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Original Article

Insilico and *Invitro* anthelmintic properties of phytocompounds in *Rostellularia quinquangularis (J. Koenig ex Roxb.) Nees*

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Abstract

The present study aimed to evaluate the anthelmintic activity of various extracts of *Rostellularia quinquangularis* (*R. quinquangularis*) against adult Indian earthworms (Pheretima posthuma). Petroleum ether extract (PERQ), ethyl acetate extract (RQEA), and ethanol extract (RQEE) of *R. quinquangularis* were tested at different concentrations (10, 20, 50, and 100 mg/mL), along with the positive control (albendazole) and negative control (normal saline). Anthelmintic activity was assessed based on the duration of paralysis and mortality. The RQEE extract showed significant anthelmintic activity, with the highest activity observed at a concentration of 100 mg/mL, exhibiting paralysis time of 1.62 min and death times of 19.9 min, compared to the standard albendazole. Further, HR LC-MS analysis of the RQEE extract revealed the presence of various phytoconstituents based on m/z signals. Molecular docking analysis using AutoDock Vina indicated that Columbianetin, Dunnione, Cryptochlorogenic acid, Gaylussacin, Luvangetin, and Albendazole showed docking scores of -8.1, -7.9, -7.4, -7.3, -7.2, and -6.8 Kcal/mol, respectively. These results suggest that *R. quinquangularis* possesses potent anthelmintic activity, supporting its traditional use in medicinal practices.

Keywords: Rostellularia quinquangularis; Anthelmintic; Insilico; Invitro; LC-MS; Extraction.

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1. Introduction

Millions of individuals worldwide suffer from helminthiasis, commonly known as worm infections, caused by roundworms, hookworms, whipworms, and tapeworms. According to the World Health Organization,

helminthiasis is common in tropical and subtropical areas of the world, with a high prevalence of poverty and poor sanitation [1]. An estimated 1.5 billion individuals, or one-fifth of the world's population, are thought to be infected with soil-transmitted helminths (STH). Schistosomiasis, lymphatic filariasis, and onchocerciasis are other common helminthiasis illnesses that can result in malnutrition, diarrhea, and anemia. In extreme circumstances, disability and death are conceivable [2]. Helminthiasis is still widespread, especially in low- and middle-income countries, despite efforts by international organizations to control and eradicate it by providing access to safe and effective treatments and enhancing sanitation and personal hygiene. However, many anthelmintic drugs currently on the market have negative side effects, including hepatitis, bone marrow suppression, and allergic responses, and they may not be as effective against gastrointestinal helminths. [2]; ivermectin causes severe allergic reactions, liver problems, and vision changes [3], and praziquantel causes fever, rash, and allergic reactions [4]. As a result, interest in alternative anthelmintic sources has increased. As an alternative to allopathic treatments, which may lead to drug resistance and residual issues, medicinal plants from the Acanthaceae family are being assessed for their anthelmintic activity. These plants have demonstrated potential as anti-helminthic drugs.

The genus *Rostellularia* belongs to the family Acanthaceae, which comprises approximately 250 genera and 2500 species of herbs, shrubs, and trees [5]. Acanthaceae plants are widely distributed in tropical and subtropical regions and are known for their beautiful flowers and medicinal properties. Many plants in this family have been used in traditional medicine because of their anti-inflammatory, antimicrobial, and analgesic properties [6]. *R. quinquangularis* is known as the five-angled rostellaria or snakehead plant. This plant is native to tropical regions of Asia and Africa and is often used in traditional medicine to treat various ailments such as fever, inflammation, and wounds. Based on the literature review, various primary and secondary metabolites were isolated, such as flavonoids viz., apigenin, luteolin, kaempferol, and quercetin; alkaloids viz., rostelline and rostellimine; terpenoids viz., triterpenoids and diterpenoids; phenolic acid viz., caffeic acid and chlorogenic acid; and steroids viz., stigmasterol and β-sitosterol.

In light of the growing issue of drug resistance around the world, the expense of synthetic anthelmintics, and the potential of natural medicines, we carried out this study to analyze the biochemical components of *R. quinquangularis* and determine the efficiency of its extracts against nematodes. To fulfill this purpose, we conducted an LC-MS analysis of the extract to examine the composition of the extracts and ascertain their major components. Subsequently, we performed in silico analyses and in vitro studies on *Pheretima posthuma* (Indian Earthworm).

2. Materials and Methods

2.1. Collection and authentication of plant

Plant specimens were procured from the Seshachalam forest in Andhra Pradesh. To ensure the authenticity of the plant material, the Department of Botany at Sri Venkateswara

University in Tirupati, Andhra Pradesh, conducted authentication procedures and assigned Voucher No. 0428.

2.2. Preparation of Extracts

The whole plants were cleansed with water, segregated, and dried in the shade to eliminate impurities and other extraneous substances. The dried plant material was then pulverized into a coarse powder and sieved through a No. 14 mesh size. The resulting powder was placed in the thimble tube of a Soxhlet apparatus and subjected to solvent extraction with various solvents such as petroleum ether, ethyl acetate, and ethanol for 6 hours. The extracted samples were filtered, heated, and dried through rotary vacuum evaporation. The dried extracts were stored at low temperatures in a refrigerator for further analysis.

