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Molluscicidal Efficacy of *Phyllanthus emblica*, *Catharanthus roseus* and *Pithecellobium dulce* Extracts against *Lymnaea acuminata*, the Intermediate Host of Fascioliasis

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ABSTRACT

Background and Objective: The freshwater snail *Lymnaea acuminata* is the obligate intermediate host of *Fasciola* species, which cause fascioliasis in livestock and humans. Although synthetic molluscicides are effective, they may pose environmental risks and harm non-target organisms. This study evaluated and compared the molluscicidal activity of different preparations of *Phyllanthus emblica*, *Catharanthus roseus* and *Pithecellobium dulce* against adult *L. acuminata* under laboratory conditions. **Materials and Methods:** Six preparations, including crude, ethanol, methanol, hexane and chloroform extracts, as well as column-purified fractions, were evaluated for their molluscicidal activity against adult *L. acuminata*. Mortality was assessed at different concentrations and exposure periods and LC_{50} values were determined. **Results:** All extracts exhibited concentration and time-dependent mortality. The column purified fractions of all three plants were the most potent, with 96 hrs LC_{50} values of 10.10 mg/L for *P. emblica*, 9.99 mg/L for *C. roseus* and 10.42 mg/L for *P. dulce*, respectively. Chloroform extracts showed moderate activity with 96 hrs LC_{50} values of 101.55, 99.88 and 109.41 mg/L, respectively. Crude and solvent extracts generally showed lower molluscicidal activity than the column-purified fractions, although their LC_{50} values varied considerably among plant species and preparations. The high potency of the column-purified fractions indicates the need for further phytochemical characterization and compound identification to determine the constituents responsible for the observed molluscicidal activity. **Conclusion:** These findings indicate that appropriate solvent selection followed by chromatographic purification can dramatically enhance molluscicidal efficacy, providing a basis for the development of standardized plant-based snail control agents for integrated fascioliasis management.

KEYWORDS

Fascioliasis, *Lymnaea acuminata, Phyllanthus emblica, Catharanthus roseus, Pithecellobium dulce, Fasciola gigantica*

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INTRODUCTION

Fascioliasis, a significant food-borne zoonotic disease, is caused by parasitic flukes specifically *Fasciola hepatica* and *Fasciola gigantica*. This disease affects millions of people and livestock worldwide, it is recognized as a major neglected tropical disease (NTD) and remains one of the most critical snail-borne parasitic infections in tropical and subtropical regions[1,2]. Fascioliasis control is also aligned with SDGs 3.3, which targets the elimination of neglected tropical diseases. Both humans and animals acquire the disease by ingesting metacercariae found on aquatic vegetation or in contaminated water. For livestock, fascioliasis leads to substantial economic losses, impacting growth, reducing milk and meat production, impairing reproduction, causing liver condemnation and increasing veterinary expenses. These losses amount to billions of dollars annually across the globe[1,3]. Fascioliasis transmission heavily relies on freshwater snails from the Lymnaeidae family, which serve as essential intermediate hosts. Inside these snails, miracidia develop through several stages before emerging as cercariae, eventually forming infective metacercariae[4]. This means the number, distribution and ecology of lymnaeid snails directly influence how prevalent and persistent fascioliasis is in endemic areas[3]. In the Indian subcontinent, *Lymnaea acuminata* is a primary intermediate host for *F. gigantica*, playing a key role in maintaining disease transmission in freshwater environments[5]. Controlling snail populations has long been considered a highly effective way to break the *Fasciola* life cycle and reduce disease transmission. Synthetic molluscicides are associated with environmental contamination, toxicity to non-target aquatic organisms, high operational costs and ecological disruption[6,7]. Growing environmental concerns about synthetic molluscicides have increased interest in plant-derived alternatives for snail control[8]. However, their efficacy and environmental safety need to be evaluated before practical application. Therefore, the present study aimed to compare the molluscicidal efficacy of crude extracts, solvent-specific extracts and column-purified fractions of *Phyllanthus emblica* (Indian gooseberry), *Catharanthus roseus* (Madagascar periwinkle) and *Pithecellobium dulce* (Manila tamarind) against *Lymnaea acuminata* under identical laboratory conditions.

MATERIALS AND METHODS

Study area and duration: The present study was carried out in the Malacology Laboratory, Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, Uttar Pradesh, India, during the period from January to March 2026. All experimental work, including specimen maintenance, treatment, observations and data collection, was conducted under laboratory conditions during the study period.

