Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 8 Issue 4 November 2026

Research Article

Molluscicidal Efficacy of *Artemisia annua*: Influence of Plant Part and Extraction Method Against *Lymnaea acuminata* Snail

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DOI: 10.31080/ASVS.2026.08.1054

Received: October 10, 2026

Published: November 04, 2026

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Abstract

Background: *Lymnaea acuminata* is an important intermediate host of fascioliasis affecting humans and livestock, and controlling it is essential to interrupt disease transmission.

Objective: To evaluate the molluscicidal activity of crude aqueous, ethanolic, and column-purified extracts of different parts of *Artemisia annua* against *L. acuminata* under laboratory conditions.

Methods: Adult *L. acuminata* were exposed to different extract concentrations for 24–96 h, using six aquaria with 10 snails each per concentration. Mortality was recorded, and LC_{50} values were determined using probit analysis.

Results: Molluscicidal activity varied with plant part, extraction method, and exposure duration. The column-purified leaf extract showed the lowest 96-h LC_{50} (0.84 mg/L), followed by the artemisinin reference compound (3.37 mg/L), ethanolic leaf extract (7.14 mg/L), and ethanolic flower extract (9.49 mg/L). Among crude aqueous extracts, the leaf extract (417.19 mg/L) showed a lower LC_{50} than the flower extract (765.61 mg/L). No observable mortality occurred with the tested stem extract at any exposure period.

Conclusion: *Artemisia annua*, particularly its column-purified leaf extract, demonstrated molluscicidal potential against *L. acuminata*. Further studies are required to evaluate its environmental safety and practical applicability.

Subtitle: Molluscicides from the Plant *Artemisia annua* against the Harmful Snail *Lymnaea acuminata*.

Keywords: *Artemisia annua*; *Lymnaea acuminata*; Fascioliasis; Molluscicidal Activity; Plant-Derived Molluscicides; Dose-Response Relationship

Introduction

Freshwater gastropods play a crucial role in the life cycle of several parasitic organisms, particularly trematodes responsible for important zoonotic diseases [1,2]. Among them, *Lymnaea acuminata* is recognised as a major vector snail involved in the transmission of fascioliasis and other trematode infections affecting humans and livestock [3]. Consequently, regulating the population of such vector snails is considered one of the most practical methods for disrupting parasite transmission. Traditionally, snail control has relied heavily on synthetic molluscicidal chemicals.

Although these compounds are effective, their prolonged and repeated application has resulted in serious ecological issues, including contamination of aquatic environments, accumulation in biological systems, and harmful impacts on non-target organisms [4]. These drawbacks have encouraged researchers to search for safer and environmentally sustainable alternatives. Plant-based molluscicides have recently gained attention as promising substitutes for synthetic chemicals [5,6]. Plants produce a wide range of secondary metabolites such as alkaloids, flavonoids, terpenoids and phenolic compounds, many of which possess bioactive properties capable of affecting pest organisms [7,8]. Species belonging to the genus *Artemisia* are widely known for their medicinal value and diverse biological activities, including antimicrobial, insecticidal and antiparasitic effects [9,10]. *Artemisia annua* L. is a medicinal species of the genus *Artemisia* and is particularly recognised as a natural source of artemisinin, a bioactive sesquiterpene lactone recognised for its potent pharmacological properties [11]. Although *A. annua* is associated with artemisinin production, the presence and concentration of artemisinin in the extracts evaluated in the present study were not confirmed using a specific analytical method. Previous studies have investigated the molluscicidal potential of *Artemisia annua* against different molluscan species. Preet [12] evaluated leaf extracts against different age groups of the freshwater snail *Indoplanorbis exustus* and reported LC_{50} values of 8.14, 8.65, and 5.93 ppm for immature, young mature, and adult snails, respectively, with the carbon tetrachloride extract showing the highest toxicity. Amirmohammadi., *et al*. [13] also reported toxic effects of *A. annua* essential oil against the terrestrial slug *Agriolimax agrestis*. However, comparative information on the molluscicidal efficacy of different plant parts and extraction methods of *A. annua* against *Lymnaea acuminata* remains limited. Therefore, the present study was undertaken to evaluate and compare the molluscicidal activity of crude aqueous extracts and organic solvent extracts prepared from different parts, viz., leaf, flower and stem of *Artemisia annua* against *Lymnaea acuminata*. The investigation also aimed to determine which plant part and extraction method provide the most effective formulation for environmentally sustainable snail control strategies.