2.3. Qualitative phytochemical tests

An examination of the phytochemical constituents in various *R. quinquangularis* extracts was performed, and identification tests were utilized to detect the presence of various primary and secondary phytoconstituents.

2.4. In-vitro Anthelmintic activity

2.4.1. Earthworms Collection

The earthworms were thoroughly cleaned of fecal matter by washing with a normal saline solution. For the experiments, earthworms of P. posthuma species, measuring 5-6 cm in length, 0.10-0.20 cm in width, and weighing 1-3 g, were utilized. These earthworms were selected because of their anatomical and physiological similarities to intestinal roundworms, making them a suitable model for anthelmintic activity studies [7, 8].

2.4.2. Preparation of Extracts and reference drug

Extracts were prepared for in vitro study at 10, 20, 50, and 100 mg/mL concentrations. EERQ, EARQ, and PERQ extract samples were prepared by dissolving 100, 200, 500, and 1000 mg of crude extract in 1 mL of dimethyl sulfoxide and then diluting with 10 mL of normal saline solution. The final concentrations of the samples were 10 mg/mL, 20 mg/mL, and 50 mg/mL. Albendazole served as the standard drug in this study, whereas normal saline solution was used as the control [9].

2.4.3. Anthelmintic activity

To conduct in vitro studies on anthelmintic activity extracts at concentrations of 10, 20, 50, and 100 mg/mL were prepared. These extracts (EERQ, EARQ, and PERQ) by dissolving 100, 250, 500, and 1000 mg of crude extract in 1 mL of dimethyl sulfoxide and then diluting with 10 mL of normal saline solution to obtain final concentrations of 10, 20, 50, and 100 mg/mL. This study used Albendazole as the standard drug, and a normal saline solution was used as the control [9, 10].

2.5. Statstical Analysis

Results were expressed as the mean±SEM, and statistical analysis was conducted using

GraphPad Software (Version 3, USA). Dunnett's multiple comparison test was used to compare data with ANOVA. Statistical significance was defined as P< 0.05.

2.6. Identification of Bioactives by High-Resolution Liquid Chromatography -Mass Spectroscopy (HR LC-MS)

HR-LC-MS analysis was conducted on EERQ to identify the potential anthelmintic phytoconstituents. The UHPLC-PDA-Detector (6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3)) was used for analysis, and the compounds were identified based on their mass spectra and fragmentation patterns. Metabolomic identification was carried out using Compound Discoverer 2.0 and MF 7.0 SR3 [11, 12].

2.7. In-silico Studies

2.7.1. Drug Likeliness and ADMET Analysis

To assess the drug-likeliness of the compound, the DrugLiTo tool, Lipinski's rule of five, was used to gauge the compounds [13]. ADMET values of the selected active phytoconstituents were predicted using the admetSAR server.

2.7.2. Molecular Docking

Based on the results of previous studies [14, 15], molecular docking was used to explore the interaction mechanisms between ligands and enzymes. The tubulin-colchicine stathmin-like domain complex (PDB ID:1SA0) (**Figure 1**) was used as the simulation target [16]. The RSCB Protein Data Bank provided the crystallized structures, and PubChem provided 3D structural information on the ligands. (**Figure 2**) The 1SA0 structure was optimized, and the grid box parameters are listed in **Table 1**.

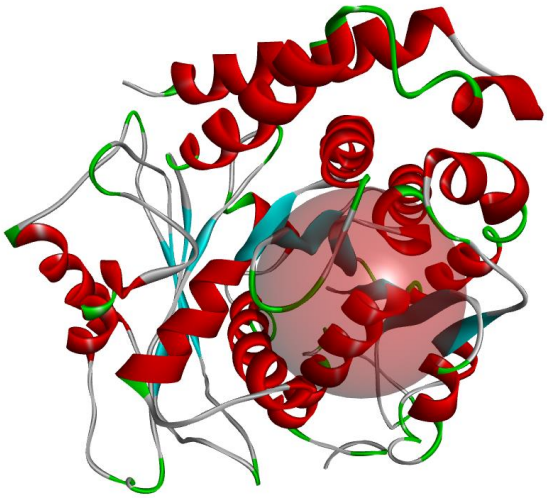

Figure 1. 3D ribbon-type representation of the tubulin-colchicine: stathmin-like domain complex (PDB ID:1SA0) with the active site (highlighted in red).