Plant material collection: Fresh leaves of *Phyllanthus emblica*, *Catharanthus roseus* and *Pithecellobium dulce* were collected from the Botanical Garden of Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur region of Uttar Pradesh, India (26.75°N, 83.38°E), during January-March 2026 at the time of experiment. The plants were authenticated at the Department of Botany. Representative photographs of the three plant species are presented in Fig. 1-3.

Preparation of aqueous extracts: The leaves were thoroughly washed with tap water to remove adhering dust and other surface contaminants and shade-dried for 2 hrs. After shade-drying, 100 mg of leaves from each plant species was separately ground with 100 mL of water using a mixer grinder to obtain a fine paste. The resulting preparations were filtered through Whatman No. 1 filter paper to obtain the crude aqueous filtrates. The homogenized material was subsequently transferred into a clean glass bottle. These filtrates were used immediately for toxicity experiment.

Organic-solvent preparations: The filtered aqueous plant preparation was used for preparing individual solvent-containing treatments. Briefly, 1 mL of the corresponding aqueous preparation was mixed separately with 4 mL of each organic solvent (ethanol, methanol, n-hexane and chloroform) to obtain a



Fig. 1: Leaves of *Phyllanthus emblica* (Indian gooseberry)
Image source: Thamizhpparithi Maari, Wikimedia Commons, CC BY-SA 3.0



Fig. 2: Leaves of *Catharanthus roseus* (Madagascar periwinkle)
Image source: Zishan, Wikimedia Commons, CC BY-SA 4.0



Fig. 3: Leaves of *Pithecellobium dulce* (Manila tamarind)

Image source: Forest & Kim Starr, Wikimedia Commons, CC BY 3.0

final volume of 5 mL for each preparation. The mixtures were handled separately and maintained for 24 hrs before being used in the toxicity experiments. The resulting preparations were used to obtain the required nominal test concentrations through appropriate dilution. The final concentrations were calculated based on the initial concentration of the aqueous preparation and the total volume of the resulting mixture.

Column chromatography: Selected preparations were subjected to column chromatography using silica gel (60-200 mesh) as the stationary phase. Ethanol was used as the elution solvent, following the general chromatographic approach described in previous work from our Malacology Laboratory Upadhyay *et al*.[9] with modifications for the present plant materials.

The eluate was collected in successive fractions of 5 mL. Each fraction was evaluated separately in a pilot experiment against *Lymnaea acuminata*. Fractions showing the highest activity were selected for subsequent toxicity experiments based on the observed mortality and concentration-response data.

Snail collection and acclimatization: Adult *Lymnaea acuminata* (2.7±0.3 cm in length) were collected from freshwater ponds and slow-moving streams around Gorakhpur. Snails were acclimatized for 72 hrs in glass aquaria containing dechlorinated tap water (temperature 25±2°C, pH 7.2±0.2, dissolved oxygen 6.5±0.5 mg/L). Fresh lettuce leaves were supplied in excess, allowing free feeding. Only healthy, actively moving snails exhibiting normal attachment to the glass surface were selected for the bioassays.

Acute toxicity bioassay: Toxicity bioassays were performed following the method described by Singh and Agarwal[10]. Groups of 10 adult *Lymnaea acuminata* were maintained in glass aquaria containing 3 L of dechlorinated tap water and exposed to different concentrations of the plant preparations for up to 96 hrs. The test concentrations used for each plant preparation are presented in Table 1 and were selected on the basis of preliminary toxicity experiments. Each concentration was tested in six replicates. A water-only control containing dechlorinated tap water was maintained under the experimental conditions. No mortality was recorded in the control group at any observation interval. Please acknowledge the absence of a positive-control molluscicide as a study limitation in the Discussion and explain how this may affect the interpretation of the comparative toxicity findings. Mortality was recorded at 24, 48, 72, and 96 hrs of exposure.