Materials and Methods

All the experiments mentioned below were carried out in the Malacology Laboratory, Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur.

- **Experimental Animals:** Adult *Lymnaea acuminata* (2.1–2.6 cm in shell length) were collected from nearby freshwater habitats in Gorakhpur. The specimens were identified using established taxonomic keys and maintained in laboratory aquaria before experimentation. The snails were acclimatised for at least 72 hours at room temperature in dechlorinated water under natural light conditions and were fed aquatic vegetation during this period. Only active and healthy snails were selected for the experiments. For each experimental set, 10 snails were maintained in an aquarium containing 3 L of water.
- **Plant Material**: Whole plants of *Artemisia annua* were obtained from a local nursery in Warangal, Telangana, and transported to the laboratory. The plant identity was confirmed by a taxonomist from the Department of Botany, Deen Dayal Upadhyaya Gorakhpur University. A voucher specimen (No. 118/25) was deposited in the herbarium of the Botany Department. The plants were subsequently propagated locally for further experiments. The plant material was thoroughly washed to remove adhering soil particles and other impurities. The leaves, stems, and flowers were separated and shade-dried at room temperature. The dried material was ground into a fine powder using an electric grinder and stored in airtight containers until extraction.
- **Preparation of Crude Extracts:** Aqueous crude extracts were prepared using the maceration extraction method by immersing a measured quantity of plant part powder in distilled water. The mixture was kept for approximately 24 hours with occasional agitation to facilitate the extraction of soluble constituents. After incubation, the mixture was filtered first through muslin cloth and subsequently through Whatman No. 1 filter paper to obtain a clear filtrate. This filtrate served as the stock extract, which was diluted with distilled water to prepare the required experimental concentrations.
- **Preparation of Organic Solvent Extracts:** Dried powder of each plant part was extracted with an organic solvent such as ethanol. Standard extraction procedures, such as maceration, were employed for 24 h to isolate the phytochemical constituents. The obtained extracts were concentrated under reduced pressure to remove excess

solvent and yield a semi-solid residue. The resulting extract was subsequently dispersed in water to prepare the required test concentrations for the molluscicidal bioassays.

- **Preparation of Column-Derived Fractions for Bioassay:** The crude extracts were subjected to column chromatography using silica gel (60–120 mesh) as the stationary phase, with 95% ethanol as the eluent. The extract was carefully loaded onto the 5 × 45 cm glass column. Eluted fractions were collected separately, monitored visually, and fractions with similar visual characteristics were pooled. The pooled column-derived material was dried, weighed, and used to prepare stock solutions for molluscicidal bioassays. Test concentrations were expressed as milligrams of dried column-derived material per litre (mg/L).
- **Reference Compounds:** A purified compound of artemisinin derived from floral parts was procured as a research sample from Hebei Kuisheng Trading Co., China, under academic collaboration and used as a reference compound for comparative evaluation in the present study. The purity of the supplied sample was not independently verified.

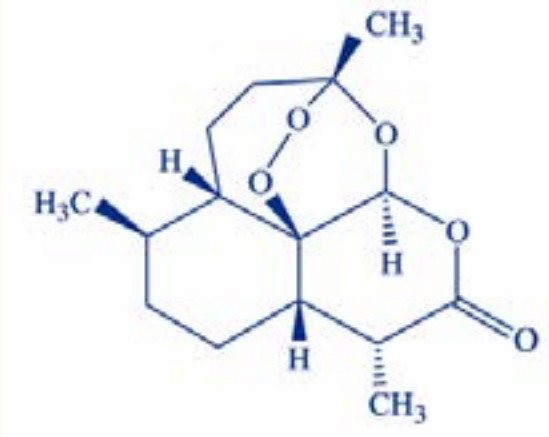


Figure a: Chemical Structure of artemisinin.

Thin layer chromatography

Thin-layer chromatography (TLC) was performed using the methods described by Singh and Singh [14] and Mohammed., *et al*. [15] to analyse the phytochemical constituents of *Artemisia annua* leaf and flower extracts. Pre-coated silica gel G_{60} F_{254} plates (20 × 20 cm) were used, and samples, along with column fractions, were spotted using a micropipette. The plates were developed in an n-hexane: ethyl acetate: acetic acid (2:1:0.1) solvent system, air-dried, and visualised using iodine vapours. The spots were recorded, and retention factor (Rf) values were calculated to compare and identify similar components in different fractions.