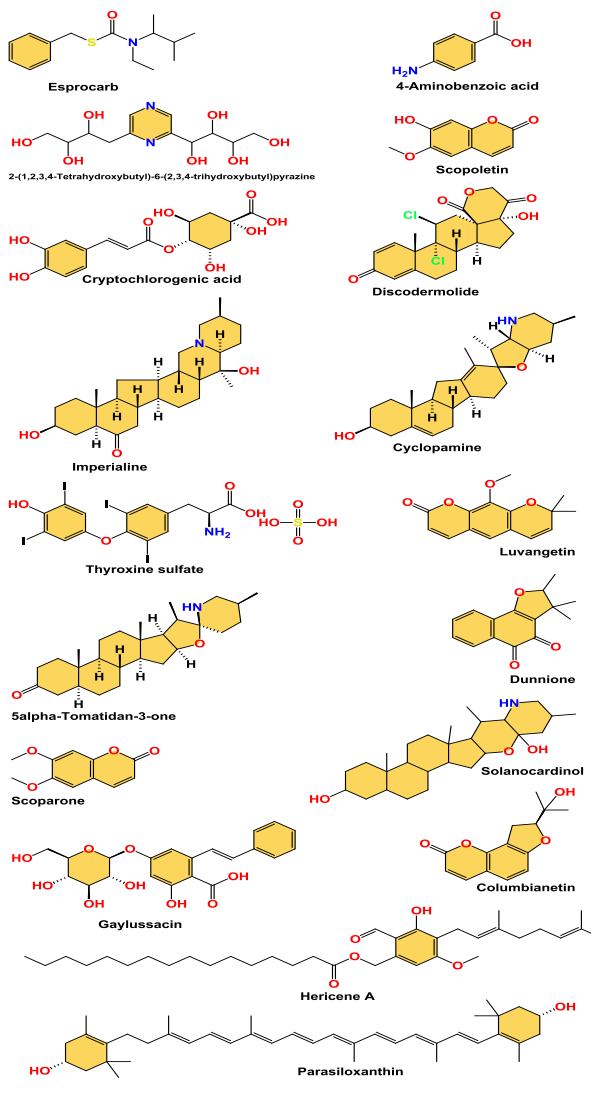

Figure 2. 2D shows the phytoconstituents retrieved from the LC-MS analysis of EERQ.

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)	127.059219	95.344719	13.796594
Size	**x**	**y**	**z**
	10	10	10
Exhaustiveness		8	

3. Results and Discussion

3.1. Qualitative Phytochemical Analysis

The whole-dried plant of *R. quinquangularis* was powdered and sieved, and the resulting powder was soaked in petroleum ether, ethyl acetate, and ethanol for 24 hours. The filtrate obtained after filtration through Whatman filter paper was subjected to phytochemical analysis using standard methods. The analysis revealed the presence of steroids, fixed oils, and volatile oils in PERQ, while EARQ contained alkaloids, flavonoids, and tannins. The ethanol extracts contained the majority of primary and secondary metabolites, as shown in **Table 2**.

3.2. Anthelmintic activity

This study investigated the effects of different concentrations of *R. quinquangularis* extract and albendazole on P. posthuma, a type of earthworm. The results showed that the extract-treated earthworms exhibited dose-dependent paralysis and mortality, whereas those treated with albendazole as the reference drug showed similar effects. EERQ displayed the best anthelmintic activity among the three extracts tested, comparable to the standard drug, albendazole (**Table 3**).

Table 2: Preliminary phytochemical analysis of various extracts of *R. quinquangularis*.

S. No	Phytochemical	Test Name	Results		
			PERQ	EARQ	EERQ
1	Alkaloids	Mayers	-	+	+
		Wagners	-	+	+
2	Flavonoids	Shinoda	-	+	+
		Alkaline	-	+	+
3	Tannins	$Fecl_3$	-	+	+
		Lead acetate	-	+	+
4	Steroids	Salkowski	+	-	+
		Liebermann- Burchard	+	-	+
5	Volatile oils	-	+	-	+
6	Saponins	Foam	-	-	-
7	Glycosides	-	-	-	+
8	Carbohydrates	Molisch	-	-	+
9	Proteins	Biuret	-	-	+
		Millions	-	-	+
10	Amino acids	Ninhydrin	-	-	+
11	Fixed oils	-	+	-	-

(+) Present (-) Absent

Table 3: Anthelmintic activity of various extracts of *Rostellularia quinquangularis* against *Pheretima Posthuma*.

Treatment	Dose	Paralysis Time	Death Time
Control	-	-	-
Albendazole	10	5.36±1.34*	29.85±3.57*
	20	3.82±0.6$	20.52±1.46$
	50	1.76±0.31*	13.89±3.31*
	100	1.64±0.26#	9.77±2.2#
PERQ	10	16.68±2.93	69.78±4.9
	20	12.62±1.6*	60.75±4.64*
	50	9.97±2.63	53.79±2.84
	100	8.64±1.51$	44.17±3.94$
EARQ	10	10.13±1.24*	37.75±1.2*
	20	6.76±0.3*	30.14±3.34*
	50	5.82±0.91	24.74±3.8
	100	4.65±0.74	21.78±3.9
EERQ	10	7±1.62	43.82±1.31
	20	4.04±1.3#	29.16±3.9#
	50	3.8±0.75	23.71±3.26
	100	1.62±0.23$	19.9±2.54$

