Original Article

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Anthelmintic activity of *Mansoa alliacea* against *Pheretima posthuma*: *In vitro* and *In silico* approach

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ABSTRACT

Objectives: *Mansoa alliacea* has been utilized to remedy many afflictions of humans. Literary works illustrate that it possesses numerous biological activities. Our analysis work aims to distinguish phyto-derived anthelmintic substances from *M. alliacea* against the enzyme β-tubulin and to consider the cause of its function in the molecular basis on *Invitro* and *Insilico* methods. **Materials and Methods:** In this study, *Manosa alliacea* was subjected to extraction using various solvents based on polarity and the extracts were analyzed by GC-MS. Then using Pheretima posthuma, *in-vitro* studies were done, and *insilico* studies have been conducted using PyRx tool. Subsequently, DruLiTo software was used to study drug-like predictions. **Results:** Tests showed that methanolic extract has the most important dose-dependent anthelmintic efficacy at various levels. By insilico studies, it shows that the four phytochemicals of *M. alliacea* are very likely against the β-tubulin. Utilizing contemporary strategies, these phyto-compounds from a natural origin might establish a reliable medication or support lead identification. **Conclusion:** Utilizing contemporary strategies, these phyto-compounds from a natural origin might establish a reliable medication or support lead identification. Identified hit compounds could be further taken for *in vitro* studies to examine their effectiveness versus helminths.

Keywords: Absorption; Distribution; Metabolism; Excretion and Toxicity, AutoDock, *Mansoa alliacea*, PASS, *Pheretima posthuma*, β-Tubulin

INTRODUCTION

Considering that the beginning of the human world, alternative medicine with healing has actually been made use of in the therapy of numerous disorders.[1] According to the WHO, 80 percentile of the populace of few Asian countries rely on conventional medicine in their day-to-day elements of healthcare.[2] About 25% of the prescribed drugs consist of plant-derived components, and also about 121 active substances are presently made use of in pharmaceutical products.[3]

The past 50 years of research study have offered a couple of medications made use of to treat human helminthiases infection; nevertheless, in lasting usage, lots of parasites are revealing resistance to these medications. The factor given for

the reduced activity can be either due to the heritable changes (epigenetic or genetic) lack of ability of anthelmintic versus a populace of parasites or decrease in time to which medical therapy uses its impact. The usage of the plant can play an essential function in antihelmintic drug-target recognition.[4,5]

In the age of modern innovations, the analog system based technique is made use of in the evaluation of phytoconstituents of therapeutic plant essences with the help of molecular docking as well as likewise molecular attributes, which are essential devices in biology in addition to computer-assisted medication design.[6] In docking, the goal is to anticipate the primary binding site in betwixt a ligand, and a protein of three-dimensional structure, as well as individual ligand conformation in the dynamic scoring of the protein, is placed using racking up feature.[7] In the 1990s, inadequate pharmacokinetic, as well as toxicity properties, triggers pricey last phase failings in drug development. In drug development procedures, *in silico*, ADMET modeling obtained a substantial interest in reasonable drug-design layout. Promising substances are explored for their pharmacokinetic properties, metabolic process, possible toxicity, and also unfavorable results.

The chemical constituents and efficiency of extracts from *Mansoa alliacea* had been intensively explored in earlier research. The plant *M. alliacea* has been reported to contain several phytoconstituents including β-amyrin, β-sitosterol, ursolic acid, β-sitosteryl-d-glucoside, apigenin, luteolin, 7-O-methylscutellarein, apigenin-7-glucuronide,[8,9] alliin,[10] 9-methoxy-α-lapachone, 4-hydroxy-9-methoxy-α-lapachone, 19-hydroxy hexatriacontane-18-one,[11] glycyrrhetol, β-peltoboykinolic acid, 3β-hydroxyurs-18-en-27-oic acid[12] and possess anti-hyperlipidemic,[13] antifungal,[14] antimicrobial,[15] and biocide.[16]

Our research study intended to evaluate the anthelmintic activity of various portions of *M. alliacea* extract as well as identify the molecular interactions existing between various phytoconstituents with the beta-tubulin enzyme.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plants of *M. alliacea*, Bignoniaceae, were gathered from Tirupati, Andhra Pradesh (13° 37' 44.6340" N and also 79° 25' 28.0056" E), acknowledged and also confirmed by Prof. K. Madhava Chetty, Plant Taxonomist, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. They were washed after which cleaned by regular water, air-dried, ground into powder in a home appliance, and also preserved for pharmacognostical study.

Preparation of Extracts

The shade-dried root was powdered with the assistance of a miller, as well as a crude powder was acquired. The coarse powder (1000 g) was drawn out with petroleum ether, chloroform, ethyl acetate, and methanol utilizing a Soxhlet device. The extract was filtered, concentrated by vaporizing the solvent in a rotating evaporator, and also maintained in the fridge.[17,18]

Preliminary Phytochemical Screening[19]

The various extract of *M. alliacea* underwent initial phytochemical testing for the recognition of chemical components according to the standard operating procedures.[20-23]

Collection of Worms

The Indian earthworms, *Pheretima posthuma* (Annelida) utilized in today's research study, were gathered from damp soil of Vaddeswaram Village, Guntur District, Andhra Pradesh, India.