Molluscicidal bioassay

The molluscicidal potential of crude aqueous and ethanolic extracts was assessed using laboratory bioassay techniques as described by Singh and Agarwal [16]. Groups of 10 snails (shell length: 2.1–2.6 cm) were exposed to different concentrations of crude leaf extracts (300, 500, 700, 900 mg/L), ethanolic leaf extracts (5, 7, 9, 11 mg/L), crude flower extracts (600, 700, 800, 900 mg/L), and ethanolic flower extracts (7, 9, 11, 13 mg/L) in glass aquaria containing 3 L of dechlorinated water. Similar groups were exposed to various concentrations of column-purified leaf fractions (0.7, 0.9, 1, 3 mg/L), while the reference pure compound (flower-derived artemisinin) was tested at concentrations of 1, 3, 5, and 7 mg/L. Six aquaria were maintained for each concentration. Mortality was recorded at 24, 48, 72, and 96 h, and the average percentage mortality was calculated for each concentration (Table 1). The bioassays were conducted at room temperature under static exposure conditions for 96 h, without water renewal or feeding during the exposure period. A normal water control was maintained alongside the experimental treatments, and no mortality was observed in the control group. Niclosamide (0.30 mg/L) was used as the positive control, producing 50% mortality at 24 h and 100% mortality at 72 h. Water pH was not measured or recorded during the experiments.

Snails were considered dead when they showed no movement and did not respond to mechanical stimulation.

Statistical analysis

Mortality data were recorded separately for six replicates, with 10 snails per replicate. Mortality was expressed as a percentage, and replicate variability was assessed using the mean and standard deviation (SD). Median lethal concentrations (LC_{50}) and their 95% confidence limits were estimated by probit analysis using POLO software [17]. Percentage mortality data were transformed into probit values and analysed against the logarithm of the test

Extracts/Duration	24 h	48 h	72 h	96 h
Leaf (Crude)				
300	6.67%	13.33%	23.33%	46.67%
500	13.33%	21.67%	31.67%	58.33%
700	21.67%	33.33%	43.33%	65%
900	40%	50%	61.67%	91.67%
Leaf (Ethanolic)				
5	5%	10%	20%	31.67%
7	15%	23.33%	31.67%	45%
9	23.33%	31.67%	43.33%	60%
11	41.67%	51.67%	63.33%	80%
Leaf (column-purified)				
0.7	3.33%	10%	20%	41.67%
0.9	13.33%	21.67%	31.67%	50%
1	21.67%	31.67%	41.67%	61.67%
3	41.67%	51.67%	61.67%	83.33%
Flower (Crude)				
600	6.67%	16.67%	26.67%	41.67%
700	11.67%	21.67%	33.33%	48.33%
800	18.33%	28.33%	43.33%	56.67%
900	45%	55%	70%	83.33%
Flower (Ethanolic)				
7	5%	13.33%	23.33%	33.33%
9	15%	23.33%	35%	45%
11	25%	33.33%	43.33%	53.33%
13	38.33%	48.33%	61.67%	75%
Pure Compound				
1	5%	15%	23.33%	33.33%
3	10%	20%	30%	38.33%
5	25%	33.33%	41.67%	57%
7	46.67%	55%	65%	75%

Table 1: The average percentage mortality recorded at different concentrations (in mg/L) and exposure periods (h).

concentrations. A regression line was fitted to the transformed data to estimate LC_{50} values, and model goodness-of-fit was evaluated using the chi-square (χ^2) test. The slope of the regression line, standard error (SE), chi-square (χ^2), degrees of freedom (df), heterogeneity factor, and 95% confidence limits were determined for each treatment and exposure period (Table 2). The degrees of freedom were 22 for all treatments and exposure periods, as obtained from the POLO output.