EERQ displayed the best anthelminthic activity among the three extracts, which was comparable to the standard. **Figure 3** shows that the paralysis times were 7, 4.04, 3.8, and 1.62 min for the 10, 20, 50, and 100 mg/mL concentrations, respectively, and the death times were 43.82, 29.16, 23.71, and 19.9 min, respectively. Compared with albendazole, it causes paralysis and death within a short period at high concentrations. Among the albendazole-treated groups with concentrations of 10, 20, 50, and 100 mg/mL, the paralysis times were 5.36, 3.82, 1.76, and 1.64 minutes, and the death times were 29.85, 20.52, 13.89, and 9.77 minutes. These results indicate that the EERQ extract caused paralysis and death within a shorter period at higher concentrations than albendazole. Earthworms treated with the standard saline solution remained active with whole-body movements and displayed no physical changes.

In conclusion, this study demonstrated dose-dependent paralysis and mortality effects of *R. quinquangularis* extract and albendazole on *P. posthuma.* The EERQ extract exhibited the best anthelmintic activity, comparable to the standard drug albendazole. These findings support the potential of *R. quinquangularis* as a source of anthelmintic compounds and warrant further investigation of potential anthelmintic compounds using HR LC-MS analysis.

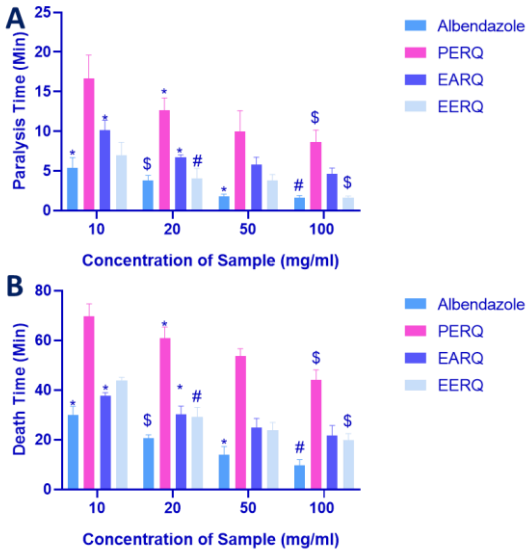

Figure 3. Paralysis and death times for various extracts of *R. quinquangularis* using albendazole as the standard.

3.3. HR LC-MS analysis of EERQ

The *in vitro* anthelmintic activity of the *R. quinquangularis* (EERQ) ethanol extract was superior to that of the other extracts tested in this study. High-resolution liquid chromatography-mass spectrometry (HR-LCMS) has been employed to understand its chemical composition better [17]. HR-LCMS separates and detects phytoconstituents using retention time, experimental m/z, MS/MS fragments, metabolite classes, and suggested compounds (**Figure 4**) [18].

EERQ extract HR-LCMS identified in the HR-LCMS EERQ extract. The top eight compounds were selected for screening based on the extract concentrations. **Table 4** lists the substances and their concentrations, and **Figure 5** shows the chromatographic profiles.

Understanding the possible bioactive components that may be present in EERQ extracts will benefit from the identification of these compounds. Their chemical components significantly influence natural products' biological activity and therapeutic potential. Researchers might clarify the mechanisms of action by correlating the presence of the chemicals in EERQ with the anthelmintic activity that has been noticed.

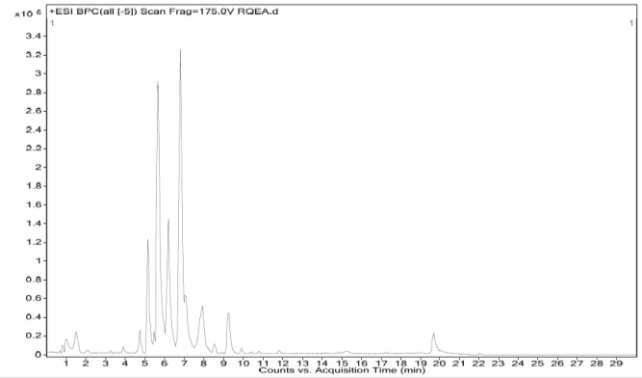

Figure 4. HR-LC-MS spectrum of EERQ.