Anthelmintic activity

A total amount of 48 earthworms had collected as well as were divided up into groups, each made up of six worms. Various concentrations (5 mg/ ml, 10 mg/ml, 15 mg/ml, as well as 20 mg/ml) of the extracts, i.e., *M. alliacea* petroleum ether extract (MAPE), *M. alliacea* chloroform extract (MACE), *M. alliacea* ethyl acetate extract (MAEE), and *M. alliacea* methanol extract (MAME), as well as standard (Mebendazole), had been prepared in distilled water of 10 ml. The earthworms have been formerly washed in regular water before these were launched into 10 ml of the corresponding Petri dish. Promptly after releasing the earthworms in the concerned Petri dishes, the time of launching was stated, and also consequently, the motility of the earthworms was observed. Time of paralysis was seen if the earthworms revealed no activities apart from when worms have trembled very and also the time of death was seen after finding that earthworms neither relocated as soon as shiver extremely neither when showered with hot water (40–50°C).[24,25]

C-MS Analysis

Centered on anthelmintic function, MAME displays intense action in all extracts. MEMA experienced review of gas chromatography–mass spectrometry (GC-MS). The work was performed with Agilent Technology 6890 Series gas chromatography coupled with (an Agilent) 5973 Mass Selective Detector operated by Agilent Chemstation Capillary panel HP-5MS. The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min and linear velocity 37 cm/s. Temperature injector was 250°C. The initial oven temperature was 60°C, which was projected to rise to 280°C at 10°C/min with 4 min for each point. 2 μL injections are made with 20:1 split ratio, operated in 70 eV electron ionization rendered and 1859 V electron multiplier voltage as follows: Ion source temperature 230°C, quadruple temperature 150°C, solvent pause 4 min, and scan scale 50–700 amu. The compounds were identified by direct comparing processing times with mass spectral data and separation levels in The National Institute of Standards and Technology (NIST) database.

Computational Study

The molecular docking was done utilizing on Beta-Tubulin enzyme versus the potent substances gathered from the literary works testimonial.

Preparation of ligand structures

Structures of ligands [Figure 1] were downloaded in the Statutory Declaration Form (SDF) documents style from the

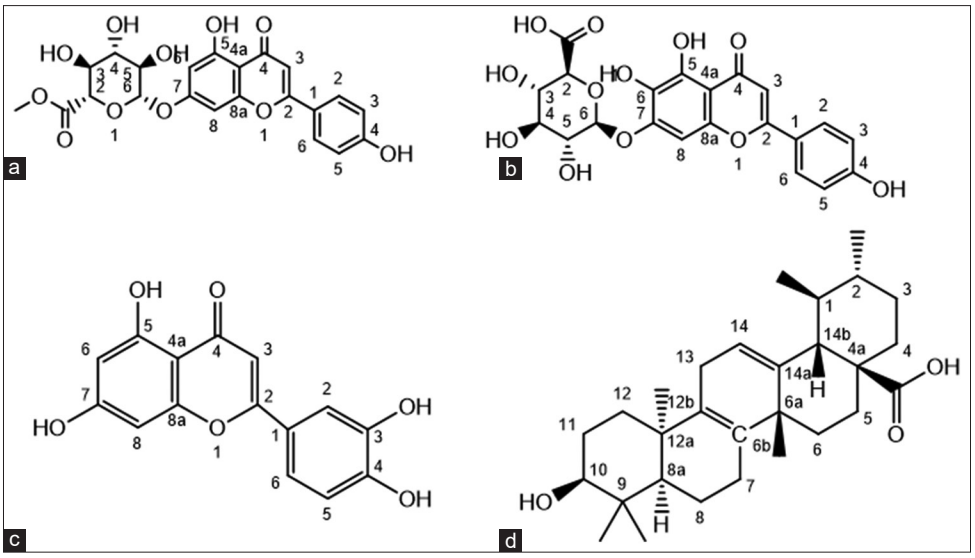

Figure 1: The three-dimensional structures of *in silico* active ligands. (a) Apigenin-7-O-ethyl glucuronide; (b) Scutellarin; (c) Luteolin; (d) Ursolic acid

Table 1: Phytochemical analysis of various extracts of the *M. alliacea*

Phytoconstituents	Method	MAME	MACE	MAEE	MAPE
Flavonoids	Shinoda test	+	−	+	−
	Zn. hydrochloride test	+	−	+	−
	Lead acetate test	+	−	+	−
Volatile oil	Stain test	+	−	−	+
Alkaloids	Wagner test	+	+	−	−
	Hager's test	+	+	−	−
Tannins and Phenols	$FeCl_3$ test	+	−	+	−
	Potassium dichromate test	+	−	+	−
Saponins	Foaming test	+	−	−	−
Steroids	Salkowski test	+	+	−	+
Carbohydrates	Molish test	+	−	−	−
Acid compounds	Litmus test	−	−	−	−
Glycoside	Keller-Kiliani test	+	−	−	−
Amino acids	Ninhydrin test	+	−	−	−
Proteins	Biuret test	+	−	−	−

"+": Present; "−": Absent. *M. alliacea: Mansoa alliacea*, MAPE: *M. alliacea* petroleum ether extract, MACE: *M. alliacea* chloroform extract, MAEE: *M. alliacea* ethyl acetate extract, MAME: *M. alliacea* methanol extract

PubChem Compound Database. Physicochemical abilities of the ligands satisfied the standards of Lipinski's guideline of five or else understood as Lipinski's guideline of drug-likeness [Table 1].[26,27]

Chemical structures in the SDF layout were transformed into the protein database (PDB) layout making use of Discovery Studio Biovia (DSB). Autodock tools (ADT) was then used to test the ligand structure for amalgamations with non-polar hydrogens, improvements of gasteiger modifications, and even rotatable bonds. Structures in the ligand. PDB file format was after that transformed into the ligand. PDBQT file format by utilizing ADT, making it possible for users with AutoDock 4.0.[28]

Preparation of β-tubulin-colchicine protein

The protein β-Tubulin (1SA0) was computed and installed from the RCSB protein database and also was inscribed with the PDB code 1SA0 [Figure 2].[19] The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run, and analyze the docking simulations. Kollman atom charges, solvation parameters, and polar hydrogens were added to the receptor for the preparation of protein in docking studies. Since ligands are not peptides, Gasteiger charge was assigned, and then non-polar hydrogens were merged. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked as it stores the potential energy arising. This grid

must surround the region of interest (active site) in the macromolecule.[29-31]