Treatment	Exposure (h)	LC_{50} (mg/L)	95% Confidence limits (mg/L)	Slope ± SE	χ^2	Heterogeneity
Leaf (crude)	24	1232.58	959.56-2228.73	2.649 ± 0.609	7.460	0.339
	48	988.45	798.41-1565.03	2.329 ± 0.527	6.169	0.280
	72	749.99	622.54-1041.73	2.077 ± 0.485	6.352	0.289
	96	417.19	334.69-484.06	2.673 ± 0.490	16.877	0.767
Leaf (ethanol)	24	12.78	10.99-17.52	4.100 ± 0.857	6.915	0.314

	48	11.21	9.84-14.38	3.723 ± 0.754	8.514	0.387
	72	9.36	8.34-11.23	3.359 ± 0.688	9.451	0.430
	96	7.14	6.31-7.93	3.662 ± 0.675	17.106	0.778
Leaf (CP)	24	3.63	2.58-7.21	1.889 ± 0.374	11.162	0.507
	48	2.63	1.96-4.69	1.716 ± 0.353	8.708	0.396
	72	1.81	1.41-2.81	1.584 ± 0.346	6.532	0.297
	96	0.84	0.66-1.00	2.210 ± 0.411	10.915	0.496
Flower (crude)	24	956.12	898.40-1076.02	10.381 ± 1.950	16.521	0.739
	48	913.77	855.29-1033.44	7.773 ± 1.500	12.306	0.559
	72	853.76	798.95-962.56	6.239 ± 1.343	8.439	0.384
	96	765.61	714.22-833.16	5.580 ± 1.286	10.936	0.497
Flower (ethanol)	24	14.97	13.13-20.02	4.885 ± 1.070	6.267	0.285
	48	13.67	12.03-18.21	3.936 ± 0.919	4.772	0.217
	72	11.40	10.26-13.64	3.650 ± 0.856	4.759	0.216
	96	9.49	8.45-10.53	3.807 ± 0.843	7.218	0.328
Pure	24	9.51	7.09-16.97	1.992 ± 0.398	16.950	0.770
	48	8.34	5.80-17.91	1.318 ± 0.298	11.467	0.521
	72	5.46	3.94-9.65	1.184 ± 0.274	12.046	0.548
	96	3.37	2.31-5.01	1.097 ± 0.264	14.472	0.658

Table 2: LC_{50} (in mg/L) estimates and probit regression statistics for different *Artemisia annua* extracts and the reference compound against *Lymnaea acuminata* at different exposure periods (h).

Treatment	96-h LC_{50} (mg/L)	95% confidence limits (mg/L)	Relative potency
Column-purified leaf extract	0.84	0.656-1.000	1.00
Artemisinin (reference compound)	3.37	2.312-5.008	4.01
Ethanolic leaf extract	7.14	6.307-7.932	8.50
Ethanolic flower extract	9.49	8.447-10.528	11.30
Crude aqueous leaf extract	417.19	334.693-484.063	496.65
Crude aqueous flower extract	765.61	714.219-833.162	911.44

Table 3: Comparative relative potency of *Artemisia annua* extracts and the reference compound against *Lymnaea acuminata* based on 96-h LC_{50} estimates.

Relative potency was calculated as the ratio of the 96-h LC_{50} of each treatment to that of the column-purified leaf extract, used as the reference treatment (Table 3). A higher ratio indicates lower potency relative to the reference. The 95% confidence limits are reported for individual LC_{50} estimates; formal confidence limits for relative-potency ratios were not calculated.

Results

The molluscicidal activity of crude aqueous, ethanolic, and column-purified leaf preparations of *Artemisia annua*, together with the reference compound artemisinin, was evaluated against *Lymnaea acuminata* at 24, 48, 72, and 96 h of exposure. Mortality

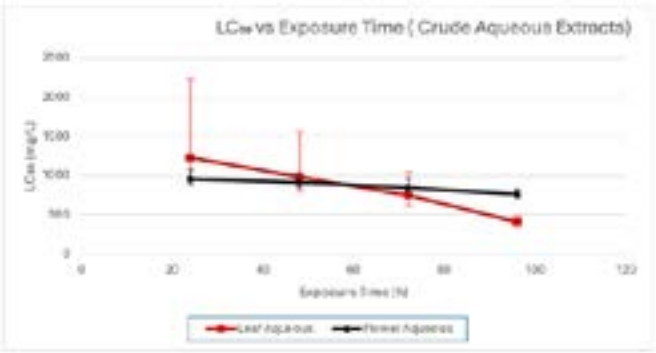


Figure 1: Comparative LC_{50} values of crude aqueous leaf and flower extracts of *Artemisia annua* against *Lymnaea acuminata* at different exposure durations (24–96 h). Vertical error bars represent the 95% confidence limits (lower and upper confidence limits) of the LC_{50} estimates.