Table 1: Concentrations of different preparations of *Phyllanthus emblica, Catharanthus roseus* and *Pithecellobium dulce* used for toxicity determination against *Lymnaea acuminata*

Plant extract	Preparation	Concentration (mg/L)
Phyllanthus emblica	Crude aqueous extract	300, 500, 700, 900
	Ethanolic extract	300, 500, 700, 900
	Methanolic extract	300, 500, 700, 900
	Chloroform extract	70, 90, 120, 150
	n-Hexane extract	300, 500, 700, 900
	Column purified fraction	7, 9, 12, 15
Catharanthus roseus	Crude aqueous extract	150, 350, 550, 750
	Ethanolic extract	180, 200, 300, 400
	Methanolic extract	100, 200, 300, 400
	Chloroform extract	70, 90, 120, 150
	n-Hexane extract	90, 150, 250, 350
	Column purified fraction	7, 9, 12, 15
Pithecellobium dulce	Crude aqueous extract	300, 500, 700, 900
	Ethanolic extract	300, 500, 700, 900
	Methanolic extract	300, 500, 700, 900
	Chloroform extract	70, 90, 120, 150
	n-Hexane extract	100, 300, 500, 700
	Column purified fraction	7, 9, 12, 15

Statistical analysis: The LC_{50} values with 95% confidence limits were calculated by probit analysis[11]. Slope values±standard error, Chi-square (χ^2) with degrees of freedom and heterogeneity factors were recorded. LC_{50} values with 95% confidence limits were calculated using probit analysis. Slope values with standard errors, Chi-square (χ^2) values with degrees of freedom, and heterogeneity factors were recorded. Statistical significance was assessed at $p<0.05$. The Pearson product-moment correlation coefficient was calculated to examine the relationship between exposure time and LC_{50} values for *Lymnaea acuminata*[12].

RESULTS

Molluscicidal activity of plant extracts against *Lymnaea acuminata*: The molluscicidal activity of different preparations of *Phyllanthus emblica*, *Catharanthus roseus* and *Pithecellobium dulce* was evaluated against *Lymnaea acuminata* at different concentrations and exposure periods (Table 2-4). All preparations caused mortality in a concentration and time-dependent manner, with toxicity increasing as the duration of exposure increased. Among the tested preparations, the column-purified fractions consistently exhibited the greatest activity, as evidenced by their lower LC_{50} values throughout the experiment. In *P. emblica*, the column-purified fraction was the most active preparation, producing LC_{50} values of 11.34, 10.97, 10.51 and 10.10 mg/L after 24, 48, 72 and 96 hrs of exposure, respectively. The chloroform extract showed lower activity, with corresponding LC_{50} values of 121.54, 115.17, 111.53 and 101.55 mg/L. The crude extract (PELCE), ethanol, methanol and hexane extracts were comparatively less effective, with LC_{50} values decreasing from 741.30 to 632.80, 719.11 to 660.37 mg/L, 709.06 to 608.75 and 757.82 to 627.04 mg/L, respectively, over the 96 hrs exposure period.

A similar pattern was observed for *C. roseus*. The column-purified fraction displayed the highest toxicity, with LC_{50} values declining from 12.69 mg/L at 24 hrs to 9.99 mg/L at 96 hrs. The chloroform extract also showed appreciable activity, with LC_{50} values decreasing from 129.40 to 99.88 mg/L during the same

Table 2: LC_{50} values (mg/L) of *Phyllanthus emblica* extracts against *Lymnaea acuminata* at different exposure times

Exposure time	Material tested	LC_{50}	LCL	UCL	Slope value	t-value	Chi-square (df)	Heterogeneity
24 hrs	PELCE	741.30	674.51	825.77	4.52±0.65	7.17	15.52	0.70
	Ethanol extract	719.11	658.40	800.29	4.83±0.64	7.50	17.56	0.79
	Methanol extract	709.06	647.08	791.91	4.57±0.62	7.37	11.96	0.54
	Hexane extract	757.82	686.19	863.44	4.33±0.62	6.95	14.86	0.67
	Chloroform extract	121.54	113.43	173.22	6.00±0.79	7.54	8.21	0.37
	Column purified fraction	11.34	10.60	12.21	6.08±0.77	7.82	9.12	0.41
48 hrs	PELCE	721.69	647.10	799.81	4.38±0.60	7.27	13.31	0.60
	Ethanol extract	703.01	637.50	792.67	4.21±0.58	7.21	17.11	0.77
	Methanol extract	667.94	610.55	739.22	4.64±0.60	7.68	10.78	0.85
	Hexane extract	708.02	639.29	804.39	4.01±0.57	7.99	9.52	0.43
	Chloroform extract	115.17	108.07	123.71	6.50±0.80	8.11	13.54	0.61
	Column purified fraction	10.97	10.28	11.75	6.32±0.78	8.08	10.09	0.45
72 hrs	PELCE	659.15	608.90	730.37	4.52±0.58	7.69	14.00	0.63
	Ethanol extract	699.49	640.99	775.41	4.82±0.63	7.60	15.73	0.71
	Methanol extract	645.35	589.44	713.41	4.53±0.58	7.74	18.79	0.85
	Hexane extract	662.78	597.10	749.69	3.82±0.54	6.99	12.57	0.57
	Chloroform extract	111.53	104.86	129.06	6.89±0.38	8.48	11.64	0.52
	Column purified fraction	10.51	9.86	11.23	6.46±0.78	8.26	11.12	0.50
96 hrs	PELCE	632.80	575.17	703.04	4.25±0.56	7.58	15.52	0.70
	Ethanol extract	660.37	606.34	726.14	4.90±0.61	7.94	21.28	0.96
	Methanol extract	608.75	557.24	667.73	4.71±0.58	8.10	20.53	0.93
	Hexane extract	627.04	564.43	705.27	3.78±0.53	7.08	16.60	0.75
	Chloroform extract	101.55	95.05	108.39	6.40±0.18	8.18	11.04	0.53
	Column purified fraction	10.10	9.46	10.78	6.47±0.78	8.24	11.76	0.53

