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***Research Article** (Open Access)*

ABUTILON INDICUM LEAF POWDER AND ITS ORGANIC EXTRACTS ARE USED AS TOXICANTS AGAINST THE HARMFUL SNAIL *LYMNAEA ACUMINATA*

Durg Vijay Ram [1] and *Pradeep Kumar[2]
[1]Department of Zoology, S.G.N. Government P.G. College,
Muhammadabad Gohana, Mau U.P. 276403
[2]Department of Zoology, Government Degree College
Sukrauli, Kushinagar U.P.274207
**Author for correspondence: pkumar_gpu@yahoo.co.in*

ABSTRACT
The serious zoonotic parasite disease fascioliasis affects both humans and herbivorous animals. This waterborne disease is caused by *Fasciola hepatica* and *F. gigantica*. Snail *Lymnaea acuminata* is an intermediate host of this fluke. One an important strategy for lowering liver fluke infections is to keep snail populations below threshold levels. The goal of the present study is to determine how *Abutilon indicum* dried leaf powder and various organic extracts work as molluscicides against the host snail *L. acuminata*. The toxic studies of leaf powder of *A. indicum*, their acetone extract, chloroform extract, ether extract, and column extract purified were exposed at different concentrations against *L. acuminata.* They continuously observed from 24h up to 96h. Mortality was observed for 24, 48, 72, and 96h exposure period. Six aquariums were setup for each concentration. The control group animals were kept in an equal volume of water under similar conditions without treatment. The dried leaf powder of *A. indicum* at 24h and 96h LC_{50} against *L. acuminata* was 18.32, and 180.38 mg/l, respectively. Among different formulations of molluscicides the column extract purified was more toxic than other extract. The column extract purified of *A. indicum* was more toxic (24h and 96 h, LC_{50}-160.30 and 151.63 mg/l, respectively) against *L. acuminata.* The results of this investigation showed that the different powdered *A. indicum* leaf preparations have strong molluscicidal effects, and their phytochemicals could be powerful molluscicide sources.

***Keywords*:** *Abutilon indicum, Lymnaea acuminata, Phytochemicals, Fascioliasis, Molluscicides*

INTRODUCTION
Fascioliasis is recognized as a significant parasitic zoonotic disease and affects approximately 2.4 to 7 million human populations worldwide (Loan *et al.,* 2025). The fluke is common among cattle, sheep, goat buffalo, and other vertebrates which have significant importance on the developments, growth rate, and productivity of animals that have greater economic loss (Kuchai *et al.,* 2011; Mas-Coma *et al.,* 2014; Eshetu *et al.,* 2017; Kumar, 2020). Liver fluke was primarily associated with lowland paddy field regions, while highland and coastal areas exhibited lower prevalence rates (Loan *et al.,* 2025). *Fasciola* infected livers showed several pathological lesions, including bile duct hyperplasia, dilation of lymphatic vessels in portal regions with extensive fibrosis, necrosis of the liver parenchyma, and glission's capsule thickening with fibrosis (Hassan *et al.,* 2025). It is caused by the two trematode species *Fasciola*

hepatica and *F. gigantica* (Singh and Agarwal, 1981; Mas-Coma *et al.*, 2005; Kumar and Singh, 2006; Kumar *et al.*, 2016) for transmission, both species of *Fasciola* require an intermediary host, which is a *Lymnaeidae* snail (Kumar, 2020) worldwide, there are about twenty species of *Lymnaeidae* snails that serve as intermediate host of *Fasciola* species. The freshwater host snail species *Lymnaea acuminata* is widely distributed in the eastern region of Uttar Pradesh, India (Singh and Agarwal, 1981; Kumar and Singh, 2006; Jaiswal and Singh, 2008; Kumar *et al.*, 2009) and secondary host are *Fasciola hepatica* and *F. gigantica* (Vishwakarma and Kumar, 2021). The best strategy to control fascioliasis is to manage the population of the vector snail (Kumar and Singh, 2006). Snails can be controlled by using synthetic molluscicides (Singh *et al.*, 2020), but they may be harmful for the environment and non-target aquatic organisms. Natural products or phyto-products are more effective against harmful snails, (Agarwal and Singh, 1988) and are biodegradable, easily available, and eco-friendly.