Docking methodology

Molecular docking was carried out utilizing the AutoDock program. Ligands were anchored independently to the receptor with grid collaborates (grid facility) as well as grid boxes of particular dimensions for each receptor. The arrangement data were involved by opening up the note pad to run AutoDock. ADT was needed to prepare the input. PDBQT documents for β-Tubulin and also to establish the dimension and even the facility of the grid box. The grid dimension was set at 60 × 60 × 60 (x, y, as well as z) factors, and also the grid facility was assigned at x, y, as well as z measurements of 124.37, 96.859, as well as 14.124, precisely, with a grid spacing of 0.375 Å. Post-docking evaluations were pictured, making use of DSB, which revealed the dimensions, as well as places of binding sites, hydrogen-bond communications, hydrophobic communications, as well as bonding, ranges as communication distance <5 Å of from the placement of the docked ligand.[28]

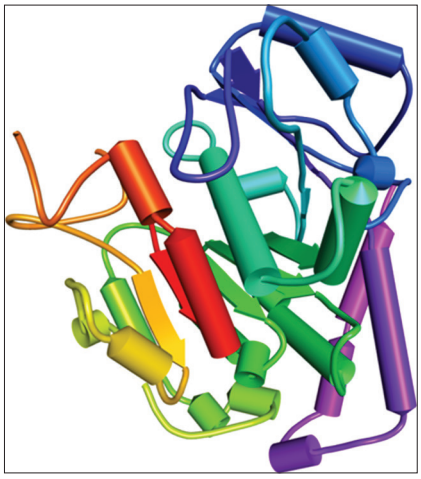

Figure 2: Three-dimensional structure of the molecular target, Tubulin-Colchicine: Stathmin-Like Domain Complex (1SA0)

Drug Likeliness

The physicochemical properties of the chosen four active compounds were studied on DruLiTo software.[32]

ADMET Analysis

ADMET of the ligands is their pharmacokinetic properties that are required to be examined to establish their function inside the body. The ADMET inheritance of the ligands was examined, making use of Molinspiration Cheminformatics software.

PASS Computer Program

PASS is a computer system based program utilized for the prognosis of various sorts of physiological actions for multiple compounds consisting of phytoconstituents. The estimated activity of a substance is predicted as probable activity (Pa) and probable inactivity (Pi). The substances revealing Pa higher than Pi are actually the only components thought about as feasible for a specific medical activity.[33-35]

Statistical Analysis

All information was revealed as the mean ± SD; information went through one-way ANOVA adhered to by Tukey examination. The analytical evaluation executed with GraphPad Prism (Version 3, USA) software program. $P < 0.05$ was taken into consideration statistically considerable.

RESULTS

Phytochemical Screening of the Extract

Initial phytochemical testing of *M. alliacea* exposed different phytoconstituents detailed in Table 1.

Anthelmintic Activity

All the extracts displayed substantial dose-dependent anthelmintic activity in concentration as contrasted to standard, mebendazole

Table 2: Biological active compounds derived from *M. alliacea*

Retention time	Compound name	Area (%)	Molecular formula	Molecular weight
6.434	1,2-Dimethyl-3-nitro-4-nitroso benzene	1.83	$C_8H_8N_2O_3$	180.16
8.222	Scutellarin	2.39	$C_{21}H_{18}O_{12}$	462.4
9.863	1,2,3-Propanetriol	0.36	$C_3H_8O_3$	92.09
10.950	Trans-Chrysanthemal	0.23	$C_{10}H_{16}O$	152.23
11.467	Luteolin	0.76	$C_{15}H_{10}O_6$	286.24
12.575	Isosorbide	0.27	$C_6H_{10}O_4$	146.14
13.238	2,3-Dihydro-Benzofuran	0.50	C_8H_8O	120.15
14.520	Apigenin 7-O-methylglucuronide	1.01	$C_{22}H_{20}O_{11}$	460.4
15.252	Ursolic acid	0.22	$C_{30}H_{48}O_3$	456.7
17.095	Fucosterol	0.20	$C_{29}H_{48}O$	412.7
17.431	Neophytadiene	0.17	$C_{20}H_{38}$	278.5
17.634	Myristic acid vinyl ester	0.10	$C_{16}H_{30}O_2$	254.41

M. alliacea: Mansoa alliacea

[Table 2]. At higher concentration, loss of mobility and also death was noticeable against *P. posthuma*. MAME, MAEE, MACE, and MAPE in concentrations 5, 10, 15, and 20 mg/ml showed paralysis of worms within 3.89–12.55, 5.47–15.68, 8.36–19.22, and 11.11–24.67 min and death within 6.22–20.15, 7.36–4.58, 11.77–29.21, and 16.52–36.91 min depending on the concentration. More time was taken by MAPE, in comparison with other extracts. The standard drug, Mebendazole showed paralysis at 1.85 ± 0.31 min and death after 2.78 ± 0.11 min at 20 mg/ml concentration [Figures 3 and 4 and Table 3].