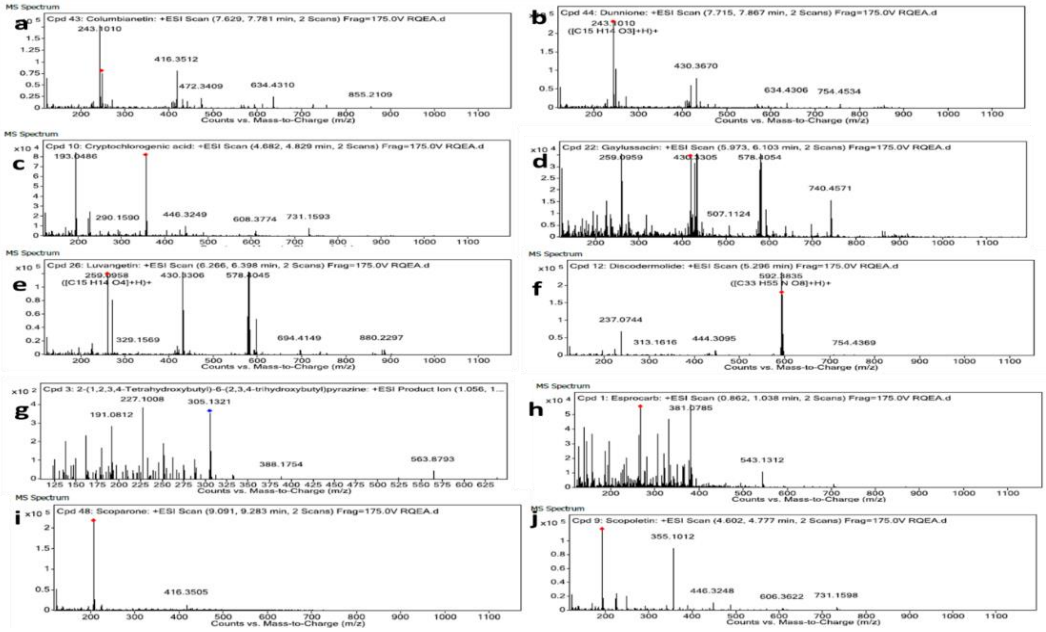

Figure 5. HR-LC-MS spectrum of EERQ.

Table 4: Anthelmintic activity of various extracts of *Rostellularia quinquangularis* against *Pheretima Posthuma*.

Name	Formula	Mass	Base Peak	m/z	Start	RT	End
Esprocarb	$C_{15}H_{23}NOS$	265.1519	266.1597	266.159	0.875	0.98	1.084
4-Aminobenzoic acid	C_7HNO_2	137.0477	138.0546	138.0549	1.054	1.126	1.197
2-(1,2,3,4-Tetrahydroxybutyl)-6-(2,3,4-trihydroxybutyl)pyrazine	$C_{12}H_{20}N_2O_7$	304.1267	227.1008	305.1333	1.056	1.129	1.201
Scopoletin	$C_{10}H_8O_4$	192.0413	193.049	193.0486	4.664	4.727	4.79
Cryptochlorogenic acid	$C_{16}H_{18}O_9$	354.0939	193.0488	355.101	4.695	4.769	4.843
Discodermolide	$C_{33}H_{55}NO_8$	591.3802	574.3723	592.3835	5.309	5.309	5.309
Imperialine	$C_{27}H_{43}NO_3$	429.3233	412.3199	430.3303	5.509	5.586	5.662
Cyclopamine	$C_{27}H_{41}NO_2$	411.3133	412.3197	412.32	5.614	5.697	5.779
Solanocardinol	$C_{27}H_{45}NO_3$	431.3389	412.3193	432.3462	5.832	5.832	5.832
Thyroxine sulfate	$C_{15}H_{11}I_4NO_7S$	856.6255	430.3305	429.3208	5.866	5.866	5.866
Luvangetin	$C_{15}H_{14}O_4$	258.0887	199.0753	259.0959	5.999	6.057	6.115
Gaylussacin	$C_{21}H_{22}O_9$	418.1255	257.0797	419.1327	5.988	6.06	6.131
Hericene A	$C_{35}H_{56}O_5$	556.4182	578.4037	579.4077	6.417	6.499	6.581
Scopoletin	$C_{10}HO_4$	192.0415	193.0482	193.0487	6.826	6.911	6.995
5alpha-Tomatidan-3-one	$C_{27}H_{43}NO_2$	413.3284	414.3355	414.3357	6.886	6.981	7.077
Parasiloxanthin	$C_{40}H_{58}O_2$	569.4316	592.4229	592.4212	7.136	7.257	7.378
Columbianetin	$C_{14}H_{14}O_4$	246.0887	229.085	247.0959	7.643	7.719	7.795
Dunnione	$C_{15}H_{14}O_3$	242.0937	135.0447	243.101	7.735	7.807	7.879
Scoparone	$C_{11}H_{10}O_4$	206.0573	207.0643	207.0646	9.112	9.204	9.296

3.4. Drug Likeliness

Using DruLiTo software, the physicochemical characteristics of the active compounds eluted from EERQ by HR LC-MS were investigated. Of the compounds identified, Thyroxine sulfate, 5alpha-Tomatidan-3-one, Solanocardinol, Hericene A, and Parasiloxanthin were found to deviate from Lipinski's rule, while all the remaining compounds adhered to the rule as demonstrated in **Table 5**.

3.5. Molecular Docking Studies

Docking experiments further explored the anthelmintic action of EERQ to understand the substances that block -tubulin and their binding mechanisms (**Table 6**). With a binding energy of -8.1 kcal/mol, the coumarin derivative columbinetin showed strong binding affinities with -tubulin. According to docking research, Columbianetin made two hydrogen bond contacts with GLY A:146 and TYR A:224 in the active region of the receptor, which demonstrated how well it fit there. This Columbianetin molecular docking study of an anthelmintic target implies that more research into its anthelmintic action is necessary.

