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Various Organic Solvent Extracts from Leaves of Indian Medicinal Plant *Leucas aspera* are used against the Liver Fluke Host Snail *Indoplanorbis exustus*

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Abstract

The two well-known species that cause fascioliasis, or liver rot disease, are *Fasciola hepatica* and *F. gigantica*. Although it has spread to every continent, South America, Southeast Asia, the Middle East, and Oceania have been particularly affected. Worldwide, fasciolosis infection is a serious health issue for animals. In addition to causing unfavorable conditions that impact animal health, the infection may lower the amount and quality of animal products, such as meat, milk, and wool. An intermediate host for liver flukes is the freshwater snail *Indoplanorbis exustus*. One important strategy for lowering fascioliasis may be to keep the snail population below threshold levels. In this study, the effects of *Leucas aspera* leaf powder and its organic extracts (acetone, chloroform, and ether) were investigated. The column extract was used as a molluscicide against the snail *I. exustus*. Six replicates were used to observe host snail mortality at 24, 48, 72, and 96 hours of exposure. Without any treatment, the control snail group was maintained in an identical volume of water (three liters) with ten snails. The LC_{50} of *L. aspera* dried leaf powder against *I. exustus* was 192.31 mg/l at 24 hours and 183.13 mg/l at 96 hours. The ether extract of *L. aspera* dry leaf powder was more toxic to *I. exustus* (24-hour LC_{50}: 179.32 mg/l). Column purified fraction of dried leaf powder of *L. aspera* had LC_{50} values of 173.51 and 164.03 mg/l at 24 and 96 hours, respectively. The outcomes of this investigation showed that various leaf products of *L. aspera* had strong molluscicidal effects, and their formulations might serve as useful molluscicides.

Key words: *Indoplanorbis exustus*, *Leucas aspera*, Molluscicides, *Fasciola gigantica*, *Fasciola hepatica*

Fasciola is a significant livestock helminth parasite affecting livestock, particularly ruminants such as cattle, sheep, and goats that is becoming a significant source of zoonotic disease development [16], [18], [30-31]. One of the major global diseases of *Fasciola* in humans and ruminants is zoonotic disease [5], [7], [19]. Metacercaria found on the leaves of aquatic plants that are frequently used as vegetables can cause human fasciolosis, a plant/food borne trematode infection [19]. It is a prevalent disease that impacts the growth, development, pace, and productivity of animals with a higher economic loss, including cattle, sheep, goats, buffalo, and other vertebrates [6], [16]. The two main trematode species that cause liver fluke are *Fasciola hepatica* and *Fasciola gigantica* [12], [18]. Mammals are a primary host of *Fasciola*, while snails are the secondary host [18]. The freshwater snail *Indoplanorbis exustus* is a secondary host of the *Fasciola* [13], [15], [28]. An effective method to control liver flukes is to reduce the number of snails [12]. There have been several attempts to lower the prevalence of liver fluke by employing various plant-derived phytoproducts and synthetic molluscicides to combat harmful snails [2], [12], [14], [17]. Synthetic molluscicides have the potential to harm aquatic organisms and the environment. Numerous plant-derived substances and phytochemicals exhibiting molluscicidal properties have been identified [12], [29].

In India's traditional medical system, *Leucas aspera*, frequently referred to as "Thumbai," is an important medicinal plant that serves as an insecticidal agent, diaphoretic, stimulant, expectorant, aperient, and antipyretic [22]. In the Indian state of South Kannada, it is also used to treat jaundice [27]. In the region of Assam, the leaves of *Leucas aspera* are consumed as vegetables. It is used in ethnomedicinal applications to treat skin diseases, headaches, piles, paralysis, dry mouth, stomach ulcers, abdominal pain, and headaches associated with dysentery [11]. Some Gram-positive and Gram-negative bacteria, including *Salmonella typhi*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas pyocyanea*, *Shigella flexneri*, *Vibrio cholera*, *Staphylococcus aureus*, and *Proteus vulgaris*, are susceptible to the antibacterial properties of *Leucas aspera* essential oil [20]. Antimicrobial, antioxidant, anticancer, phytotoxic, antivenom, thrombolysis, hepatoprotective, anti-inflammatory, analgesic, antinociceptive, antiulcer, antimalarial, antipyretic, and antidiabetic properties are among the several biological actions exhibited by *Leucas aspera* extracts [20]. The aim of this work was to investigate the molluscicidal properties of dried leaf powder from *Leucas*

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aspera and its several organic extracts against the freshwater intermediate host snail, *Indoplanorbis exustus*.

MATERIALS AND METHODS

Test animals

In the district of Mau (Muhammadabad Gohana) (U.P.), India, adult test animals *Indoplanorbis exustus* (0.85±0.20 cm in length) were collected from ponds and low-lying submerged fields. The test groups of snails were acclimated to dechlorinated tap water at 25±30 °C for 72 hours.

