreen forests, typically at altitudes between 700 and 1,800 m. The plant prefers well-drained soil and is adapted to humid tropical climates (Nayar and Sastry, 1987; Ma et al., 2006). The bark of *G. bilamellata* Gagnep. in East Africa is used to cure intestinal infestations and syphilis; root infusion

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is used to combat anemia, chest pain, snakebites, colds, diarrhea, and infertility in women. The leaves of the plant are used to treat helminthiasis, antidiabetic, febrifuge, and as an anticonvulsant (Arbonnier, 2004; Quattrocchi, 2012). To date, the leaves and stems of the plant have produced four neolignans, specifically 8-*O*-4′-neolignanguaiacylglycerol-β-coniferyl ether *(threo)*, 8-*O*-4′-neolignanguaiacylglycerol-β-coniferyl ether *(erythro),* nitidanin, and bilagrewin. Lignans are receiving attention in nutrition and health research because of their association with a lower risk of cancer, heart disease, and metabolic conditions. Additionally, two coumarin lignans, namely cleomiscosin D and grewin, have been identified. Furthermore, a quinone derivative (2,6-dimethoxy-1-acetonylquinol) and two triterpene derivatives, namely 3α,20-lupandiol and 2α,3β-dihydroxyolean-12-en-28-oic acid have been also isolated. Finally, the stem bark yields a derivative sterol, daucosterol (Ma et al., 2006; Ullah et al., 2012; Kumar et al., 2022). The chloroform extract of *G. bilamellata* Gagnep. exhibited antimalarial activity against D6 and W2 clones of *Plasmodium falciparum* with IC_{50} values of 2.3 ± 0.13 and 1.7 ± 0.12 µM, respectively (Ma et al., 2006; Ullah et al., 2012).

On the other hand, molecular docking could be defined as the study of the interaction or binding of two or more molecular structures involving different types of drugs as well as enzymes or proteins and how they are fitted together in the relevant simulations and computational-based analyses (Abbasi et al., 2021; Anita Margret et al., 2022). This powerful approach serves as a key tool and a reliable criterion in a variety of scientific disciplines from structural molecular biology to those upon the computer-assisted drug design (Shahriari et al., 2021). The objective of the performance of molecular docking is basically for the prediction of the major binding mode(s) of a ligand with a protein possessing 3D structure (Sarkar et al., 2023).

Our study represents a groundbreaking effort to explore the therapeutic potential of *G. bilamellata* Gagnep. by using advanced analytical techniques and computational methods. Using high-resolution liquid chromatography-mass spectrometry (HR-LC-MS) and molecular docking with β-tubulin as the target, we identified the phytoconstituents of *G. bilamellata* Gagnep. and predicted its anthelmintic activity. This novel approach provides valuable insights into the medicinal properties of *G. bilamellata* Gagnep. and paves the way for further investigation of its potential use in healthcare.

2. Experimental

2.1. Collection and authentication of plants

On October 25, 2022, a total of 5 kg of whole *Grewia bilamellata* Gagnep. plant was collected from the Seshachalam forest (latitude: 14.3333; longitude: 78.2500; altitude: 700 m) in Andhra Pradesh. The collected plant material was authenticated by Dr. K. Madhava Chetty, a plant taxonomist from the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh (voucher number 0428).

2.2. Preparation of *G. bilamellata* Gagnep. extracts

Whole plants (*G. bilamellata* Gagnep.) were first washed with water and dried in the shade to remove any dirt and other foreign matter. A coarse powder was subsequently prepared from the dried plant material and passed through a No. 14. After drying, the powdered material was placed in the thimble tube of a Soxhlet apparatus and extracted for 6 h with various solvents, *e.g.*, petroleum ether, ethyl acetate, and ethanol. For further study, the dried extract samples were kept in a refrigerator at low temperature after being filtered and dried using rotary vacuum evaporation.

2.3. Qualitative phytochemical tests

The phytochemical analysis of the whole plant extracts of *G. bilamellata* Gagnep. Viz., *G. bilamellata* petroleum ether extract (GBPE), *G. bilamellata* ethyl acetate extract (GBEA), and *G. bilamellata* ethanol extract (GBEE) was performed using standard methods (Khandelwal, 2008; Senguttuvan et al., 2014).

2.4. *In vitro* antihelmintic activity

2.4.1. Earthworms collection

Adult earthworms were procured from Prem Sai Organic Farm, Mellampudi, Guntur District, Andhra Pradesh for the purpose of conducting the assay. The use of adult earthworms was preferred owing to their anatomical and physiological resemblance to the intestinal roundworm parasites that are found in humans. Earthworms are widely used for the preliminary *in vitro* evaluation of anthelmintic activity primarily because of their accessibility (Pillai and Nair, 2011).

2.4.2. Preparation of extracts and reference drug

Crude extracts (100, 200, 500, and 1000 mg) were dissolved in 1 mL of dimethyl sulfoxide (DMSO) and diluted with 10 mL of normal saline to prepare diluted extract samples (10, 20, 50, and 100 mg/mL) of G. bilamellata Gagnep. for the *in vitro* studies. Albendazole was used as the standard drug, whereas a normal saline solution was used as the control (Das et al., 2011).

2.4.3. Anthelmintic activity

In the anthelmintic assay, *G. bilamellata* Gagnep. extracts (10, 20, 50, and 100 mg/mL) were used against Indian earthworms (*P. posthuma*). In this study, six groups of Indian earthworms were totally studied. Moreover, different concentrations of the extracts and albendazole (10, 20, 50, and 100 mg/mL) were used, while using normal saline as control. This study was conducted to observe the anthelmintic activity of earthworms and the time taken by earthworms to paralyze and become fatal was recorded (DSNBK et al. 2021).

2.5. Statistical analysis

The results obtained in the study are expressed as mean

± SEM. Dunnett's multiple comparison tests were also conducted to compare the data with ANOVA. Statistical analyses were performed using the GraphPad Software (Version 3, USA). The main criterion for the significance of our statistical evaluations was $p < 0.05$.

2.6. Identification of bioactive compounds by HR-HR-LC-MS

HR-LC-MS) analysis was performed on the GBEE samples using a ChipCube G6550A iFunnel Q-TOF mass spectrometer with an electrospray ionization source. Separation of phytochemicals was achieved using a Hypersil GOLD C-18 column (2.1 × 100 mm, 3 µm particle size) as the stationary phase. A gradient mobile phase of "solvent A" (0.1% formic acid in water) and "solvent B" (90% acetonitrile, 0.1% formic acid and 10% water) was used at a flow rate of 300 µL/min. The injection volume of GBEE was 3 µL at an injection speed of 100 µL/min with a 5.0 sample flush out factor. The gradient started with 95:5 (H_2O/CH_3CN) for 1 min, changed to 0:100 (H_2O/CH_3CN) for 25 min, and returned to 95:5 (H_2O/CH_3CN) for 6 min. The iFunnel MS Q-TOF instrument segment was maintained at a gas flow rate of 13 L/min with a temperature of 250 °C for a gas flow rate of 11 L/min at 300 °C for the sheath gas flow rate and 35 PSI nebulizer gas flow pressure. The acquisition method was set to MS mode with a minimum range of 125 (*m/z*) and a maximum of 1000 (*m/z*), at a scanning rate of 1 spectra/s. The analysis was performed at the Sophisticated Analytical Instrument Facility (SAIF) of the Indian Institute of Technology, Bombay (IIT Bombay), India (Noumi et al., 2020; Singh et al., 2022).