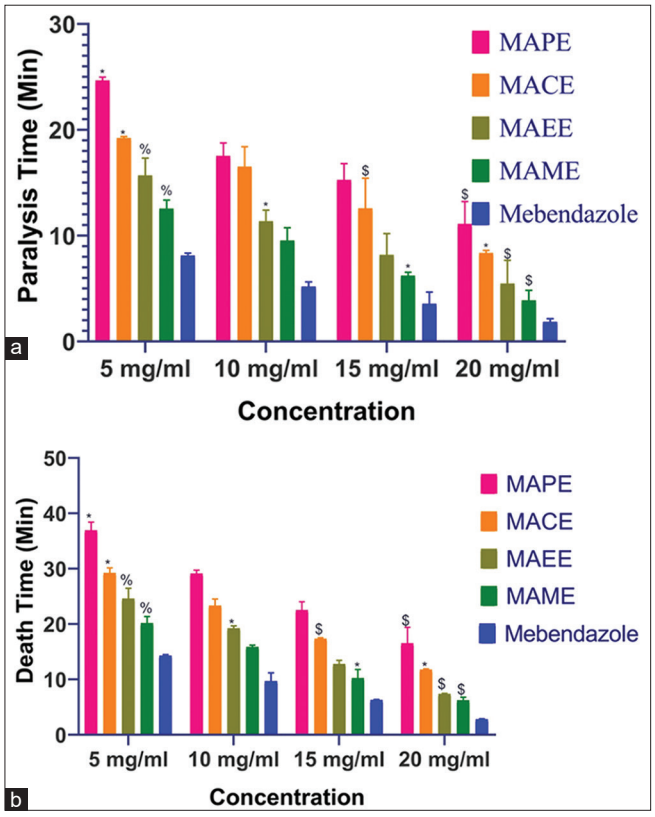

Figure 3: (a) Paralysis time for various extracts of *Mansoa alliacea* with standard mebendazole. (b) Death time for various extracts of *Mansoa alliacea* with standard mebendazole

GC–MS of Methanol Extract of *M. alliacea*

Based on the preliminary phytochemical analysis and *in vitro* anthelmintic activity studies, MAME extract was evaluated for GC analysis. The GC–MAME analysis is shown in Figure 4. Separation strategies coupled with GC–MS allowed effective separation of constituents as shown in Figure 4 GC–MS TIC trace. Phytochemical compound identifications were based on the peak area, retention and molecular formula. Centered on the projected mass spectrophotometer, four compounds are Apigenin-7-O-methylglucuronide, MAME Scutellarin, Luteolin, and Ursolic acid [Table 2].

Computational Study

In today's examination, to evaluate the possibility of substances accountable for anthelmintic activity, the docking score was made use of to verify the prospective binding energy. The molecules were additionally based on iLOG predictors utilizing online tools to identify their ADME/T properties [Figures 5-9].

Docking studies revealed that out of the four compounds; Apigenin 7-O-methylglucuronide had the best docking score of -8.8, which showed two hydrogen bond interactions (GLN11, and TYR224) and also hydrophobic interactions (ALA12) with the β-tubulin enzyme. Mebendazole revealed no hydrogen bonding. Interestingly, all the ligands were interacted with β-Tubulin, with a common amino acid, Ala12. The standard mebendazole revealed the good docking rating of -7.1, but which was lower than all the ligands. The outcomes gotten by the AutoDock 4.0 are shown in Table 4, as well as the protein-ligand interactions revealing hydrogen, hydrophobic, and electrostatic bonding are additionally published in Tables 4 and 5 [Figures 5-9].

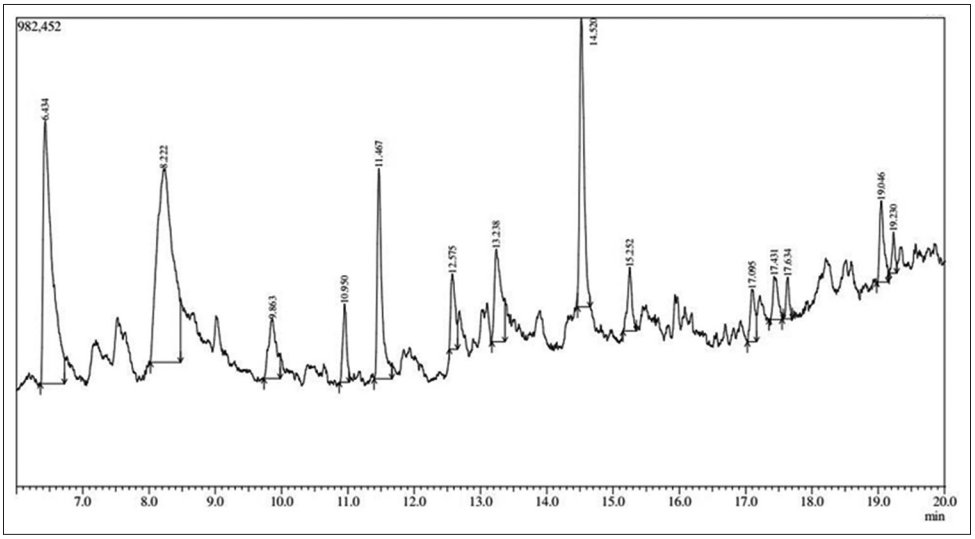

Figure 4: Gas chromatography–mass spectrometry spectral analysis of methanol extract of *Mansoa alliacea*

Table 3: Anthelmintic activity of various extract of *M. alliacea* against *P. posthuma*