Plants and their formulations

The locally grown *Leucas aspera* leaves were collected from Muhammadabad Gohana (Tahsil), District Mau, India. The electric grinder was used to crush the dried leaves of *L. aspera* into fine powders, which were subsequently sieved using fine-mesh cloth. The molluscicidal abilities of this fine powder were evaluated against the vector snail *Indoplanorbis exustus*.

Preparation of extracts

100 milliliters of 98% acetone, 99.7% chloroform, and 98% ether were used to extract one gram of dried leaf powder from *L. aspera* over the course of 24 hours at room temperature. Sterilized Whatman No.1 filter paper was used to filter each mixed solution separately. The filtered extracts were then vacuum-evaporated [9]. Molluscicidal activity has been determined using the residues that were thus produced.

Column extract

A 5×45 cm column was used to perform silica gel (60-120 mesh, Qualigens Glass, Precious Electrochemidus Private Limited, Bombay, India) chromatography on 100-gram dried leaf powder of *L. aspera* combined with 1L of 95% ethanol. Fractions of extracted solutions are collected in 5 ml bottles. The leftover particles from the vacuum-evaporation of ethanol were used to assess its molluscicidal properties.

Experimental assay

The toxicity test of various organic extracts and a column extract of *L. aspera* was conducted using Kumar and Singh [12] methods. Three liters of dechlorinated tap water were set up in a glass aquarium containing ten experimental snails. After having been exposed to various amounts for up to 96 hours, snail mortality was measured during 24, 48, 72, and 96 hours. Six aquariums were set up for each concentration in order to do statistical analysis.

The experimental setup's control groups were maintained in the same water volumes under comparable circumstances without any kind of treatment. Snail mortality was measured every 24 hours for up to 96 hours. The contraction of the snail body inside the shell was used to determine snail mortality; a lack of reaction to the needle probe was interpreted as proof of snail death.

Calculation

The POLO computer program was used to calculate the lethal concentration (LC_{50}) values, slope values, t-ratio, "g" value, lower and upper confidence limits (LCL and UCL), and heterogeneity factor [24].

RESULTS AND DISCUSSION

The toxicity of *Leucas aspera* dry leaf powder, as well as its different organic extract fractions and column extract, against *Indoplanorbis exustus*, varied with time and concentration. The *Leucas aspera* dry leaf powder's LC_{50} values were 192.31 mg/l at 24 hours and 183.13 mg/l at 96 hours (Table 1). The dried leaf powder ethanol extract of *Leucas aspera* was the most effective of all the organic solvent extracts (Table 1). The ether extract of *Leucas aspera* dry leaf powder has a 24-hour LC_{50} of 179.32 mg/l and a 96-hour LC_{50} of 170.23 mg/l against *Indoplanorbis exustus*. All of the organic solvent extract fractions have extremely hazardous column extract fractions. After 24 hours, the column extract of *Leucas aspera* dried leaf powder had an LC_{50} value of 173.51 mg/l. At 96 hours, the LC_{50} of the column extract of *Leucas aspera* dried leaf powder was 164.03 mg/l (Table 1).

The individual estimations of LC based on each of the six replicates were found to be within the 95% confidence ranges of LC_{50}, and the slope values provided in (Table 1) were steep. The heterogeneity factor was less than 1.0, and the t-ratio was more than 1.96. At all probability levels (90, 95, and 99), the g-value was less than 0.5 (Table 1).

The results of this investigation show that *L. aspera* dried leaf powder is a powerful source of molluscicides. Because *L. aspera* molluscicidal components dissolve in water and penetrate the host's bodily fluids, they have a lethal impact on *I. exustus*. The results section makes it abundantly evident that the harmful effects depend on both concentration and time. The uptake of the active moiety, which progressively raises the quantity of harmful active components in the snail body with a rise in exposure duration, is one possible explanation for the time-dependent toxic action of *Leucas aspera* products. The phyto-active ingredient can react with the bodily fluids of the animal and function at the enzyme level. Molluscicidal components of *Leucas aspera* are more soluble in ethanol, as evidenced by the highest toxicity of ethanol extract compared to other organic extracts.

Molluscicidal capacities against *Indoplanorbis exustus* are represented by the organic extract of dried leaf powder of *Leucas aspera*. It is likely that the molluscicidal component of *Leucas aspera* builds up over time and kills snails. The ethanol extract of *Leucas aspera* demonstrated 80% antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* [23]. Additionally, these extracts demonstrated activity against *Shigella dysenteriae* [1], *Pseudomonas aeruginosa*, and *Escherichia coli* [25]. In comparison to Gram-positive bacteria like *Bacillus cereus*, *B. subtilis*, *B. megaterium*, and *Staphylococcus aureus*, the ethanolic extract of *Leucas aspera* leaf demonstrated greater antibacterial activity against Gram-negative bacteria like *Salmonella paratyphi*, *S. typhi*, *S. dysenteriae*, *Escherichia coli*, *Vibrio cholera*, and *Pseudomonas aeruginosa* [21]. However, the methanolic extract of *L. aspera* leaves exhibited potent antibacterial qualities in its dichloromethane fraction [26]. *Leucas aspera* leaves have antiplasmodial action against *Plasmodium falciparum* chloroquine-sensitive (3D7) stain [3]. Carbohydrate, flavonoid, terpenoids, phenol, alkaloids, saponins, and tannins are all found in the phytochemical screening of *Leucas aspera* leaf extract [11]. The anthelmintic activity of condensed tannins has been documented in a number of anthelmintic plants [4], [10], and it is known to block the activities of endogenous enzymes [8]. According to Ayoub and Yankov [2], a number of tannins from various plant groups have molluscicidal effects.