To further understand the anthelmintic action of EERQ that has been reported, docking experiments were conducted to examine the substances that block α-tubulin and their binding mechanisms, as indicated in **Table 7**.

Table 5: Drug Likeliness Properties of different compounds eluted from HR LC-MS of EERQ.

Sr. No.	Compound	MW	logp	Alogp	HBA	HBD	TPSA	AMR	Violated Lipinski's rule
1	Esprocarb	241.97	3.156	2.62	2	0	45.61	83.24	No
2	4-Aminobenzoic acid	129.99	-0.143	-0.229	3	0	17.07	40.5	No
3	2-(1,2,3,4-Tetrahydroxybutyl)-6-(2,3,4-trihydroxybutyl)pyrazine	283.97	-5.377	-3.936	9	0	24.72	71.8	No
4	Scopoletin	183.98	0.97	-0.031	4	0	35.53	53.85	No
5	Discodermolide	403.93	1.67	0.568	5	0	60.44	102.82	No
6	Cryptochlorogenic acid	335.95	-1.129	-1.194	9	0	43.37	85.8	No
7	Cyclopamine	375.03	3.646	0.683	3	0	9.23	117.93	No
8	Imperialine	394.05	4.011	-1.792	4	0	20.31	113.41	No
9	Thyroxine sulfate	765.6	2.306	1.046	5	0	26.3	131.48	Yes
10	Luvangetin	243.98	2.135	0.798	4	0	44.76	76.06	No
11	5alpha-Tomatidan-3-one	376.04	6.701	0.423	3	0	26.3	114.32	Yes
12	Dunnione	227.98	1.487	1.071	3	0	43.37	72.17	No
13	Scoparone	195.98	1.291	0.034	4	0	44.76	58.89	No
14	Solanocardinol	385.99	6.509	-0.148	4	0	9.23	116.25	Yes
15	Columbianetin	231.98	1.057	0.38	4	0	35.53	69.92	No
16	Gaylussacin	395.95	1.84	-0.769	9	0	35.53	111.7	No
17	Hericene A	499.97	11.997	-1.065	5	0	52.6	149.92	Yes
18	Parasiloxanthin	511.99	10.276	8.147	2	0	0	194.8	Yes

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

Ligands	Highest to Lowest mode of conformation with corresponding RMS binding affinities in ΔG (Kcal/mol)								
	1	**2**	**3**	**4**	**5**	**6**	7	8	9
Columbianetin	-8.1	-7.2	-7.1	-6.8	-6.8	-6.7	-6.6	-6.5	-6.2
Dunnione	-7.9	-7.3	-6.8	-6.5	-6.5	-6.3	-6.3	-6.2	-6.1
Cryptochlorogenic acid	-7.4	-6.6	-6.2	-6	-5.8	-5.5	-	-	-
Gaylussacin	-7.3	-7.2	-6.8	-5.8	-5.6	-4.8	-4.5	-	-
Luvangetin	-7.2	-6.6	-6.6	-6.4	-6.1	-6.1	-5.8	-5.5	-5.3
Discodermolide	-6.5	-6.2	-4.8	-4.2	-4.1	-3.7	-	-	-
2-(1,2,3,4-Tetrahydroxybutyl)-6-(2,3,4-trihydroxybutyl)pyrazine	-6.4	-6.4	-6.4	-6.3	-6.3	-6.3	-6.2	-6.1	-6.1
Esprocarb	-6.3	-6.0	-5.9	-5.7	-5.6	-5.5	-5.5	-5.5	-5.4
Scoparone	-6.2	-6.1	-5.9	-5.6	-5.5	-5.5	-5.5	-5.4	-5.3
Scopoletin	-6.1	-6	-5.9	-5.9	-5.8	-5.8	-5.5	-5.4	-5.4
Albendazole	-6.8	-6.4	-6	-5.8	-5.7	-5.6	-5.5	-5.3	-5.2

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

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	**Hydrophobic Interactions**	**Electrostatic Interactions**
Columbianetin	-8.1	GLY A:146 (3.71), TYR A:224 (5.07)	ALA A:12 (5.76)	-
Dunnione	-7.9	ASN A:206 (4.80)	GLN A:11 (5.94), ALA A:12 (3.68, 4.04), TYR A:224 (4.78, 5.78)	-
Cryptochlorogenic acid	-7.4	GLN A:11 (4.26), ASN A:101 (4.16), GLY A:144 (3.59), ILE A:171 (4.04)	-	-
Gaylussacin	-7.3	ASN A:101 (5.27), TYR A:224 (5.64)	ALA A:12 (5.36), ILE A:171 (6.30)	-
Luvangetin	-7.2	ASN A:206 (4.81)	ALA A:12 (4.31, 4.89), ILE A:171 (5.86), SER A:178 (6.77)VAL A:177 (5.71)	-
Albendazole	-6.8	SER A:140 (3.68)	ALA A:12 (4.43, 5.72), ILE A:16 (5.59), ILE A:171 (5.29), TYR A:224 (6.72)	GLU A:183 (7.67), TYR A:224 (6.71)

With a binding energy of -8.1 Kcal/mol, the coumarin derivative columbinetin demonstrated strong binding interactions with -tubulin. As seen in **Fig. 6a**, Columbianetin successfully occupied the receptor's active site and established two hydrogen bonds with GLY A:146 (3.71) and TYR A:224 (5.07). The binding energy and important interactions indicate that more research into the anthelmintic activity of columbianetin is necessary, as this is the first molecular docking examination of an anthelmintic target.