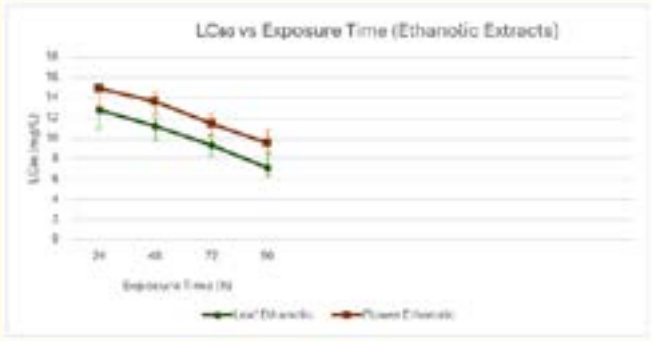


Figure 2: Effect of exposure time (24, 48, 72, and 96 h) on the LC_{50} values of ethanolic leaf and flower extracts of *Artemisia annua*, with 95% confidence-limit error bars.

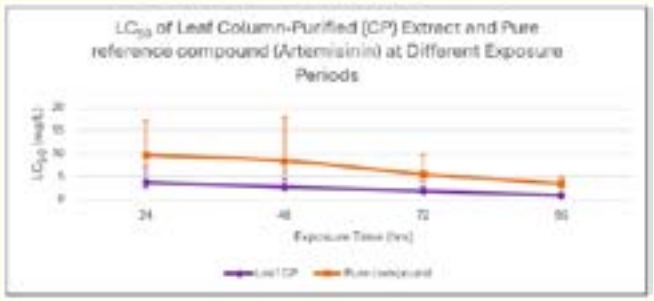


Figure 3: Comparison of LC_{50} values of leaf column-purified extract and reference pure compound (Artemisinin) at different exposure times, with 95% confidence-limit error bars.

generally increased with increasing concentration and exposure duration, although the response varied among treatments (Table 1).

The LC_{50} estimates and associated probit regression statistics for all treatments are presented in Table 2. For the crude leaf extract, the LC_{50} decreased from 1232.58 mg/L at 24 h to 417.19 mg/L at 96 h. The corresponding values for the crude flower extract decreased from 956.12 to 765.61 mg/L. For the ethanolic leaf extract, the LC_{50} decreased from 12.78 mg/L at 24 h to 7.14 mg/L at 96 h, while the ethanolic flower extract showed a decrease from 14.97 to 9.49 mg/L over the same exposure period. The column-purified leaf preparation showed LC_{50} values of 3.63, 2.63, 1.81, and 0.84 mg/L at 24, 48, 72, and 96 h, respectively. The reference artemisinin preparation showed corresponding LC_{50} values of 9.51, 8.34, 5.46, and 3.37 mg/L. The decreasing LC_{50} values across exposure periods indicate that lower concentrations were associated with the estimated median lethal response at longer exposure durations. Among the tested preparations, the column-purified leaf preparation had the lowest estimated LC_{50} at each exposure period. At 96 h, its LC_{50} was 0.84 mg/L, compared with 3.37 mg/L for the reference artemisinin preparation, 7.14 mg/L for the ethanolic leaf extract, and 9.49 mg/L for the ethanolic flower extract. The crude leaf and flower extracts had higher LC_{50} estimates of 417.19 and 765.61 mg/L, respectively. These comparisons describe the estimated LC_{50} values; they do not, by themselves, establish statistically significant differences between treatments.

The 95% confidence limits, slope estimates with standard errors, chi-square statistics, and heterogeneity values obtained from the probit analyses are reported in Table 2. The reported degrees of freedom for the probit analyses were 22. These statistics provide additional information on the estimated concentration–response relationships and the fit of the probit models. Thin-layer chromatography of the leaf and flower preparations showed a spot with an Rf value of approximately 0.27, comparable to the reported Rf value of the artemisinin reference under the chromatographic conditions used. This observation indicates a similarity in chromatographic migration; however, the Rf value alone does not conclusively confirm the identity of artemisinin in the tested preparations.

In contrast to the leaf and flower preparations, the tested stem extract did not produce observable mortality in *Lymnaea*

acuminata at any of the tested concentrations during the 24–96 h exposure period. Thus, no measurable molluscicidal activity was detected for the stem extract under the experimental conditions used.