The results section makes it clear that snail mortality is significantly increased by even slight increases in the concentration of various preparations (Table 1). The regression is considered significant if the t-ratio value is higher than 1.96. Since the mean value is smaller than 0.5, the index of significance of the potency estimating values shows that the values are within the limit at all probability levels (90, 95, and 99). When the heterogeneity factor is less than 1.0, it indicates that the model fits the data sufficiently because the concentration response lines would lie within the 95% confidence limits in repeat testing of the random sample.

Table 1 The toxicity of *Leucas aspera* leaf powder (LP) and their various organic solvent extracts against the snail *Indoplanorbis exustus* at different exposure times

Formulation of molluscicides	Exposure Time (Hours-h)	LC_{50} (mg/l)	Limits		Slope value	t- ratio	g-value	Heterogeneity
			LCL	UCL				
LP	24	192.31	173.03	212.40	3.22 ± 0.14	2.16	0.45	0.12
AC-Ex		183.72	164.93	207.92	1.25 ± 0.35	3.56	0.21	0.30
CH-Ex		185.42	163.29	210.38	2.20 ± 0.21	3.34	0.32	0.25
ET-Ex		179.32	154.12	203.86	2.12 ± 0.15	2.18	0.20	0.20
CE		173.51	151.03	196.42	2.11 ± 0.74	2.70	0.26	0.23
LP	48	190.11	170.29	212.51	1.54 ± 0.23	2.35	0.40	0.56
AC-Ex		181.44	163.21	196.17	2.20 ± 0.31	3.30	0.14	0.26
CH-Ex		183.21	160.18	199.34	2.43 ± 0.14	2.17	0.38	0.62
ET-Ex		176.29	154.77	191.33	2.40 ± 0.31	3.26	0.20	0.46
CE		170.48	151.76	190.28	1.15 ± 0.31	2.30	0.14	0.23
LP	72	187.86	160.33	194.43	2.23 ± 0.55	3.27	0.35	0.28
AC-Ex		177.45	155.62	191.24	1.23 ± 0.56	3.61	0.18	0.31
CH-Ex		180.16	162.81	203.18	1.30 ± 0.51	2.89	0.27	0.42
ET-Ex		173.22	151.43	193.29	2.18 ± 0.32	3.18	0.34	0.34
CE		167.28	148.76	180.17	2.45 ± 0.31	2.70	0.29	0.15
LP	96	183.13	151.24	198.43	2.60 ± 0.24	3.25	0.31	0.20
AC-Ex		173.28	154.13	189.33	1.12 ± 0.29	3.55	0.42	0.13
CH-Ex		177.23	153.77	196.83	1.38 ± 0.08	2.28	0.25	0.52
ET-Ex		170.23	148.26	198.06	2.70 ± 0.51	3.15	0.59	0.13
CE		164.03	151.26	189.33	2.17 ± 0.23	2.82	0.42	0.18

LC-Lethal concentration, LCL-Lower confidence limits, UCL-Upper confidence limits, LP (Leaf powder), AC-Ex (Acetone extract), CH-Ex (Chloroform extract), ET-Ex (Ether extract), and CE (Column extract).

Ten *Indoplanorbis exustus* host snails (In six batches) were exposed to different concentrations of the above formulated molluscicides. Snail mortality was determined every 24h

CONCLUSION

Leucas aspera dry leaf powder and its various organic solvent extracts exhibit potent molluscicidal activity against *Indoplanorbis exustus*, with toxicity increasing in a time- and concentration-dependent manner. Among the tested formulations, the ethanol extract and column extract of *Leucas aspera* displayed the highest efficacy, indicated by the lowest LC_{50} values across all exposure durations. The consistency of the LC_{50} values within 95% confidence intervals, along with favorable t-ratio and heterogeneity factor values, confirms the reliability and significance of the results. The increasing toxicity over time may be attributed to the cumulative uptake of active phytochemicals such as tannins, flavonoids, and alkaloids, known to interfere with enzymatic systems in snails. In addition to its molluscicidal potential, *Leucas aspera* also shows broad-spectrum antibacterial and antiplasmodial activities, further enhancing its value as a multipurpose biocontrol agent. These findings support the potential of *Leucas aspera* as an eco-friendly, plant-based molluscicide and warrant further investigation into its active compounds for integrated pest and disease management strategies.

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