A naphthoquinone derivative called Dunnione, on the other hand, had a binding energy of -7.9 Kcal/mol, established hydrogen bonds with ASN A:206 (4.80), and interacted hydrophobically with GLN A:11, ALA A:12, and TYR A:224 (**Fig. 6b**).

The standard, albendazole, displayed a binding energy of -6.8 kcal/mol and established contacts with SER A:140 (3.68), ALA A:12 (4.43, 5.72), ILE A:16 (5.59), ILE A:171 (5.29), TYR A:224 (6.72), GLU A:183 (7.67), and TYR A:224 through hydrophobic and electrostatic interactions (6.71). According to docking experiments, only albendazole formed electrostatic contacts with GLU A:183 and TYR A:224, while other critical amino acids implicated in the suppression of -tubulin included ALA A:12, ILE A:171, and TYR A:224 (**Fig. 6F**).

Docking experiments shed essential light on the substances that block -tubulin and their binding pathways. Favorable binding interactions with -tubulin were observed for columbinetin, dunnione, and albendazole, with albendazole displaying additional electrostatic interactions. The main amino acids involved in the inhibition of tubulin were identified in this study. These results encourage further research on the anthelmintic activity of the tested compounds and imply that *R. quinquangularis* could be a potential source of anthelmintic substances.

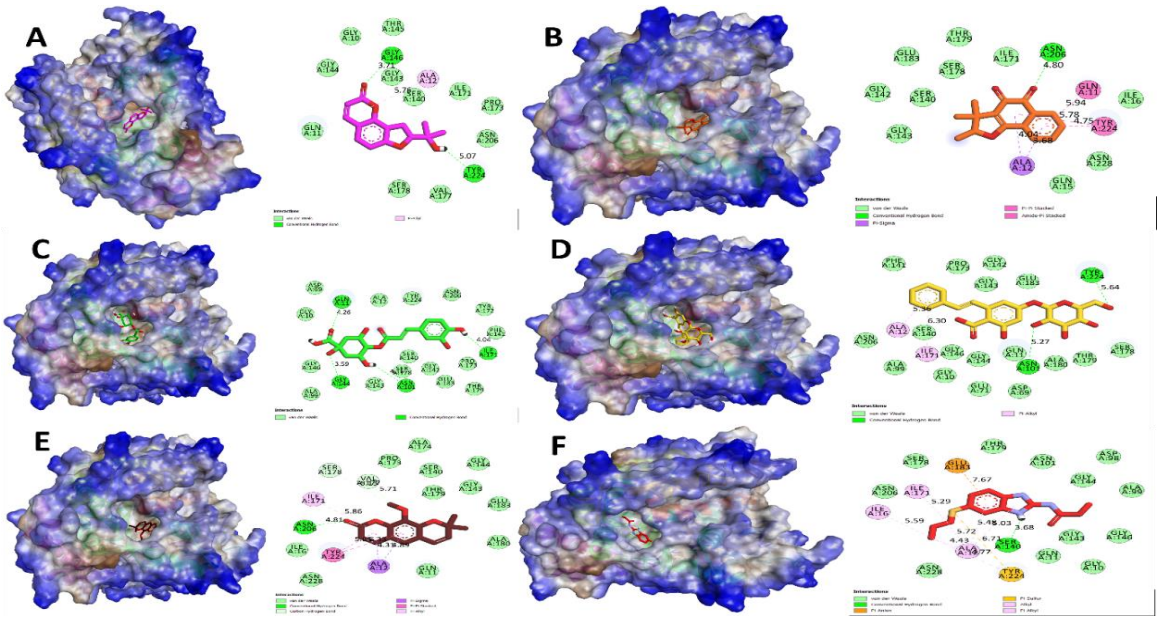

Figure 6. Molecular overlay and 2D representation of the top five docking scores retrieved from EERQ with Tubulin-Colchicine: Stathmin-Like Domain Complex (1SA0) by Autodock Vina. (A) Columbianetin, (B) Dunnione, (C) Cryptochlorogenic acid, (D) Gaylussacin, (E) Luvangetin, (F) Albendazole.

3.5. 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.

During drug development, evaluating the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile is essential [19]. This assessment helps to identify potential issues, such as toxicity, that may lead to drug withdrawal from the market. By analyzing these characteristics, researchers can determine whether a compound is likely to be absorbed, distributed, metabolized, and excreted and whether it exhibits any harmful effects.