Since plant products are less expensive, more widely accepted, safer, and perhaps biodegradable than different synthetic molluscicides, they are utilized as natural molluscicides (Harston and Hostettmann, 1985; Kumar and Singh, 2006; Kumar *et al.*, 2013). The phytochemical components of the medicinal plant *Abutilon indicum* (also known as Atibala in Hindi) are galactose, fructose, leucine, histidine, gossypetin-8, 7-glucosides, cyaniding-3 rutinoside, p-hydroxy benzoic acid, gossypetin-8-glucoside, and caffeic acid. The plants are found in tropical and subtropical regions of India (Roshan and Shekshavali, 2016). The aqueous extracts of *A. indicum* leaf have potent properties to reduce blood glucose levels in rats (Seetharam *et al.*, 2002). However, β-sitosterol compounds are isolated from the petroleum ether extract of *A. indicum* which showed mosquito larvicidal activity (Rahuman *et al.*, 2008). The medicinal plant *A. indicum* is widely used to treat pharmacological illnesses and maladies because of its hepatoprotective, anticancer, antioxidative, antidiabetic, antifungal, antibacterial, larvicidal, hypoglycemia, and wound-healing qualities (Sharma *et al.*, 2013). The goal of this study is to assess the molluscicidal activity of column purified extract, various organic extracts, and dried leaf powder of *A. indicum* against the vector snail *L. acuminata*.

MATERIALS AND METHODS

Collection of host snails

Adult *L. acuminata* (2.0±0.3 cm in length) were collected from ponds and low-lying submerged fields in Muhammadabad Gohana, Mau (U.P.) India. The snails were acclimatized for 72 hours in dechlorinated tap water at 25±3^{0}C. The dissolved oxygen, free carbon dioxide, bicarbonate alkalinity, and pH of water were 6.3-7.2 mg/l, 5.2-6.2 mg/l, 104.0-106.0 mg/l, and 7.3-7.2, respectively.

Preparation of crude leaf powder

The dried *Abutilon indicum* leaves were pulverized in the electric grinder and crude powders thus obtained, were then sieved with the help of fine mesh cloth. This fine leaf powder was then used separately for toxicity/molluscicidal experiments against the host snail *L. acuminata*.

Preparation of crude extracts

Five-gram dried leaf powder of *A. indicum* was extracted with 100 ml of 98% acetone, 99.7% chloroform, and 98% ether at room temperature for 24h. Each extract preparation was filtered separately through sterilized Whatman No-1 filter paper (Jaiswal and Singh, 2008), and the filtered extracts were subsequently evaporated under a vacuum. The residues, thus obtained,

were used for the determination of toxicity (LC_{50}) value. The leaf powder of *A. indicum* yielded 230 mg acetone, 235 mg chloroform, and 233 mg ether extracts.

Preparation of column extract purified (CEP)

One hundred milliliters of ethanol solvent was mixed with a 50gram extract fraction of dried leaf powder of *A .indicum* were subjected to silica gel (60-120 mesh, Qualigens Glass, Precious Electrochemidus Private Limited, Bombay, India) chromatography through a 5×45 cm column purifier. Ten-milliliter fractions eluted with ethanol (95%) solvent were collected. The organic solvent was evaporated under a vacuum machine and the remaining solids extract obtained was used for the determination of the toxicity activity of each fraction against snails.

Toxicity experiment

The toxicity experiment of different organic extracts and column purified extract of *A. indicum* was performed by the method of Kumar and Singh, (2006). Ten experimental animals were kept in a glass aquarium containing 3 liters of dechlorinated tap water. Snails were exposed continuously for 96h to different concentrations and preparation of *A. indicum* and mortality was observed for 24, 48, 72, and 96h. Six aquariums were setup for each concentration. The control groups of animals were kept in an equal volume of water under similar conditions without treatment. The mortality of snails was recorded at intervals of 24h each up to 96h. Mortality of snails was established by the contraction of the snail body within the shell, no response to the needle probe was taken as evidence of snail death. The mortality data were observed every 24h up to 96h.

Experimental analysis

Lethal concentration (LC_{50}) values lower and upper confidence limits (LCL and UCL), slope values, t- ratio, 'g' value and heterogeneity factor were calculated using POLO computer program (Robertson *et al.,* 2007).

RESULTS

The lethal concentration value of dried leaf powder of *Abutilon indicum* and their different organic extract against host snail *L. acuminata* were concentration and time-dependent. The LC_{50} value of dried leaf powder of *A. indicum* at 24h was 186.32 mg/l and at 96h 180.38 mg/l (Table-1). Among all the organic solvent extract fractions, the ether extract of dried leaf powder of *A. indicum* was more toxic (Table-1) (Fig-1). The 24h and 96h LC_{50} of ether extract of dried leaf powder of *A. indicum* against *L. acuminata* were 175.32 and 168.24 mg/l, respectively. The ethanolic column extract purified of all the extract fractions was highly toxic. The LC_{50} values of the column extract purified of dried leaf powder of *A. indicum* at 24h were 160.30 mg/l, whereas at 96h exposure, the LC_{50} values were 151.63 mg/l (Table-1) (Fig-1).