2.7. *In silico* studies

2.7.1. Drug likeliness

To assess the drug-like properties of the phytoconstituents identified in GBEE, a drug-likeness tool (DruLito) was employed. DruLito evaluated the chemical compounds based on Lipinski's rule of five, which examines parameters such as molecular weight, log P, and the number of hydrogen bond donors and acceptors. This analysis provides insight into the likelihood that the identified phytoconstituents possess drug-like properties (Setlur et al., 2017).

2.7.2. Molecular docking

The docking study was implemented using Auto Dock Vina, and the relevant input files for Auto Dock Vina were generated using the Auto Dock program. The tubulin-colchicine stathmin-like domain complex (PDB ID:1SA0) (Prasanth et al., 2020b) was used as the target for the current study (Fig. 1). The RSCB protein data bank provided crystallized structures, and PubChem provided 3D structural information on the ligands. Incorporating polar hydrogen atoms and gesture charges is necessary to prepare files through Auto Dock. Table 1 presents the measurements of the coordinates of the grid box and size of the grid box. Vina is implemented with a shell script supplied by the Auto Dock Vina developers. The strength of the bond between the ligand and receptor was represented as a negative score in kilocalories per mole. The Autodock Vina script produced nine distinct positions of the ligand with different binding energies for each ligand. A Perl script was used to obtain the ligand with the highest binding affinity for docked complexes (Sharma et al., 2009). Fig. 2 represents the 2D representation of the top five phytoconstituents having the highest docked scores against β-tubulin eluted from HR-LC-MS analysis of GBEE, as well.

2.7.3. ADMET analysis

To evaluate the potential pharmacokinetic properties and toxicity risks associated with the identified phytoconstituents, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction tool (ADMETSAR) was employed. ADMETSAR utilizes computational models to predict parameters such as aqueous solubility, blood-brain barrier permeability, cytochrome P_{450} inhibition, hepatotoxicity, and mutagenicity. This analysis aids in assessing the overall drug-like characteristics and potential safety concerns of the phytoconstituents. The ADMETSAR results provided valuable insights into the ADMET properties and potential risks associated with the identified compounds, guiding further investigations for the development of safe and effective anthelmintic drugs (Prasanth et al., 2020a).

3. Results and Discussion

Medicinal plants are of prime significance since they can be considered as the proper alternatives of a wide spectrum of synthesized drugs having harmful impacts for the human beings' health (Mohammadhosseini et al., 2019a, 2019b). A simple perusal of the scientific databases displays that a large number of valuable natural compounds have been characterized in different organs of plant materials within the past few decades. These compounds have been reported to display promising therapeutic activities and biological properties (Mohammadhosseini et al., 2022).

3.1. Extractive values of extracts of *G. bilamellata* Gagnep. and their qualitative phytochemical analysis

The percentage yields of GBPE, GBEA, and GBEE extracted from *G. bilamellata* Gagnep. were determined to be 2.52, 4.12, and 9.61 (w/w%), respectively. An initial phytochemical screening of the GBPE, GBEA, and GBEE extracts was performed, and the results are shown in Table 2.

3.2. Anthelmintic activity

The anthelmintic potencies of GBEE, GBEA, and GBPE were determined in *Pheretima posthuma* worms. According to our finding, as the concentration of the extract increased, the anthelmintic activity increased. The anthelminthic activity of GBEE was the highest among the three extracts, and was similar to that of the standard. Using concentrations of 10, 20, 50, and

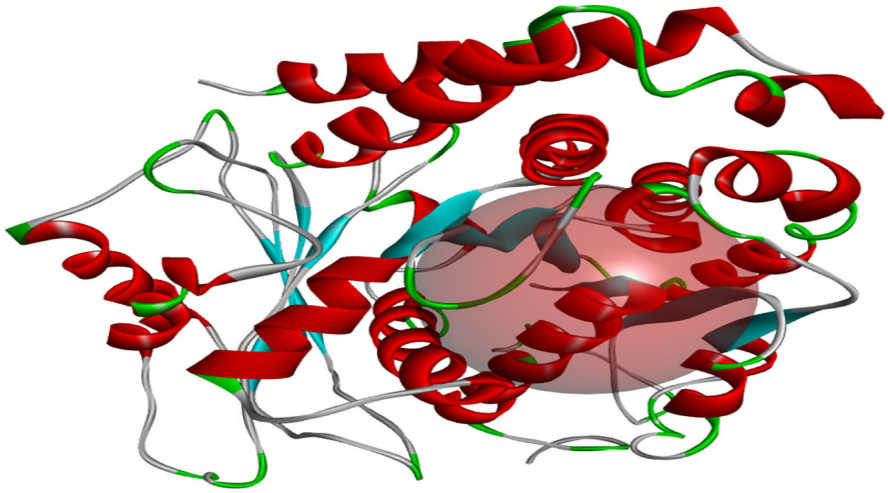

Fig. 1. 3D ribbon-type representation of the tubulin-colchicine: Stathmin-like domain complex (PDB ID:1SA0) with the active site (highlighted in red).

Table 1
Grid-Box coordinators used in autodock vina for molecular docking.

Centre	x	y	z
Tubulin-colchicine: stathmin-like domain complex (PDB ID: 1SA0)	127.059	95.345	13.797
Size	x	y	z
	10	10	10
Exhaustiveness	8		