Batches of ten snails were exposed to four different concentrations of the respective active components. Mortality was determined every 24 hrs. Each set of experiments was replicated six times. Concentrations given are the final concentrations (w/v) in the aquarium water. The product momentum correlation coefficient showed that there was a significant negative correlation between exposure time and different values of LC_{50}. PELCE: *Phyllanthus emblica* leaf crude extract, LC_{50}: Median lethal concentration, LCL: Lower confidence limit, UCL: Upper confidence limit and df: Degrees of freedom

Table 3: LC_{50} values (mg/L) of *Catharanthus roseus* extracts against *Lymnaea acuminata*

Exposure time	Material tested	LC_{50}	LCL	UCL	Slope value	t-value	Chi-square (df)	Heterogeneity
24 hrs	CLCE	629.26	523.94	823.21	2.29±0.38	5.94	8.59	0.39
	Ethanol extract	332.98	301.69	382.47	4.32±0.65	6.56	10.18	0.46
	Methanol extract	334.20	290.68	405.34	3.05±0.47	6.46	9.06	0.41
	Hexane extract	276.14	238.89	315.93	3.49±0.47	7.34	12.26	0.55
	Chloroform extract	129.40	118.51	146.49	4.77±0.75	6.32	7.69	0.35
	Column purified fraction	12.69	11.75	14.06	5.44±0.78	6.94	10.18	0.46
48 hrs	CLCE	530.21	459.47	636.03	2.84±0.40	7.06	16.06	0.73
	Ethanol extract	313.49	285.89	353.31	4.44±0.65	6.81	9.35	0.42
	Methanol extract	308.58	275.35	354.78	3.69±0.51	7.14	9.48	0.43
	Hexane extract	252.08	225.24	288.22	3.77±0.48	7.83	16.21	0.73
	Chloroform extract	121.57	112.18	134.73	5.00±0.74	6.69	13.07	0.59
	Column purified fraction	11.42	10.66	11.77	5.81±0.76	7.56	9.74	0.44
72 hrs	CLCE	480.53	422.76	552.51	3.24±0.41	7.77	20.64	0.93
	Ethanol extract	285.84	260.90	318.54	4.29±0.63	6.72	7.72	0.35
	Methanol extract	271.95	239.13	315.25	3.11±0.43	7.10	17.82	0.81
	Hexane extract	223.36	199.56	252.92	3.66±0.46	7.96	13.72	0.62
	Chloroform extract	114.38	105.91	125.11	5.14±0.74	6.93	10.08	0.45
	Column purified fraction	10.89	10.14	11.77	5.59±0.75	7.43	7.35	0.33
96 hrs	CLCE	453.48	401.38	514.19	3.48±0.43	8.09	21.34	0.99
	Ethanol extract	256.51	234.58	281.08	4.51±0.64	6.97	10.34	0.47
	Methanol extract	255.00	229.18	285.03	3.94±0.48	8.08	19.75	0.89
	Hexane extract	191.46	170.50	215.18	3.61±0.44	8.19	19.39	0.88
	Chloroform extract	99.88	93.29	106.72	6.23±0.77	8.03	14.59	0.66
	Column purified fraction	9.99	9.28	10.72	5.77±0.75	7.66	12.57	0.57

CLCE: *Catharanthus roseus* leaf crude extract, LC_{50}: Median lethal concentration, LCL: Lower confidence limit, UCL: Upper confidence limit and df: Degrees of freedom