Treatment/Dose	Paralysis time	Death time
Control (Water)	0.00	0.00
Mebendazole (5 mg/ml)	8.12±0.23	14.26±0.22
Mebendazole (10 mg/ml)	5.18±0.46	9.66±1.53
Mebendazole (15 mg/ml)	3.56±1.11	6.25±0.13
Mebendazole (20 mg/ml)	1.85±0.31	2.78±0.11
MAPE (5 mg/ml)	24.67±0.32*	36.91±1.51*
MAPE (10 mg/ml)	17.54±1.22	29.11±0.63
MAPE (15 mg/ml)	15.26±1.55	22.51±1.52
MAPE (20 mg/ml)	11.11±2.12$	16.52±2.91$
MACE (5 mg/ml)	19.22±0.15*	29.21±0.93*
MACE (10 mg/ml)	16.52±1.89	22.31±1.23
MACE (15 mg/ml)	12.58±2.85$	16.32±0.22$
MACE (20 mg/ml)	8.36±0.25*	11.77±0.15*
MAEE (5 mg/kg)	15.68±1.65%	24.58±1.89%
MAEE (10 mg/kg)	11.36±1.06*	19.22±0.46*
MAEE (15 mg/kg)	8.18±2.02	12.77±0.68
MAEE (20 mg/kg)	5.47±2.21$	7.36±0.12$
MAME (5 mg/kg)	12.55±0.81%	20.15±1.22%
MAME (10 mg/kg)	9.54±1.22	15.86±0.33
MAME (15 mg/kg)	6.22±0.32*	10.22±1.59*
MAME (20 mg/kg)	3.89±0.94$	6.22±0.56$

Values are expressed as mean±SEM (n=6), *P<0.05, %P<0.01 and $$P$<0.001 versus Standard. MAPE= *Manosa alliacea* petroleum ether extract; MACE: *Manosa alliacea* chloroform extract; MAEE: *Manosa alliacea* ethyl acetate extract; MAME: *Manosa alliacea* methanol extract. *M. alliacea: Mansoa alliacea, P. posthuma: Pheretima posthuma*

Drug Likeliness

The physicochemical properties of the chosen four active compounds were studied on DruLiTo software. Except, Luteolin, reaming all the compounds had one deviation with Lipinski's rule [Table 6].

PASS Predictions for Antiviral Activity

The biological activity spectra of previously identified phytoconstituents were obtained by online PASS version. These predictions were interpreted and used in a flexible manner and given in Table 7.

ADMET Analysis

The ADME properties of selected ligands are shown in Table 8. Here, estimated characteristics of all substances were in the range to fulfill the five-potential rule of Lipinski to be identified as drug-like.

DISCUSSION

Plant-derived natural compounds have gotten interested as a possible resource of brand-new healing representatives. Many of the scientifically active medications are from all-natural items, which show the significance of medicines having all-natural remedies in medicine exploration procedure. With this sight, the plant, *M. alliacea*, has been checked out for the assessment of anthelmintic task utilizing earthworm complied with by *in silico* molecular docking research study as well as ADME/T evaluation.[36,37]

Helminths illness is considered to be an important problem in humans as well as pets that contribute to a catastrophic and chronic illness that eventually leads to

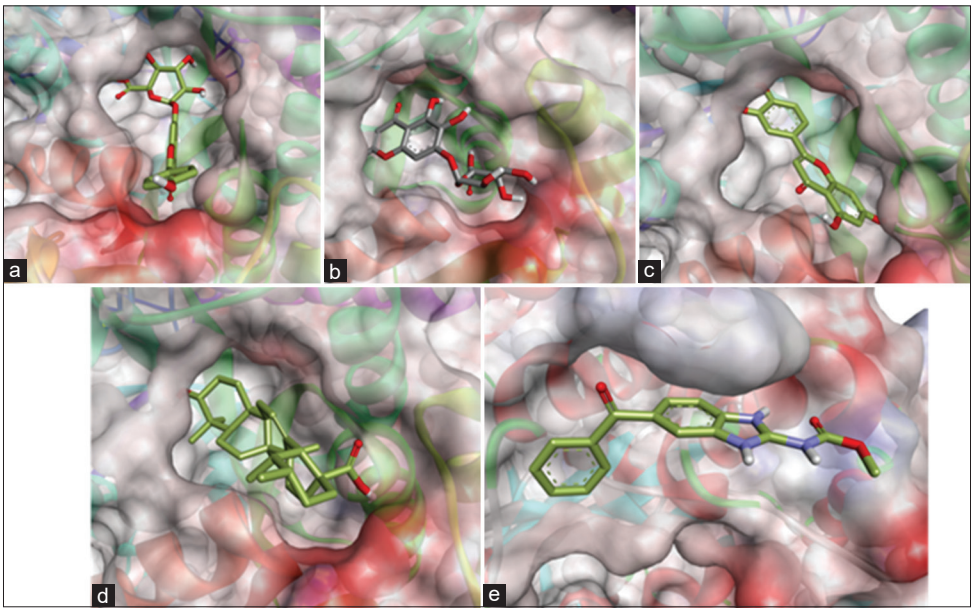

Figure 5: *In silico* docked complexes of ligand (Ball and Stick representation) with Tubulin-Colchicine: Stathmin-Like Domain Complex (1SA0) (Molecular representation) by AutoDock 4.0.(a) Apigenin-7-O-methylglucuronide; (b) Scutellarin; (c) Luteolin; (d) Ursolic acid; (e) Mebendazole

Table 4: Binding affinities of isolated compounds and standard at the active site of β-Tubulin

Ligands	Highest to the lowest mode of conformation with corresponding RMS binding affinities in ΔG (Kcal/mol)								
	1	2	3	4	5	6	7	8	9
Apigenin-7-O-methylglucuronide	−8.8	−7.5	−7.3	−7.1	−6.9	−6.9	−6.8	−6.7	−6.6
Scutellarin	−8.5	−8.4	−8	−8	−7.8	−7.7	−7.7	−7.7	−7.7
Luteolin	−8.4	−8.2	−8.1	−8	−7.6	−7.4	−7.2	−7.1	−7
Ursolic acid	−8.6	−7.5	−7	−7	−6.9	−6.8	−6.7	−6.7	−6.6
Mebendazole	−7.1	−7.1	−7.1	−6.9	−6.7	−6.7	−6.7	−6.6	−6.4