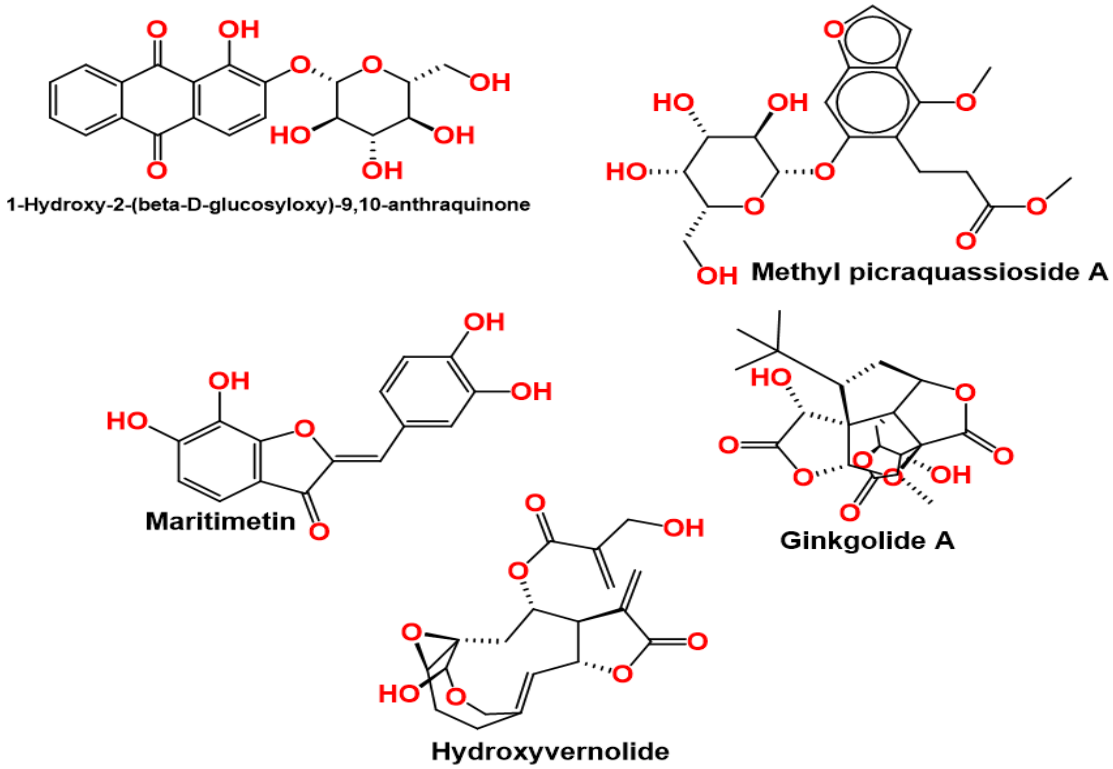

Fig. 2. 2D representation of the top five phytoconstituents with the highest docked scores against β-tubulin eluted from HR-LC-MS analysis of GBEE.

Table 2
Preliminary phytochemical analysis of various extracts of *G. bilamellata* Gagnep.

S. No	Phytochemical	Test Name	Results		
			PEGB	EAGB	GBEE
1	Alkaloids	Mayers	-	+	+
		Wagners	-	+	+
2	Flavonoids	Shinoda	-	+	+
		Alkaline	-	+	+
3	Tannins	Ferric Chloride	-	+	+
		Lead acetate	-	+	+
4	Steroids	Salkowski	+	-	+
		Liebermann-Burchard	+	-	+
5	Volatile oils	-	+	-	+
6	Saponins	Foam	-	-	+
7	Glycosides	-	-	-	+
8	Carbohydrates	Molisch	-	-	+
9	Proteins	Biuret	-	-	+
		Millons	-	-	+
10	Amino acids	Ninhydrin	-	-	+
11	Fixed oils	-	+	-	+

(+) Present (-) absent

100 mg/mL revealed that GBEE had paralysis times of 6.50, 3.54, 3.30, and 1.12 min, with death times of 42.19, 27.53, 22.08, and 18.27 min, respectively (Table 3; Fig. 3). However, the standard drug, albendazole, showed a paralysis time of 1.14 min and death time of 8.14 min when subjected to 100 mg/mL.

Table 3
Anthelmintic activity of various extracts of *G. bilamellata* Gagnep. against *Pheretima posthuma*.

Treatment	Dose	Paralysis Time	Death Time	IC_{50} (mg/mL)
Control	-	-	-	
Albendazole	10	5.56 ± 1.65*	28.22 ± 2.89*	181.947
	20	2.54 ± 0.52$	18.89 ± 0.78$	
	50	1.26 ± 0.23*	12.26 ± 2.63*	
	100	1.14 ± 0.18#	8.14 ± 1.52#	
GBPE	10	16.18 ± 2.85	68.15 ± 4.22	310.337
	20	12.12 ± 1.52*	59.12 ± 3.96*	
	50	9.47 ± 2.55	52.16 ± 2.16	
	100	8.14 ± 1.43$	42.54 ± 3.26$	
GBEA	10	9.63 ± 1.16*	36.12 ± 3.52*	270.488
	20	6.26 ± 0.22*	28.51 ± 2.66*	
	50	5.32 ± 0.83	23.11 ± 3.12	
	100	4.15 ± 0.66	20.15 ± 3.22	
GBEE	10	6.50 ± 1.54	42.19 ± 0.63	223.468
	20	3.54 ± 1.22#	27.53 ± 3.22#	
	50	3.30 ± 0.67	22.08 ± 2.58	
	100	1.12 ± 0.15$	18.27 ± 1.86$	

Values are expressed as mean ± SEM (n = 3), *p < 0.05, #p < 0.01, and $$p$ < 0.001 versus standard. GBPE: *G. bilamellata* Gagnep. petroleum ether extract; GBEA: *G. bilamellata* Gagnep. ether acetate extract; GBEE: *G. bilamellata* Gagnep. ethanol extract.

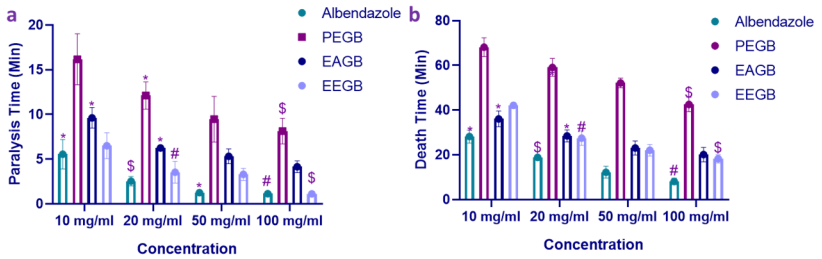

Fig. 3. Paralysis and death times for various extracts of *G. bilamellata* Gagnep. using standard albendazole.

3.3. HR-LC-MS analysis of GBEE

The *in vitro* anthelmintic activity observed revealed a higher activity of GBEE than the other extracts, and its chemical composition was determined by HR-LC-MS. This method can distinguish and recognize phytoconstituents based on retention time, experimental *m/z*, MS/MS fragments, metabolite groups, and likely compounds (Fig. 4). In this context, eight compounds with the highest concentrations in the extract were selected for further molecular evaluation (Table 4, Fig. 5).

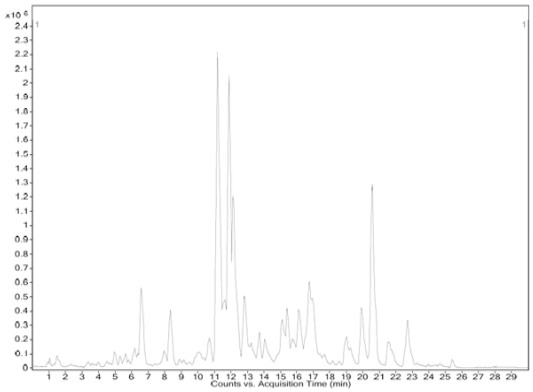

Fig. 4. Identification and profiling of bioactive compounds in GBEE using HR-LC-MS spectrum analysis.

3.4. Drug likeliness

In drug discovery and development, the assessment of drug candidates is primarily based on their pharmacological activity. This measure links physical and chemical characteristics with its influence on bioavailability when consumed orally in the human body. The DruLito program was used to analyze the physicochemical properties of the eight selected active chemicals. As shown in Table 5, out of the 38 compounds eluted from HR-LC-MS of GBEE, 16 violated Lipinski's rule, while the remaining 22 obeyed the rule.