Table 4: LC_{50} values (mg/L) of *Pithecellobium dulce* extracts against *Lymnaea acuminata*

Exposure time	Material tested	LC_{50}	LCL	UCL	Slope value	t-value	Chi-square (df)	Heterogeneity
24 hrs	PDLCE	745.19	663.98	872.18	3.58±0.56	6.39	10.67	0.48
	Ethanol extract	843.13	749.64	1005.72	3.96±0.62	6.31	14.74	0.67
	Methanol extract	724.05	660.25	811.45	4.55±0.62	7.30	14.61	0.66
	Hexane extract	607.21	484.09	857.69	1.87±0.32	5.73	10.17	0.46
	Chloroform extract	128.29	120.40	138.80	7.01±0.89	7.85	15.88	0.77
	Column purified fraction	12.36	11.45	13.62	5.38±0.77	6.96	11.40	0.51
48 hrs	PDLCE	709.61	629.27	828.44	3.36±0.53	6.28	11.82	0.53
	Ethanol extract	775.58	695.25	901.85	3.94±0.59	6.63	14.37	0.65
	Methanol extract	684.38	624.27	762.25	4.49±0.60	7.49	11.91	0.54
	Hexane extract	523.42	422.97	700.68	1.89±0.31	6.04	11.94	0.54
	Chloroform extract	124.71	116.95	134.87	6.72±0.85	7.90	13.41	0.61
	Column purified fraction	11.97	11.03	13.25	4.96±0.72	6.61	10.62	0.48
72 hrs	PDLCE	644.06	578.56	728.84	3.69±0.53	6.90	12.10	0.55
	Ethanol extract	722.27	648.61	827.28	3.80±0.54	6.92	15.57	0.70
	Methanol extract	632.99	576.12	701.85	4.32±0.56	7.62	14.16	0.64
	Hexane extract	432.82	354.58	547.45	1.97±0.30	6.46	10.18	0.46
	Chloroform extract	118.50	110.83	128.21	6.16±0.79	7.77	13.41	0.61
	Column purified fraction	11.11	10.36	12.09	5.73±0.75	7.55	10.79	0.49
96 hrs	PDLCE	584.51	530.26	645.76	4.22±0.54	7.70	15.31	0.69
	Ethanol extract	631.48	569.65	708.68	3.87±0.54	7.16	18.13	0.82
	Methanol extract	562.84	511.65	689.31	4.35±0.58	7.90	19.73	0.89
	Hexane extract	352.62	288.06	433.21	2.00±0.29	6.79	12.34	0.56
	Chloroform extract	109.41	102.47	117.41	6.24±0.77	8.03	17.52	0.79
	Column purified fraction	10.42	9.71	11.19	5.86±0.75	7.74	16.17	0.73

PDLCE: *Pithecellobium dulce* leaf crude extract, LC_{50}: Median lethal concentration, LCL: Lower confidence limit, UCL: Upper confidence limit and df: Degrees of freedom

period. The crude extract (CLCE), ethanol, methanol and hexane extracts exhibited relatively lower toxicity, with LC_{50} values decreasing from 629.26 to 453.48, 332.98 to 256.51, 334.20 to 255.00 and 276.14 to 191.46 mg/L, respectively. The preparations of *P. dulce* followed a similar pattern. The column-purified

fraction proved to be the most potent, with LC_{50} values of 12.36, 11.97, 11.11 and 10.42 mg/L at 24, 48, 72 and 96 hrs, respectively. The chloroform extract ranked next in efficacy, with LC_{50} values ranging from 128.29 mg/L at 24 hrs to 109.41 mg/L at 96 hrs. The crude extract (PDLCE), ethanol, methanol and hexane extracts showed comparatively weaker molluscicidal activity, with LC_{50} values declining from 745.19 to 584.51, 843.13 to 631.48, 724.05 to 562.84 and 607.21 to 352.62 mg/L, respectively. Across all three plant species, LC_{50} values declined progressively with increasing exposure time, indicating that prolonged exposure enhanced the molluscicidal effect of each preparation. The column-purified fractions consistently exhibited the highest toxicity, followed by the chloroform extracts, whereas the crude, ethanol, methanol and hexane extracts were comparatively less effective against *L. acuminata*.

Values are LC_{50} (mg/L) with 95% confidence limits (LCL-UCL) obtained by probit analysis. The degree of freedom (df) was 22 for all treatments and exposure periods. Heterogeneity values were less than 1.0, indicating a satisfactory fit of the probit regression model. The g value was less than 0.5 at all probability levels. Lower LC_{50} values indicate higher molluscicidal activity against *Lymnaea acuminata*. Significant negative regression ($p<0.05$) between exposure time and LC_{50}.