The slope values given in Table-1 were steep and the separate estimates of lethal concentration based on each of the six replicates were found to be within the 95% confidence limits of LC_{50}. The t- ratio was greater than 1.96 and the heterogeneity factor was less than 1.0. The g-value was less than 0.5 at all probability levels (90, 95, and 99) (Table-1).

DISCUSSION

This study shows that the dried leaf powder of *A. indicum* is a powerful molluscicide that can control harmful snails. The section on the results shows that the active phytochemical components of the leaf of *A. indicum* are readily soluble in water and progressively permeate the bodily fluids of the host snail, *Lymnaea acuminata*, causing harmful consequences. The results

Table-1. Toxicity of *A. indicum* leaf powder and different organic solvent extracts against the snail *L. acuminata* at different exposure periods.

Exposure Periods	Values	Preparations of molluscicides (mg/l) LP	AC-Ex	CH-Ex	ET-Ex	CPE
	LC_{50}	186.32	179.62	180.69	175.32	160.30
	LCL	166.31	161.25	170.84	165.32	151.80
	UCL	195.48	190.36	189.69	196.42	172.96
24h	**S-v**	2.23±0.14	1.15±0.40	1.20±0.21	1.62±0.60	2.04±0.14
	t-ratio	2.40	3.30	3.44	3.01	2.71
	g-value	0.31	0.33	0.24	0.21	0.56
	H-value	0.30	0.18	0.16	0.23	0.21
	LC_{50}	183.62	177.23	178.81	173.38	158.22
	LCL	170.31	158.32	164.30	162.73	148.63
48h	**UCL**	196.11	192.48	185.42	182.40	174.62
	S-v	1.24±0.13	1.09±0.32	1.23±0.34	2.18±0.41	1.15±0.36
	t-ratio	2.39	2.30	2.47	3.04	3.14
	g-value	0.40	0.24	0.41	0.43	0.30
	H-value	0.18	0.10	0.19	0.18	0.36
	LC_{50}	182.50	175.82	176.92	170.66	154.30
	LCL	165.40	160.85	158.43	155.36	142.69
72h	**UCL**	193.82	184.96	189.36	191.84	170.68
	S-v	1.21±0.61	1.34±0.06	1.38±0.13	2.31±0.49	1.61±0.30
	t-ratio	2.18	3.32	2.44	3.18	3.71
	g-value	0.32	0.19	0.33	0.20	0.25
	H-value	0.10	0.35	0.46	0.12	0.18
	LC_{50}	180.38	173.64	177.38	168.24	151.63
	LCL	169.43	162.40	160.98	158.40	138.62
	UCL	196.30	184.55	190.43	182.63	188.40
96h	**S-v**	2.62±0.64	1.91±0.18	1.73±0.31	2.34±0.21	2.21±0.53
	t-ratio	4.37	2.15	3.11	2.40	3.32
	g-value	0.16	0.12	0.18	0.17	0.14
	H-value	0.21	0.28	0.32	0.19	0.29

Six batches of ten L. acuminata were exposed to different concentrations of the above molluscicides. Mortality was determined after every 24h. LC-Lethal concentration, LCL-lower confidence limits, UCL-upper confidence limits, S-v (Slope value), H-value (Heterogeneity), LP (Leaf powder), AC-Ex (Acetone extract), CH-Ex (Chloroform extract), ET-Ex (Ether extract), and CEP (Column extract purified).

section illustrates their hazardous effects, which are both concentration and time-dependent. The time-dependent toxic effect of *A. indicum* leaf products may be either due to the uptake of the active moiety which progressively increases the amount of toxic active components in the host body with increase along with the exposure period or it might be possible that the active compound could change into more toxic forms in the aquarium water or the snail body fluids due to the action of various enzymes activities. The LC_{50} of the column extract purified of leaf powder of *A. indicum* at 24h exposure was 160.30mgl, and at 96h exposure, the LC_{50} was observed at 11.63 mg/l. The toxicity of *A. indicum* leaf products is time and concentration-dependent. It may be due to the uptake of the active molluscicidal components progressively increasing in *L. acuminata* body with an increase in the exposure period.