In recent years, using *in silico* methods for determining ADMET profiles has gained popularity as a faster, cheaper, and potentially life-saving alternative to traditional *Invitro* methods [20]. *In silico* methods use computational models and algorithms to predict a compound's ADMET properties based on its chemical structure and other relevant factors, bypassing the need for animal models and reducing the time and expense associated with traditional methods [21].

Table 8: ADMET analysis of phytoconstituents eluted from HR LC-MS of RQEE.

	Swiss ADME							**ADMETSAR**							**PROTOX-II**				
Phytocompounds	**log P o/w**	**Water Solubility**	**GI Absorption**	**Lipinski Rule**	**Veber's Rule**	**PAINS Alert**	**TPSA**	**Lead Likeliness**	**HIA**	**CaCO2**	**BBB**	**CYP1A2**	**CYP2C19**	**CYP2C9**	**CYP2D6**	**LD50 (mg/kg)**	**Hepatotoxicity**	**Carcinogenicity**	**Immunotoxicity**	**Mutagenicity**	**Cytotoxicity**
Columbianetin	2.37	Soluble	High	Yes	Yes	0	59.67	No	0.9881	0.7636	0.8415	0.6717	0.7313	0.7486	0.8270	832 (Class4)	Inactive	Inactive	Inactive	active	Inactive
Dunnione	2.43	Soluble	High	Yes	Yes	0	43.37	No	1	0.6846	0.9348	0.8528	0.5949	0.7947	0.8905	8000 (Class 6)	Inactive	active	Inactive	Inactive	Inactive
Cryptochlorogenic acid	-0.64	Soluble	Low	Yes	No	0	164.75	No	0.7433	0.8005	0.5663	0.9045	0.9069	0.9071	0.8976	5000 (Class 5)	Inactive	Inactive	active	Inactive	Inactive
Gayllussacin	1.55	Moderately Soluble	Low	Yes	Yes	0	156.91	No	0.5131	0.8659	0.5977	0.9266	0.8903	0.8632	0.9150	2190 (Class 5)	Inactive	Inactive	Inactive	Inactive	Inactive
Luvangetin	2.79	Soluble	High	Yes	Yes	0	48.67	Yes	0.9848	0.7896	0.8333	0.5685	0.6453	0.9240	0.8756	1100 (Class 4)	Inactive	active	active	active	Inactive

In addition to being non-toxic, ideal drug candidates should exhibit acceptable ADME characteristics. Based on SwissADME, ProTox-ii, and admetSAR, we examined the ADME profiles of the identified molecules, including drug-likeness, partition coefficients, solubility, HIA, BBB, and cytochrome P450 inhibition (**Table 8**) [22].

One of the most important properties of ADMET is its ability to absorb drugs from the human gut [HIA]. The ability of drugs to be absorbed from the human gut, known as human intestinal absorption (HIA), is a crucial property of ADMET [23]. HIA plays a pivotal role in the transport of drugs to target cells [24]. A higher HIA improved intestinal absorption of the compound [20]. In addition to Gaylussacin, all compounds showed HIA values greater than 0.9, indicating good membrane permeation.

The blood-brain barrier (BBB) is a significant challenge in drug development for the central nervous system (CNS). The brain capillary endothelium forms the BBB and acts as a barrier restricting the entry of various cells and molecules into the CNS. The lack of pores on the cell surfaces of CNS vessels makes it extremely difficult to transport drugs to the brain [23]. This poses a significant challenge in the delivery of compounds to the CNS. Columbianetin, Dunnione, and Luvangetin displayed better BBB penetration, with values greater than 0.9.

A Pan-Assay Interference Structural (PAINS) alert was used to determine the toxicity of compounds with desirable physicochemical properties. This assay is also referred to 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 [25]. PAINS analysis provides information on the potential toxicity of a molecule. However, all the phytocompounds had 0 PAINS structural alerts, indicating their non-toxic nature (**Table 8**).

These compounds have been evaluated for their hepatotoxic, carcinogenic, and mutational potential [26]. The ProTox II results revealed that except for Dunnione and Luvangetin, the rest of the compounds were non-carcinogenic. They can also be used as drugs to treat helminthiasis. Because these compounds cannot accumulate in the body, they are less likely to cause cancer if treated long. Except for Cryptochlorogenic acid and luvangetin, the other compounds exhibited no immunotoxicity. No hepatotoxicity or cytotoxicity was observed for any of the compounds tested. ADMET studies often use these properties to analyze drug behavior [27].

4. Conclusion

Different extracts of *R. quinquangularis* have demonstrated anthelmintic effects against *Pheretima posthuma*, with the RQEE exhibiting comparable activity to the reference drug albendazole. RQEE resulted in the lowest paralysis and death rates in this model. HR LC-MS chemical analysis of RQEE identified various phytoconstituents, including Columbianetin, Dunnione, Cryptochlorogenic acid, Gaylussacin, Luvangetin, and Dunnione, which could be responsible for the observed anthelmintic activity. The isolation and testing of individual compounds are necessary to identify potential candidates for new anthelmintic drugs.

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None.

Conflict of interest

The authors declare to have no conflict of interest.

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