3.5. Molecular docking studies

This study involved the selection of 22 compounds that adhered to Lipinski's rule and eluted from the HR-LC-MS analysis of GBEE. These compounds were subsequently subjected to molecular docking studies and the results are presented in Table 6. As can be seen in this table, molecular docking studies revealed that 1-hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone exhibited the highest docking score (-8.3 kcal/mol against Tubulin-Colchicine. In comparison, the standard drug, albendazole, yielded a docking score of -6.1 kcal/mol. Among the 22 compounds, 20 displayed superior binding energy compared to that of the standard drug albendazole, indicating that GBEE contains potential anthelmintic compounds that inhibit β-tubulin. The interactions between the ligands and Tubulin-Colchicine enzymes are illustrated in Fig. 6 and detailed in Table 7. Interestingly, the anthraquinone, 1-hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone revealed good binding interactions of -8.3 kcal/mol with the β-tubulin. As noted in Fig. 6a, the anthraqionone fits well at the active site of the receptor and constructed three hydrogen bond interactions with GLN A:11 (3.78), GLU A:71 (5.25), GLY A:146 (3.83). It should be noted that this is the first simulated molecular docking investigation of 1-hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone in an anthelmintic target, where the binding energy and key interactions of 1-hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone with β-tubulin are promising and may provide guidance for a more detailed investigation of its anthelmintic activity. In contrast, methyl picraquassioside A (Fig. 6b) showed a binding energy of -8.1 kcal/mol. It formed a hydrogen bond with PHE A:141 (5.30), SER A:178 (4.45), ASN A:206 (4.86), and hydrophobic interactions with ALA A:12 (6.33), GLY A:143 (5.11), GLU A:183 (5.79). The standard albendazole (Fig. 6e) exhibited binding energy of -6.7 kcal/mol and formed a hydrogen bond with SER A:140 (3.67), THR A:145 (3.69), GLY A:146 (4.22), hydrophobic interactions with ILE A:16 (5.66), ILE A:171 (5.27), ALA A:12 (4.56, 4.80, 5.74) and electrostatic interactions with GLU A:183 (7.64), TYR A:224 (6.77).

Docking studies indicated that key amino acids, namely ALA A:12, GLY A:146, SER A:140, GLU A:183, and TYR A:224, play crucial roles in the formation of various bonds for the inhibition of β-tubulin. These findings highlight the significance of these amino acids in ligand binding and provide insights into the potential targets for anthelmintic activity. These results suggested that *G. bilamellata* Gagnep. possesses promising anthelmintic properties warranting further investigation to explore its potential as a source of novel therapeutic compounds.

Table 4
Biological active compounds derived from GBEE through HR-LC-MS analysis.

Name	Formula	Mass	Base peak	m/z	RT	Height
Neuraminic acid	$C_9H_{17}NO_8$	267.094	136.06	268.101	1.49	34730
3α,4,7,7α-Tetrahydro-1H-isoindole-1,3(2H)-dione	$C_8H_9NO_2$	151.063	174.052	174.053	1.571	30022
Piperolein B	$C_{21}H_{29}NO_3$	343.217	125.058	344.225	4.526	31508
Hexamethylphosphoramide	$C_6H_{18}N_3OP$	179.116	202.104	202.105	4.962	40944
Lotaustralin	$C_{11}H_{19}NO_6$	261.119	202.105	262.126	5.018	44387
Istamycin C1	$C_{19}H_{37}N_5O_6$	431.270	126.090	432.277	5.309	35719
Progeldanamycin	$C_{27}H_{41}NO_6$	475.296	488.150	476.303	5.667	30844
Auriculine	$C_{31}H_{45}NO_8$	559.316	520.330	560.324	5.826	30014
3,4-bis(Methylene)-hexanedioic acid	$C_8H_{10}O_4$	170.059	193.047	193.048	6.141	95159
2,4,6-Triethyl-1,3,5-trioxane	$C_9H_{18}O_3$	174.126	179.105	197.116	6.48	280007
1-Hydroxy-2-(β-D-glucosyloxy)-9,10-anthraquinone	$C_{20}H_{18}O_9$	402.092	207.026	403.099	7.846	57635
Hexadecanedioic acid	$C_{16}H_{30}O_4$	286.214	309.130	309.203	8.251	121550
6-Methyl-2-methylene-6-octene-1,3,8-triol	$C_{10}H_{18}O_3$	186.126	209.117	209.115	8.3	47100
8-Acetylegelolide	$C_{16}H_{20}O_6$	308.123	309.131	309.131	8.382	289776
Maritimetin	$C_{15}H_{10}O_6$	286.045	287.052	287.052	8.853	69458
5,8-Dihydroxy-3-(4-hydroxybenzyl)-7-methoxy-4-chromanone 8-acetate	$C_{19}H_{18}O_7$	358.103	326.076	359.110	9.752	35270
Coriandrone *E*	$C_{13}H_{12}O_5$	248.068	271.057	271.058	9.825	100895
Methyl 3,4,5-trimethoxycinnamate	$C_{13}H_{16}O_5$	252.100	233.079	275.089	9.829	43537
Methylpicraquassioside A	$C_{19}H_{24}O_{10}$	412.136	289.067	435.125	10.24	68564
p-Coumaroyl quinic acid	$C_{16}H_{18}O_8$	338.100	361.088	361.089	10.467	31116
Kamahine C	$C_{14}H_{20}O_5$	268.131	220.071	291.120	10.5	69351
2-Propenyl 2-aminobenzoate	$C_{10}H_{11}NO_2$	177.080	200.069	200.069	10.679	38692
Trinexapac-ethyl	$C_{13}H_{16}O_5$	252.100	233.078	275.089	10.688	63846
1-*O*-E-Cinnamoyl-(6-arabinosylglucose)	$C_{20}H_{26}O_{11}$	442.146	263.086	465.136	10.876	44738
5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone	$C_{11}H_{12}O_5$	224.068	217.010	247.058	10.963	86121
Sinensetin	$C_{20}H_{20}O_7$	372.118	343.078	373.125	11.069	773627
Methadyl acetate	$C_{23}H_{31}NO_2$	353.240	345.085	376.229	11.101	42367
Ginkgolide A	$C_{20}H_{24}O_9$	408.141	401.083	431.130	11.414	41851
Hydroxyvernolide	$C_{19}H_{22}O_8$	378.131	371.071	401.120	11.669	56358
Gibberellin A75	$C_{19}H_{24}O_8$	380.146	373.089	403.135	11.929	884832
(9Z,11E,13E,15*Z*)-4-*Oxo*-9,11,13,15-octadecatetraenoic acid	$C_{18}H_{26}O_3$	290.185	291.119	291.193	11.93	165860
C16 Sphinganine	$C_{16}H_{35}NO_2$	273.264	274.271	274.272	12.014	273032
Conchosin B	$C_{17}H_{20}O_6$	320.126	313.068	343.115	12.035	669692
Butyl 3-*O*-caffeoylquinate	$C_{20}H_{26}O_9$	410.157	403.100	433.146	12.232	308595
5-Acetoxydihydrotheaespirane	$C_{15}H_{26}O_3$	254.188	137.058	277.177	12.513	41715
N-(Heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide	$C_{15}H_{21}NO_3$	263.152	201.053	286.141	12.837	49849
Gabapentin	$C_9H_{17}NO_2$	171.126	445.208	194.115	13.426	33715
Sphinganine	$C_{18}H_{39}NO_2$	301.295	302.303	302.303	13.68	144304
Limonoate	$C_{26}H_{34}O_{10}$	506.217	238.120	507.225	15.329	195496
Cycloate	$C_{11}H_{21}NOS$	215.131	238.121	238.121	15.34	45576
Geranyl 2-ethylbutyrate	$C_{16}H_{28}O_2$	252.209	275.199	275.198	15.854	117698
Teasterone	$C_{28}H_{48}O_4$	448.355	279.228	471.343	15.981	43149
Palmitic Acid	$C_{16}H_{32}O_2$	256.240	279.230	279.230	16.189	191520
Citronellyl hexanoate	$C_{16}H_{30}O_2$	254.225	277.214	277.214	16.631	252049
16-Hydroxy hexadecanoic acid	$C_{16}H_{32}O_3$	272.235	277.214	295.224	16.709	207244