A. indicum leaf methanolic extract was tested using the disc diffusion method against *B. subtilis*, *S. aureus*, and *E. coli*. *S. aureus* showed a stronger inhibitory effect than the other microorganisms (Prathibaraj and Manjunath, 2014). The chloroform, ethanol, and aqueous

extracts of *A. indicum* leaves were investigated for their antibacterial activity against *B. subtilis*, *S. aureus*, *K. pneumonia*, *P. aeruginosa*, *E. coli*, and *Sal. Typhi*. The maximum bacterial growth inhibition was exhibited by ethanol extract which was followed by chloroform extract while aqueous extract did not show any activity (Poonkothai, 2006). The aqueous and methanolic leaf

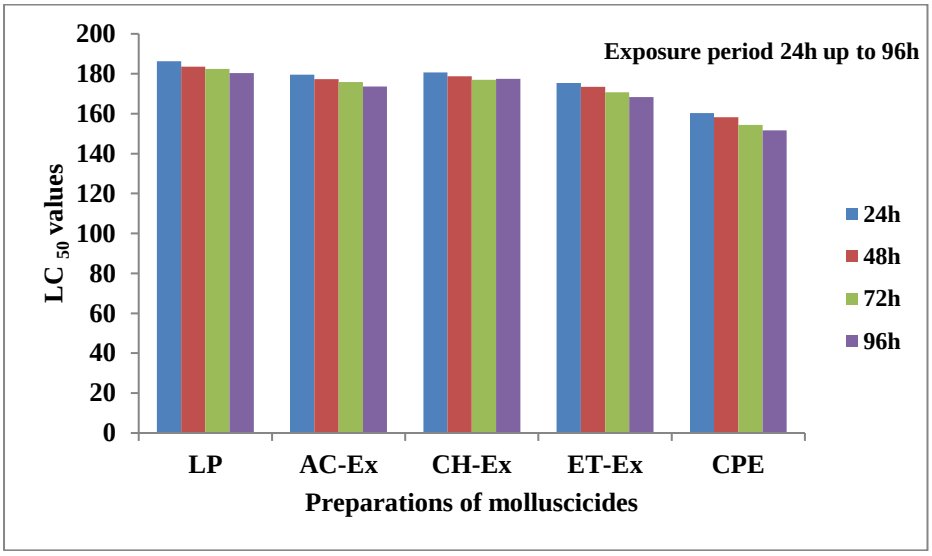

Figure 1: Histogram shows the toxic effect of leaf powder of *A. indicum* and their organic extract against *L. acuminata*. *LP (Leaf powder), AC-Ex (Acetone extract), CH-Ex (Chloroform extract), ET-Ex (Ether extract), and CEP (Column extract purified).*

extracts of *A. indicum* showed significant antidiarrhoeal activity. These extracts were reported to reduce diarrhea by inhibiting intestinal peristalsis, gastrointestinal motility, and PGE2-induced enteropooling (Chandrashekhar *et al.*, 2004). It has been discovered that the ethanolic extract of the *A. indicum* plant protects rats from acetaminophen-induced nephrotoxicity. It also shows a significant reduction in serum creatinine, alkaline phosphatase, and uric acid levels in rat (Vshakiram *et al.*, 2013). The aqueous extract of *A. indicum* seed possessed significant diuretic and natriuretic activities. In contrast to purosemide (20 mg/kg), oral administration of the extract at doses of 200 and 400 mg/kg resulted in a notable dieresis and enhanced salt elimination, but had no effect on urinary potassium excretion (Gunasekaran *et al.*, 2010). When given orally to rats at doses of 200 and 400 mg/kg, the aqueous and ethanol extracts of *A. indicum* leaf significantly increased urine volume and urinary electrolyte (Na^+, K^+, and Cl^-) excretion. To support the traditional usage of *A. indicum* for its diuretic activity, the aqueous extract at 400 mg/kg demonstrated a significant rise in urine volume and urinary Na^+, K^+, and Cl^-levels comparable to those of furosemide (25 mg/kg) (Chauhan and Nagori, 2014). The cytotoxicity activity of the methanolic extract of *A. indicum* was evaluated using lung aceno carcinoma (NCI-H23) and human melanoma (SK-MEL28) cell lines. With IC_{50} values of 4.71 mg/ml on SK-MEL28 and 15.8 mg/ml on NCI-H23 cell lines, it also exhibits good inhibitory effects on cancer cells (Srikanth *et al.*, 2012).

The LC_{50} values in Table 1 (Fig 1) clearly show that the different preparations of *A. indicum* leaf extract and column purified extract are dependent on time and concentration, resulting in a notable mortality rate in snails. A t-ratio value greater than 1.96 indicates that the regression is

significant. The index of significance of the potency estimating values indicates that the value of the mean is within the limit at all probability levels (90, 95, and 99) since it is less than 0.5. Values of heterogeneity factor less than 1.0 denote that in the replicate tests of random sample, the concentration-response lines would fall within the 95% confidence limits and thus the model fits the data adequately.

CONCLUSION

The phytochemicals found in *A. indicum* leaves can be utilized as a powerful molluscicide against harmful snails, and livestock keepers will find it more environmentally friendly, according to the current toxic study against host snail *Lymnaea acuminata*. To identify the active components in the leaf and to comprehend how phytochemicals carry out toxicological activity in the host body, more research is required.

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