Discussion

The results of the present study demonstrate that *Artemisia annua* possesses molluscicidal potential against *Lymnaea acuminata*. The higher activity of ethanolic and column-purified leaf extracts compared with crude aqueous extracts may be associated with differences in solvent extraction efficiency and the recovery of bioactive constituents. Solvent properties can influence the types and quantities of phytochemicals extracted from plant material [18-20]. The diverse secondary metabolites reported in *Artemisia* species, including terpenoids and flavonoids, may contribute to their biological activity [21,22]. However, the individual contribution of these compounds to the molluscicidal activity observed in the present study remains to be established. *Artemisia* species are known to contain artemisinin, a bioactive sesquiterpene lactone [11,15]. The TLC spot observed in the present study had an Rf value comparable to that reported for artemisinin; however, this similarity alone does not confirm its identity. Further chromatographic or spectrometric analysis would be required for confirmation. Although artemisinin and other phytochemicals may contribute to the observed activity, the present study did not establish whether the effects resulted from individual compounds or interactions among them. Similarly, mechanisms involving reactive oxygen species, cellular damage, or metabolic disruption have been reported or proposed in other biological contexts, but were not directly investigated in the present snail model [23]. These mechanisms should therefore be considered potential explanations rather than confirmed findings.

The greater activity of leaf-derived preparations compared with flower-derived preparations may reflect differences in the distribution or composition of bioactive constituents among plant parts. Variation in phytochemical profiles across plant tissues has been reported in studies of medicinal plants and botanical molluscicides [9,24,25]. Nevertheless, the hypothesis that leaves contain higher concentrations of the compounds responsible for molluscicidal activity requires direct phytochemical comparison. The increase in mortality with increasing concentration and exposure duration observed in this study is consistent with dose- and time-dependent responses reported for other botanical molluscicides [26-28]. Preet [12] reported LC_{50} values of 5.93–8.65 ppm for carbon tetrachloride leaf extract of *Artemisia annua* against different age groups of *Indoplanorbis exustus*. Although the present study also demonstrated molluscicidal activity of *A. annua* leaf extracts, direct comparison of LC_{50} values is limited by differences in snail species and extraction methods.

The absence of observable mortality following stem extract exposure may reflect differences in the abundance, composition, or availability of molluscicidal constituents compared with those in the leaf and flower preparations. However, this possibility requires phytochemical investigation and cannot be confirmed from mortality data alone.

Plant-derived molluscicides may provide potential alternatives to conventional snail-control agents. However, their botanical origin alone does not establish biodegradability, environmental safety, or selectivity towards target snails. These properties require specific assessment, including testing against non-target aquatic organisms and evaluation of environmental persistence. In addition, the relatively high LC_{50} values of the crude extracts and the practical costs associated with purification should be considered when assessing their potential application. Further work on chemical characterisation, non-target toxicity, production feasibility, and field performance is therefore necessary before *A. annua*-derived preparations can be recommended for practical snail control.

Conclusion

The present study demonstrates the molluscicidal potential of *Artemisia annua* against *Lymnaea acuminata*, with efficacy varying according to plant part, extraction method, and exposure duration. The column-purified leaf fraction exhibited the highest activity among the tested plant preparations, followed by the ethanolic leaf and flower extracts. TLC analysis provided preliminary evidence of a compound with an Rf value comparable to that reported for artemisinin; however, further chemical characterisation is required for confirmation. These findings suggest that *A. annua* may be a promising source of a plant-derived molluscicidal agent. Further studies addressing compound identification, non-target toxicity, environmental safety, and practical feasibility are necessary before its application in snail-control programmes can be recommended.

Competing Interests

The authors have no conflicts of interest to declare.

Authors' Contributions

PT: Conception and Design; VKS: Development of Methodology; PT: Acquisition of Data; PT and VKS: Analysis and Interpretation of Data; PT: Writing, review and revision of the manuscript; VKS: Study Supervision; Both authors are involved in drafting the manuscript, read and approved the final version of the manuscript.

Acknowledgements

The authors gratefully acknowledge Hebei Kuisheng Trading Co., Ltd., China, for providing a pure sample of artemisinin (reference compound) as research support material for this study. The authors sincerely appreciate their assistance in facilitating this work.

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