Table 4 Continued

Name	Formula	Mass	Base peak	m/z	RT	Height
3-Vinylbacteriochlorophyllide A	$C_{35}H_{36}MgN_4O_5$	616.253	479.204	639.241	18.28	30644
4*Z*,15*E*-Bilirubin IXa	$C_{33}H_{36}N_4O_6$	584.263	519.233	607.251	18.925	157017
Stearic acid	$C_{18}H_{36}O_2$	284.271	145.101	307.261	19.361	41767
Calafatimine	$C_{38}H_{40}N_2O_7$	636.278	593.274	659.267	21.297	31341
3-Hydroxyethylbacteriochlorophyllide a	$C_{35}H_{38}MgN_4O_6$	634.278	593.271	635.283	21.487	89075
Ganosporelactone A	$C_{30}H_{40}O_7$	512.278	535.267	535.267	21.612	54735
(3a,5b,7b,12a)-(1,3-dihydro-5-nitro-1,3-dioxo-2H-isoindol-2-yl) Methyl ester-3,7,12-trihydroxy-Cholan	$C_{33}H_{44}N_2O_9$	612.294	575.260	635.283	21.83	65419
Haplophytine	$C_{37}H_{40}N_4O_7$	652.287	503.240	653.294	22.535	115756
6-Deoxocathasterone	$C_{28}H_{50}O_2$	418.380	441.368	441.370	22.602	94300
Hypaconitine	$C_{33}H_{45}NO_{10}$	615.313	559.266	638.302	22.606	229919
Harderoporphyrinogen	$C_{35}H_{42}N_4O_6$	614.308	621.303	637.297	23.307	36308

Table 5
Drug likeliness properties of different compounds eluted from HR-LC-MS of GBEE.

Sr. No.	Title	MW	logp	Alogp	HBA	HBD	TPSA	AMR	Violated Lipinski's Rule
1	Cleomiscosin D	416.1	1.61	-1.57	9	2	112.9	112.8	No
2	2α, 3β-Dihydroxyolean-12-en-28-oic acid	472.4	8.24	1.907	4	3	77.76	137.1	Yes
3	Icariol A2	436.2	-0.222	-2.89	3	4	127.1	62.97	No
4	3α-20-Lupandiol	444.4	10.54	1.939	2	2	40.46	131.2	Yes
6	Nitidanin	404.2	1.324	-1.38	8	3	106.8	114.6	No
7	2,6-dimethoxy-1-acetonylquinol	226.1	-0.219	-1.5	5	1	72.83	59.31	No
8	Ciwujiatone	434.2	0.794	-2.06	9	3	123.9	118	No
9	Daucosterol	576.4	10.49	-0.45	6	4	99.38	156.3	Yes
10	Bilagrewin	402.1	1.501	-1.23	8	2	103.7	113.5	No
11	Neuraminic acid	267.1	-3.801	-3.95	9	7	173.7	52.8	Yes
12	Piperolein B	343.2	4.833	-1.86	4	0	38.77	92.43	No
13	Lotaustralin	261.1	-1.12	-2.26	7	4	123.2	58.02	No
14	Auriculine	559.3	3.305	0.654	9	4	128.9	149.6	Yes
15	1-Hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone	402.1	-0.678	-1.72	9	5	153.8	103.6	No
16	Hexadecanedioic acid	286.2	5.696	-3.87	4	2	74.6	58.48	Yes
17	6-Methyl-2-methylene-6-octene-1,3,8-triol	186.1	-0.498	-0.21	3	3	60.69	51.82	No
18	Maritimetin	286.1	1.915	-0.68	6	4	107.2	81.83	No
19	Coriandrone E	248.1	0.693	-0.94	5	1	64.99	66	No
20	Methyl 3,4,5-trimethoxycinnamate	252.1	1.662	0.084	5	0	53.99	71.9	No
21	Methylpicraquassioside A	412.1	-0.173	-2.69	10	4	144.1	101.4	No
22	*p*-Coumaroyl quinic acid	338.1	-0.837	-0.63	8	5	144.5	84.19	No
23	Kamahine C	268.1	-0.148	0.328	5	2	75.99	65.95	No
24	1-*O*-E-Cinnamoyl-(6-arabinosylglucose)	442.2	-0.713	-1.81	11	6	175.4	106.1	Yes
25	5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone	224.1	1.488	-0.81	5	3	86.99	56.57	No
26	Ginkgolide A	408.1	1.176	-0.75	9	2	128.6	90.34	No
27	Hydroxyvernolide	378.1	-0.669	-0.67	8	2	114.8	88.03	No
28	5-Acetoxydihydrotheaespirane	254.2	3.416	0.547	3	0	35.53	66.19	No
29	Sphinganine	301.3	6.268	-5.74	3	3	66.48	67.52	Yes
30	Geranyl 2-ethylbutyrate	252.2	4.948	2.091	2	0	26.3	76.27	No

Table 5 Continued

Sr. No.	Title	MW	logp	Alogp	HBA	HBD	TPSA	AMR	Violated Lipinski's Rule
31	Palmitic Acid	256.2	7.57	-3.9	2	1	37.3	55.52	Yes
32	Stearic acid	284.3	8.708	-4.47	2	1	37.3	61.34	Yes
33	Calafatimine	636.3	3.359	0.219	9	0	80.21	196.4	Yes
34	Ganosporelactone A	512.3	1.602	0.011	7	2	118	135	Yes
35	Haplophytine	652.3	1.382	-1.53	11	1	112.1	180	Yes
36	6-Deoxocathasterone	418.4	9.995	1.274	2	2	40.46	119.6	Yes
37	Harderoporphyrinogen	614.3	-0.534	2.084	10	4	161	173.6	Yes
38	Hypaconitine	615.3	0.443	-2.51	11	2	133.2	156	Yes

Table 6
Binding affinities of HR-LC-MS eluted compounds of GBEE and the standard at the active site of β-tubulin.