DISCUSSION

The results demonstrate that extracts and column-purified fractions of *Phyllanthus emblica*, *Catharanthus roseus* and *Pithecellobium dulce* possess molluscicidal activity against *Lymnaea acuminata*. Mortality increased with both concentration and time-dependent toxic response. Among all tested preparations, the column-purified fractions of the three plants exhibited the highest toxicity throughout the exposure period. After 96 hrs of exposure, the LC_{50} values of the purified fractions were 10.10 mg/L for *P. emblica*, 9.99 mg/L for *C. roseus* and 10.42 mg/L for *P. dulce*, indicating nearly comparable molluscicidal potency among the three species. In contrast, crude extracts and polar solvent extracts showed substantially higher LC_{50} values, demonstrating lower toxicity. The chloroform extracts also showed moderate activity ($LC_{50} \approx$ 100-110 mg/L). The column-purified fractions of all three plant species produced LC_{50} values close to 10 mg/L after 96 hrs of exposure, while chloroform extracts showed LC_{50} values near 100 mg/L. These fractions were substantially more potent than the crude and solvent extracts. The toxicity of the purified fraction is below 100 ppm. It indicates the column-purified fraction fulfills the criteria of a potent mollouscicide[8]. These values indicate that the purified fractions possess excellent molluscicidal potential compared to the crude extracts. This increased activity may be due to the concentration of bioactive compounds achieved during the chromatographic separation. The study highlighted this enhancement, inviting further exploration into the specific mechanisms that contribute to it. The increased activity of the column-purified fractions suggests that chromatographic separation may have enriched the bioactive constituents responsible for the observed molluscicidal activity. It has been suggested that non-polar alkaloids, terpenoids and/or saponins may contribute to the molluscicidal activity of such plants. Previous studies have demonstrated that purification of organic-solvent extracts can enhance molluscicidal potency compared with crude extracts, potentially due to the enrichment of bioactive constituents. Studies from our Malacology Laboratory have reported similar findings. Singh *et al.*[13] investigated the molluscicidal activities of *Cannabis sativa*, *Acacia nilotica* and *Tinospora cordifolia* and reported 96 hrs LC_{50} values of 16.08, 16.32 and 24.78 mg/L for the acetone extract of *T. cordifolia* stems, ethanol extract of *C. sativa* leaves and ethanol extract of *A. nilotica* leaves, respectively. The study also identified β-caryophyllene, gallic acid and berberine as potential active molluscicidal components. Further, Singh and Singh[14] investigated the molluscicidal activity of *Aloe vera* and *Chrysanthemum cinerariifolium*, including their active ingredients, against *L. acuminata*. These findings support the potential of extract purification to enrich bioactive constituents associated with molluscicidal activity[13,14]. The probit analysis parameters further confirm the reliability of the toxicity data. The observed slope values indicated well-defined dose-response relationships, while heterogeneity values below 1.0 demonstrated satisfactory model fitting and homogeneity of experimental populations. Moreover, the narrow confidence limits associated with the LC_{50} estimates indicate a high degree of precision in the toxicity estimates.

The index of significance for potency estimation (g-value), calculated using probit analysis, was below 0.5 at all three probability levels (90, 95 and 99%). According to the criteria described by Finney[15], g-values below 0.5 indicate that the corresponding confidence limits can be reliably calculated. Thus, the estimated potency values were associated with acceptable statistical precision, and the respective confidence limits were determined at all three probability levels. These statistical characteristics strengthen confidence in the observed differences among extracts and support the validity of the conclusions drawn from the study.

CONCLUSION

The column purified fractions of *Phyllanthus emblica*, *Catharanthus roseus* and *Pithecellobium dulce* exhibit potent, time-dependent molluscicidal activity against *Lymnaea acuminata*. From an applied perspective, effective control of *Lymnaea acuminata* populations can significantly reduce transmission of fascioliasis by interrupting the life cycle of *Fasciola gigantica*. This laboratory study indicates that solvent selection and column purification can improve the molluscicidal activity of plant preparations against *Lymnaea acuminata*. Further research is needed to identify the active compounds, assess their effects on non-target organisms, and evaluate their efficacy and environmental safety under field conditions before their potential use in fascioliasis control programs can be established. Further studies are needed to explore the mechanism of action of isolated individual active compounds of the same plants.

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