Table 5: 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
Apigenin-7-O-methylglucuronide	−8.8	GLN A:11 (4.55), TYR A:224 (4.96)	ALA A:12 (5.43)	ASP A:98 (6.35), MG A:501 (3.77)
Scutellarin	−8.5	ASN A:101 (3.73), ASP A:98 (4.26, 4.55), ASN A:228 (4.05)	ALA A:12 (3.90), ILE A:171 (6.16), TYR A:224 (5.42)	-
Luteolin	−8.4	VAL A:177 (4.08), TYR A:224 (5.75)	ALA A:12 (5.08), GLY A:143 (5.33)	GLU A:183 (7.35)
Ursolic acid	−8.6	ASP A:98 (4.40), ASN A:206 (4.78)	TYR A:224 (6.46)	-
Mebendazole	−7.1	-	ALA A:100 (4.79, 4.78), TRP A:407 (4.42, 5.67), VAL A:181 (6.30), ALA A:180 (7.06), VAL A:182 (5.28)	-

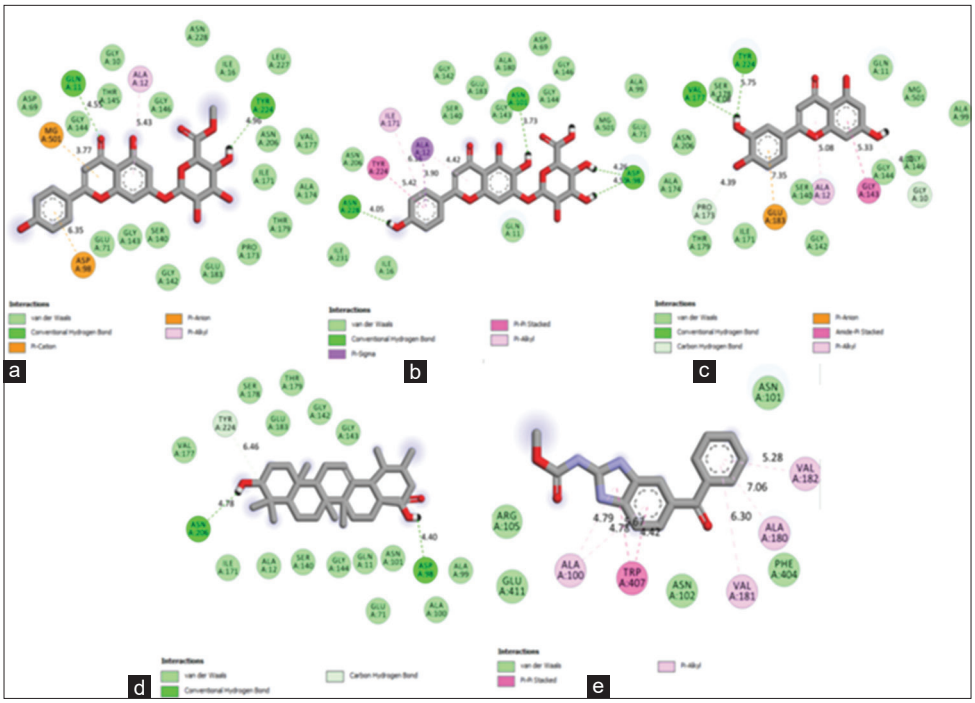

Figure 6: *In silico* 2D docked complexes of ligand (Ball and Stick representation) with Tubulin-Colchicine: Stathmin-Like Domain Complex (1SA0) by AutoDock 4.0. (a) Apigenin-7-O-methylglucuronide; (b) Scutellarin; (c) Luteolin; (d) Ursolic acid; (e) Mebendazole

fatality as well as causes medication tolerance to certain conditions. To prevent helminth contamination, research is required to focus on all-natural drugs, such as medicinal plants with novel bioactive substances with little or less unintentional impacts which are readily available to people from advanced nations and much more consistent with human

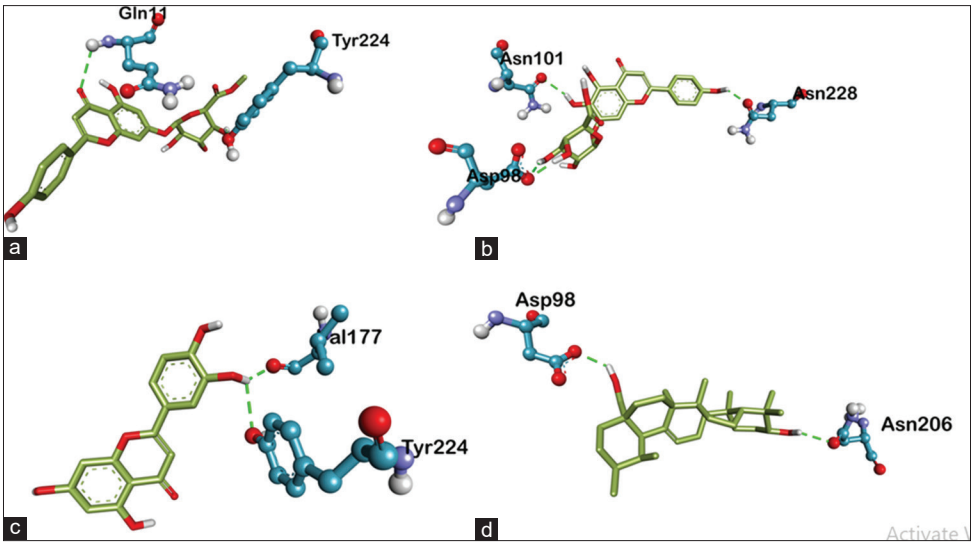

Figure 7: Various three-dimensional interactions of ligands with β-Tubulin (1SA0) through hydrogen bond. (a) Apigenin-7-O-methylglucuronide; (b) Scutellarin; (c) Luteolin; (d) Ursolic acid