Ligands	Binding Energy (kcal/mol)
1-Hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone	-8.3
Methyl picraquassioside A	-8.1
Maritimetin	-7.9
Ginkgolide A	-7.6
Hydroxyvernolide	-7.4
Kamahine C	-7.3
p-Coumaroyl quinic acid	-7.1
Bilagrewin	-7
5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone	-7
Piperolein B	-6.7
Icariol A2	-6.6
Methyl 3,4,5-trimethoxycinnamate	-6.6
Coriandrone E	-6.4
Lotaustralin	-6.4
5-Acetoxydihydrotheaespirane	-6.3
Geranyl 2-ethylbutyrate	-5.8
6-Methyl-2-methylene-6-octene-1,3,8-triol	-5.7
2,6-Dimethoxy-1-acetonylquinol	-5.6
Ciwujiatone	-5.2
Nitidanin	-5
Albendazole	-6.1

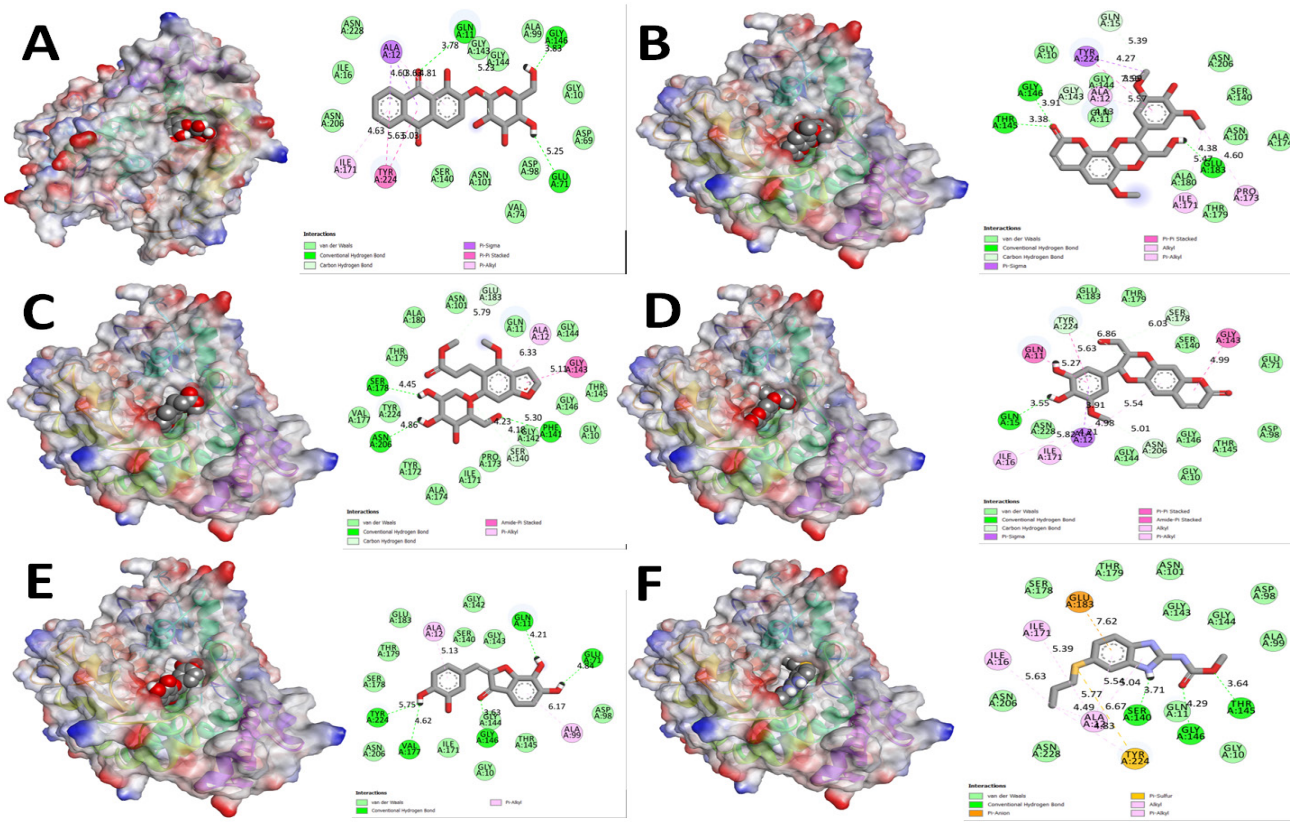

Fig. 6. Molecular overlay and 2D representation of ligands derived from HR-LC-MS analysis of GBEE with tubulin-colchicine: Stathmin-like domain complex (1SA0) by Autodock Vina. (**A**) 1-hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone, (**B**) methyl picraquassioside A, (**C**) maritimetin, (**D**) ginkgolide A, (**E**) hydroxyvernolide, (**F**) albendazole.

3.6. ADMET analysis

The ADMET attributes of the ligands were studied using Swiss ADME (http://www.swissadme.ch/), admetSAR (http://lmmd.ecust.edu.cn/admetsar2/), and Protox-II (https://tox-new.charite.de/protox_II/) web servers. Table 8 lists the predicted ADMET properties of the selected phytocompounds.

Drugs suitable for testing should not exhibit toxicity and possess desirable ADMET properties. This similarity must be considered during the early stages of drug development (Ferreira and Andricopulo, 2019). Using these descriptors, it is possible to determine whether a compound is absorbed, distributed, metabolized, excreted, or toxic. Although there are different *in vitro* methods to establish ADMET profiles, *in silico* determination is a faster, cheaper, and life-saving method to determine ADMET profiles (Norinder and Bergstrom, 2006).

In addition to being non-toxic, ideal drug candidates should exhibit acceptable ADME characteristics. Based on SwissADME, ProTox-ii, and admetSAR, the ADME profiles, including drug similarity, partition coefficients, solubility, HIA, BBB, and cytochrome P_g inhibition of the identified molecules were examined (Table 8) (Bickerton et al., 2012).

One of the most important properties of ADMET is its ability to absorb drugs from the human gut [HIA]. HIA plays a role in drug transport to the target cells (Ejeh et al., 2021). Higher HIA resulted in improved intestinal absorption of the compound. All compounds showed HIA values greater than 0.9 indicating good membrane permeation. The different features of the CNS vasculature are predicted by the blood-brain barrier [BBB]. The lack of pores on the cell surface of vessels of the central nervous system makes it extremely difficult to transport various types of cells and molecules. This makes the delivery of compounds to the central nervous system extremely difficult.

A pan-assay interference structural spectroscopic (PAINS) alert was used to determine the toxicity of compounds with desirable physicochemical properties. This assay is also known as a toxicophore test because of the presence of group elements that affect biological processes by interfering with DNA or proteins, which can cause fatal conditions, such as cancer and hepatotoxicity (Baell and Holloway, 2010). PAINS analysis provides information on the potential toxicity of a molecule. However, most of the phytocompounds had 0 PAINS structural alerts, indicating their non-toxic nature (Table 8).

These compounds were evaluated for their hepatotoxic, carcinogenic, and mutational potential (Lounkine et al., 2012). ProTox II results revealed that all compounds

Table 7
Interactions of β-tubulin amino acid residues with ligands at receptor sites.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
1-Hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone	-8.3	GLN A:11 (3.78), GLU A:71 (5.25), GLY A:146 (3.83)	ALA A:12 (3.63, 4.60, 4.81), ILE A:171 (4.63), TYR A:224 (5.03, 5.63), GLY A:143 (5.23)	-
Methyl picraquassioside A	-8.1	PHE A:141 (5.30), SER A:178 (4.45), ASN A:206 (4.86)	ALA A:12 (6.33), GLY A:143 (5.11), GLU A:183 (5.79)	-
Maritimetin	-7.9	GLN A:11 (4.21), GLU A:71 (4.84), GLY A:146 (3.63), VAL A:177 (4.62), TYR A:224 (5.75)	ALA A:12 (5.13), ALA A:99 (6.17)	-
Ginkgolide A	-7.6	ALA A:12 (3.49), GLU A:183 (5.49)	-	-
Hydroxyvernolide	-7.4	GLN A:11 (3.86), GLY A:142 (3.25)	ALA A:12 (4.16), SER A:140 (4.54)	-
Kamahine C	-7.3	GLY A:142 (3.30), TYR A:224 (5.61)	THR A:179 (4.24), SER A:140 (4.49)	-
p-Coumaroyl quinic acid	-7.1	ASP A:98 (5.01), GLY A:146 (3.37), ASN A:206 (4.00)	ALA A:12 (5.94), TYR A:224 (7.30)	-
Bilagrewin	-7	SER A:178 (4.26)	ALA A:12 (5.92), GLU A:71 (5.87), ASP A:98 (6.00), ILE A:171 (5.68), PRO A:173 (4.18), SER A:140 (4.52), GLY A:143 (4.13),	-
5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone	-7	GLY A:144 (4.11), THR A:145 (3.22), GLY A:146 (3.81), SER A:178 (3.94), GLU A:183 (5.14)	-	-
Albendazole	-6.7	SER A:140 (3.67), GLY A:146 (4.22), THR A:145 (3.69)	ILE A:16 (5.66), ILE A:171 (5.27), ALA A:12 (4.56, 4.80), ILE A:16 (5.66), ILE A:171 (5.27)	GLU A:183 (7.64), TYR A:224 (6.77)

were non-carcinogenic. They can also be used to treat helminthiasis. Because these compounds cannot accumulate in the body, they are less likely to cause cancer if they are treated for a long time. Except for kamahine C, methyl picraquassioside A, and 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone, the remaining compounds exhibited immunotoxicity. No hepatotoxicity or cytotoxicity was observed for any of the compounds tested. In ADMET studies, these properties are often used to analyze drug behavior.

4. Concluding remarks

The present study provides evidence of the anthelmintic activity of various *G. bilamellata* Gagnep. extracts against *Pheretima posthuma*. Notably, the GBEE extract demonstrated activity comparable to that of the reference drug albendazole, exhibiting the lowest rates of paralysis and death in the experimental model. HR-LC-MS analysis of GBEE revealed the presence of phytoconstituents, such as 1-hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone, methyl picraquassioside A, maritimetin, ginkgolide A, and hydroxyvernolide, which may contribute to the observed anthelmintic activity. However, future research is essential focusing on the isolation and evaluation of these individual compounds for identifying potential candidates for the development of novel anthelmintic drugs.

Conflict of interest

The authors declare that there is no conflict of interest.

Table 8

ADMET analysis of phytoconstituents eluted from HR LC-MSHR LC-MS of GBEE.

Phytocompounds	Swiss ADME								ADMETSAR							PROTOX-II					
	logPo/w	Water solubility	GI Absorption	Lipinski Rule	Veber's Rule	PAINS Alert	TPSA	Lead Likeliness	HIA	CaCO2	BBB	CYP1A2	CYP2C19	CYP2C9	CYP2D6	LD_{50} (mgkg)	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1-Hydroxy-2-(β-D-glucosyloxy)-9-10-anthraquinone	2.13	Soluble	Low	Yes	No	1	153.75	No	0.615	0.909	0.598	0.905	0.903	0.802	0.871	3000	Inactive	Inactive	Active	Active	Inactive
																(Class 5)					
Methyl picraquassioside A	2.65	Soluble	Low	Yes	No	0	148.05	No	0.506	0.799	0.800	0.723	0.816	0.812	0.835	3000	Inactive	Inactive	Inactive	Inactive	Inactive
																(Class 5)					
Maritimetin	1.7	Soluble	High	Yes	Yes	1	107.22	Yes	0.993	0.645	0.702	0.953	0.557	0.780	0.900	500	Inactive	Active	Active	Active	Inactive
																(Class 4)					
Ginkgolide A	0.38	Soluble	High	Yes	Yes	0	128.59	No	0.925	0.667	0.838	0.905	0.903	0.854	0.859	500	Inactive	Inactive	Active	Inactive	Inactive
																(Class 4)					
Hydroxyvernolide	1.83	Very Soluble	High	Yes	Yes	0	114.82	No	0.680	0.683	0.921	0.764	0.832	0.818	0.914	4	Inactive	Inactive	Active	Inactive	Inactive
																(Class 1)					
Kamahine C	1.7	Very Soluble	High	Yes	Yes	0	75.99	Yes	0.941	0.644	0.530	0.815	0.803	0.809	0.942	55	Inactive	Inactive	Inactive	Inactive	Inactive
																(Class 3)					
p-Coumaroyl quinic acid	0.83	Soluble	Low	Yes	No	0	144.52	Yes	0.788	0.578	0.787	0.942	0.92	0.929	0.951	5000	Inactive	Inactive	Active	Inactive	Inactive
																(Class 5)					
Bilagrewin	2.98	Soluble	High	Yes	Yes	0	103.68	No	0.990	0.7	0.703	0.807	0.580	0.783	0.852	5000	Inactive	Inactive	Active	Inactive	Inactive
																(Class 5)					
5-(3',4',5'-Trihydroxyphenyl)-γ-valerolactone	0.97	Soluble	High	Yes	Yes	1	86.99	No	0.522	0.662	0.678	0.649	0.561	0.768	0.846	2000	Inactive	Inactive	Inactive	Inactive	Inactive

References

Abbasi, M., Omrani, M., Raiatparvar Malieki, L., Sonboli, A., Nejad Ebrahimi, S., 2021. Phytochemical studies of *Tetrataenium nephrophyllum* and anti-acetylcholinesterase activities. Trends Phytochem. Res. 5(4), 210-217.

Anita Margret, A., Maheswari, R., Sherlin Rosita, A., Jesucastin, E., 2022. Discerning the regulated wound healing potential of *Ocimum americanum* by probing the rosmarinic acid content-a paradigm on zebrafish caudal fin regeneration. Trends Phytochem. Res. 6(2), 106-115.

Arbonnier, M., 2004. Trees, Shrubs and Lianas of West African Dry Zones. Trees, Shrubs and Lianas of West African Dry Zones, pp. 1-574.

Baell, J.B., Holloway, G.A., 2010. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J. Med. Chem. 53(7), 2719-2740.