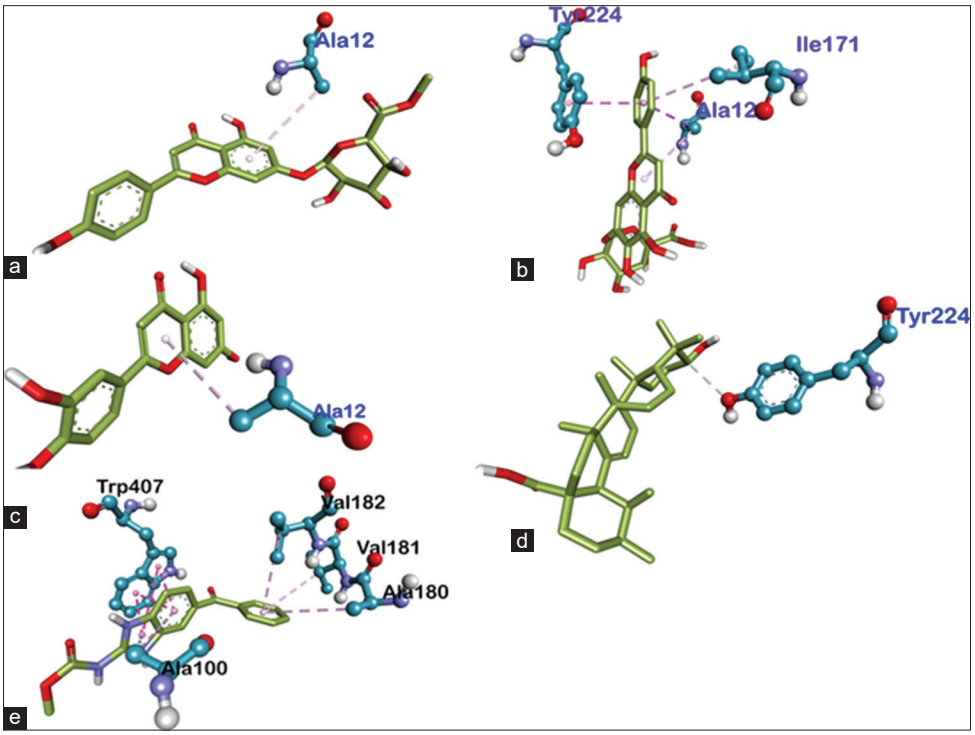

Figure 8: Various three-dimensional Interactions of ligands with β-Tubulin (1SA0) through hydrophobic interactions. (a) Apigenin-7-O-methylglucuronide; (b) Scutellarin; (c) Luteolin; (d) Ursolic acid; (e) Mebendazole

Table 6: Physicochemical properties of the active compounds and accordance with the rules of drug-likeness

Ligands	MW	logp	Alogp	HBA	HBD	TPSA	AMR	nRB	No. of Violations
Apigenin 7-O-methylglucuronide	439.94	0.31	−1.962	11	0	71.06	117.49	5	1
Scutellarin	443.94	0.337	−2.776	12	0	61.83	114.32	4	1
Luteolin	275.97	1.486	−0.787	6	0	26.3	81.76	1	0
Ursolic acid	407.98	8.954	1.484	3	0	17.07	132.26	1	1
Mebendazole	281.99	1.924	1.318	6	0	55.73	86.66	5	0

Table 7: PASS analysis of different compounds from *M. alliacea*

Main predicted activity by PASS online	Apigenin 7-O-methylglucuronide		Scutellarin		Luteolin		Ursolic acid	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
Anthelmintic activity	0.451	0.027	0.228	0.069	0.319	0.032	-	-

M. alliacea: Mansoa alliacea

Table 8: ADME/T properties of different compounds from *M. alliacea*

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Apigenin 7-O-methylglucuronide	0.02	−0.13	−0.11	0.34	−0.01	0.33
Ursolic acid	0.28	−0.03	−0.5	0.89	0.23	0.69
Scutellarin	0.08	−0.09	0.01	0.33	−0.05	0.41
Luteolin	−0.02	−0.07	0.26	0.39	−0.22	0.28
Mebendazole	0.2	0.18	0.51	−0.15	0.02	0.18

M. alliacea: Mansoa alliacea

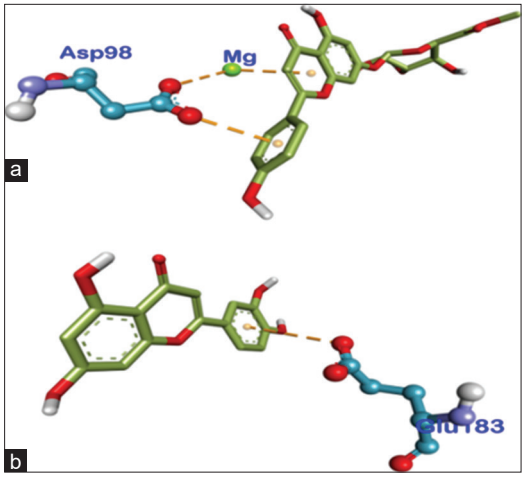

Figure 9: Various three-dimensional interactions of ligands with β-Tubulin (1SA0) through electrostatic interactions. (a) Apigenin-7-O-methylglucuronide; (b) Luteolin

biology than conventional medicines.[18,38]. In the here and now examination, monitorings were produced; the moment is considered paralysis. Furthermore, time is regarded as the fatality of earthworms versus the methanol, aqueous as well as conventional medicine, mebendazole. Several bioactive phytoconstituents such as alkaloids, flavonoids, saponins, and tannins were identified mainly during the preliminary phytochemical examination of the different *M. alliacea* extract linked to anthelmintic activities.[37]

From the above test, all *M. alliacea* extracts are valid. The plant has a substantial dose-dependent anthelmintic function relative to normal anthelmintic. However, methanol extract shows excellent effects as the time required to die is comparatively small relative to the standard drug. This function may be attributed to bioactive phytoconstituents such as alkaloids, flavonoids, tannins, and saponins. Any of these phytoconstituents, such as alkaloids, tannins, phenols, may account for the considerable anthelmintic function. Below, alkaloids can cause paralysis by acting on the central nervous system, whereas polyphenols and also tannins uniquely bind to complementary proteins in GI, sometimes elsewhere, produces fatality. On the many other ends, saponins anthelmintic potency is permeabilizing properties attributable to their membrane layer.