Bickerton, G.R., Paolini, G.V., Besnard, J., Muresan, S., Hopkins, A.L., 2012. Quantifying the chemical beauty of drugs. Nat. Chem. 4(2), 90-98.

Borah, S., Mahato, M.K., Kumar, M.M., Das, M.B., Barman, K., Kakoti, B.B., 2022. *Lasia spinosa*: An Ethnomedicinal Antidiabetic Plant of Assam. Bhumi Publishing, Maharashtra, India.

Das, S.S., Dey, M., Ghosh, A.K., 2011. Determination of anthelmintic activity of the leaf and bark extract of *Tamarindus indica* linn. Indian J. Pharm. Sci. 73(1), 104-107.

DSNBK, P., Atla, S.R., Atla, R.P., 2021. *In-vitro* Anthelmintic activity of *Aralia racemosa*. L (root) and *Argyreia pilosa* Wight & Arn.(Whole plant) against *Pheretima posthuma*. Iran. J. Pharm. Sci. 17(3), 23-32.

Ejeh, S., Uzairu, A., Shallangwa, G.A., Abechi, S.E., 2021. *In silico* design, drug-likeness and ADMET properties estimation of some substituted thienopyrimidines as HCV NS3/4A protease inhibitors. Chem. Africa 4(3), 563-574.

Ferreira, L.L.G., Andricopulo, A.D., 2019. ADMET modeling approaches in drug discovery. Drug Discov. Today 24(5), 1157-1165.

Khandelwal, K.R., 2008. Practical Pharmacognosy. Pragati Books Pvt. Ltd.

Kumar, S., Singh, B., Bajpai, V., 2022. Traditional uses, phytochemistry, quality control and biological activities of genus *Grewia*. Phytomedicine Plus 2(3), 100290.

Lounkine, E., Keiser, M.J., Whitebread, S., Mikhailov, D., Hamon, J., Jenkins, J.L., Lavan, P., Weber, E., Doak, A.K., Cote, S., Shoichet, B.K., Urban, L., 2012. Large-scale prediction and testing of drug activity on side-effect targets. Nature 486(7403), 361-367.

Ma, C., Zhang, H.J., Tan, G.T., Hung, N.V., Cuong, N.M., Soejarto, D.D., Fong, H.H., 2006a. Antimalarial compounds from *Grewia bilamellata*. J. Nat. Prod. 69(3), 346-350.

Mohammadhosseini, M., Frezza, C., Venditti, A., Akbarzadeh, A., 2019a. Ethnobotany and phytochemistry of the genus *Eremostachys* Bunge. Curr. Org. Chem. 23, 1828-1842.

Mohammadhosseini, M., Frezza, C., Venditti, A., Mahdavi, B., 2022. An overview of the genus *Aloysia* Palau (Verbenaceae): Essential oil composition, ethnobotany and biological activities. Nat. Prod. Res. 36(19), 5091-5107.

Mohammadhosseini, M., Venditti, A., Akbarzadeh, A., 2019b. The genus *Perovskia* Kar.: Ethnobotany, chemotaxonomy and phytochemistry: A review. Toxin Rev. 40(4), 484-505.

Nayar, M., Sastry, A.R.K., 1987. Red Data Book of Indian Plants, Botanical Survey of India, India.

Norinder, U., Bergstrom, C.A., 2006. Prediction of ADMET properties. ChemMedChem 1(9), 920-937.

Noumi, E., Snoussi, M., Anouar, E.H., Alreshidi, M., Veettil, V.N., Elkahoui, S., Adnan, M., Patel, M., Kadri, A., Aouadi, K., De Feo, V., Badraoui, R., 2020. HR-LC-MS-based metabolite profiling, antioxidant, and anticancer properties of *Teucrium polium* L. methanolic extract: Computational and *in vitro* study. Antioxidants (Basel) 9(11), 1089.

Pandiyan, R., Samiappan, S.C., Sugumaran, A., Sivakumar, S., 2022. Stomach-affecting intestinal parasites as a precursor model of *Pheretima posthuma* treated with anthelmintic drug from *Dodonaea viscosa* Linn. Green Process. Synth. 11(1), 492-502.

Pillai, L.S., Nair, B.R., 2011. A comparative study of the anthelmintic potential of *Cleome viscosa* L. and *Cleome burmanni* W. and A. Indian J. Pharm. Sci. 73(1), 98-100.

Prasanth, D., Panda, S.P., Rao, A.L., Chakravarti, G., Teja, N., Vani, V.B.N., Sandhya, T., 2020a. *In-silico* strategies of some selected phytoconstituents from *zingiber officinale* as sars cov-2 main protease (COVID-19) inhibitors. Indian J. Pharm. Educ. Res. 54(3), s552-559.

Prasanth, D.S.N.B.K., Panda, S.P., Rao, A.L., Teja, N., Vani, V.B.N., Sandhya, T., Rao, P.B.B., 2020b. *In vitro* Anthelmintic impact of various extracts of *Pavetta tomentosa* root on *Pheretima posthuma* and *in-silico* molecular docking evaluation of some isolated phytoconstituents. Indian J. Pharm. Educ. Res. 54(2), S251-S260.

Quattrocchi, U., 2012. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology (5 Volume Set). CRC press.

Sarkar, S., Hoda, M.U., Das, S., 2023. Anti-melanogenic, antioxidant potentialities and metabolome classification of six *Ocimum* species: Metabolomics and *in-silico* approaches. Trends Phytochem. Res. 7(1), 30-50.

Senguttuvan, J., Paulsamy, S., Karthika, K., 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pac. J. Trop. Biomed. 4, S359-S367.

Setlur, A.S., Naik, S.Y., Skariyachan, S., 2017. Herbal lead as ideal bioactive compounds against probable drug targets of ebola virus in comparison with known chemical analogue: A computational drug discovery perspective. Interdiscip. Sci. 9(2), 254-277.

Shahriari, M., Nourmandipour, F., Norouzi, S., Nejad Ebrahimi, S., 2021. Investigation of inhibitory properties of triphenyl-LasR enzyme involved in the quorum

sensing of *Pseudomonas aeruginosa* by molecular modeling. Trends Phytochem. Res. 5(3), 126-135.
Sharma, V., Pattanaik, K.K., Jayprakash, V., Basu, A., Mishra, N., 2009. A utility script for automating and integrating AutoDock and other associated programs for virtual screening. Bioinformation 4(2), 84.
Singh, P.K., Singh, J., Medhi, T., Kumar, A., 2022. Phytochemical screening, quantification, FT-IR analysis, and *in silico* characterization of potential bio-active compounds identified in HR-LC-MS analysis of the polyherbal formulation from Northeast India. ACS Omega 7(37), 33067-33078.
Sreejith, M., Kannappan, N., Santhiagu, A., Mathew, A.P., 2013. Phytochemical, anti-oxidant and anthelmintic activities of various leaf extracts of *Flacourtia sepiaria* Roxb. Asian Pac. J. Trop. Biomed. 3(12), 947-953.
Ullah, W., Uddin, G., Siddiqui, B.S., 2012. Ethnic uses, pharmacological and phytochemical profile of genus *Grewia*. J. Asian Nat. Prod. Res. 14(2), 186-195.