The effective dosage was calculated to be 20 mg/ml MAME for our research, with 3.89 and 6.22 min for the time of paralysis and death, respectively. It took more time to paralyze and death the rest of the concentration. Paralysis was induced, and the worms were put to death at all the doses measured. The extracted power was inversely proportional to the time required to paralyze the worms. Studies demonstrate quite clearly that the methanol extract has a significant anthelmintic effect. As indicated in folk medicine, the above observations support the usage of an anthelmintic.

By contrast of the mass spectrum with the NIST Library edition year 2005, the GC–MS study of MAME identity confirmation was carried out. Four mostly known compounds are Apigenin 7-O-methylglucuronide, ursolic acid, scutula, and Luteolin found in MAME, based on the mass spectrometer prediction.

On the other hand, organic medications that have been utilized given that the old times keep reduced toxicity, have far better absorption, as well as abundant. Scientists around the world remain in search of plants that have such biological activities, which will undoubtedly aid in the improvement of the existing clinical system, making less expensive, as well as reliable therapy.

Online testing utilizing molecular docking programs has ended up being a significantly prominent method to the advancement of new medicines, partly due to the desired time and also budgeting prices of *in silico* medication testing compared to standard lab experiments. In this research, we used a computational protein-ligand docking method making use of open software programs as well as virtualized. Interactions of the ligands; Apigenin 7-O-methylglucuronide, Ursolic acid, Scutellarin, Luteolin and standard mebendazole with the anti-helminthic protein β-Tubulin were visualized with the help of BIOVIA Drug Discovery Studio 2020.

We have tested the molecular docking of certain compounds to show the relationship between compounds and protein at the molecular level, which helps us to represent molecular action of these compounds at the intended protein binding site and to explain the biochemical mechanism of anthelminthic activity. It is inferred from the result [as shown in Table 5] that Apigenin-7-O-methylglucuronide (−8.8 kcal/mol), Scutellarin (−8.5 kcal/mol), Luteolin (−8.4 kcal/mol), and ursolic acid (−8.6) displayed substantial docking scores comparable to those of the reference drug mebendazole (−7.1 kcal/mol). Apigenin-7-O-methylglucuronide's docking score indicates the highest docking score compared to conventional drugs, mebendazole. It is clear from the docking study that these compounds, particularly Apigenin-7-O-methylglucuronide, can be a good candidate for a new anthelmintic agent.

Apigenin-7-O-methylglucuronide has the best correlation with β-Tubulin (1SA0) protein complexes. The β-tubulin with Apigenin-7-O-methylglucuronide complex formed two hydrogen bond, i.e., GLN A:11 (4.55), TYR A:224 (4.96) and one amino acid involved in hydrophobic interactions ALA A:12 (5.43) and ASP A:98 (6.35), MG A:501 (3.77) moieties are involved in electrostatic interactions. The standard, mebendazole, forms only hydrophobic interactions with the amino acid residues, which include ALA A:100 (4.79, 4.78), TRP A:407 (4.42, 5.67), VAL A:181 (6.30), ALA A:180 (7.06), and VAL A:182 (5.28).

The primary amino acids involved in hydrogen bonding are TYR A:224, which have typical interaction with Apigenin-7-O-methylglucuronide and luteolin and ASP A:98 shares a common interaction with Scutellarin and ursolic acid. In hydrophobic interactions, except ursolic acid and mebendazole, remaining all ligands had good interaction with amino acid ALA A:12. Interestingly, ASP A:98 has involved both in hydrogen bonding and electrostatic interactions also. Interestingly, all the isolated constitutes had the lowest binding energy in comparison to the standard drug.

Our end results disclose that electrostatic, hydrophobic, and hydrophilic communications are regulated by numerous amino acid deposits in each ligand-protein communication. Specifically, Ala12 was determined in all electrostatic interactions of all ligands with β-tubulin. All the ligands were involved in hydrogen bond and hydrophobic interactions with β-tubulin.

During the ADME study of compounds, we noted in Table 6 that two compounds, i.e., Apigenin 7-O-methylglucuronide and Scutellarin, exhibit hydrogen bond acceptor activity in excess of 10 and ursolic acid in excess of 5. From the findings of the ADME and toxicity study, it can be inferred that all the four substances were known as drug-like ability with fewer toxic effects in terms of improved pharmacokinetics properties.

CONCLUSION

The outcomes confirm the ethnomedicinal use of *M. alliacea* to anthelmintic activity, which recommends that this plant might be a possible resource for the growth of a new anthelmintic representative. The here and now molecular docking experiments suggest that Apigenin 7-O-methylglucuronide, Scutellarin, Luteolin, and Ursolic acid were prospect ligands for anthelmintic activity as well as act with interactions with β-Tubulin. High-throughput evaluating making use of molecular docking evaluation brought about the final thought that Apigenin 7-O-methylglucuronide revealed ideal fitness score as well as appropriates for personal usage, specifically. Even more, intricate measurable structure-activity relationship design is needed to guarantee its safety and also bioefficacy. In addition, *in vivo* experiments are required to verify these *in silico* outcomes.

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