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Original Article

Helminth Control: In Vitro Anthelmintic and Larvicidal Activities of *Solanum surattense* Against *Fasciola gigantica* (Sporocyst, Redia, and Cercaria) Larvae

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Abstract

Introduction: Liver flukes (*Fasciola* species) are parasitic helminths that cause fascioliasis in cattle and humans. The *Fasciola* species (*F. hepatica* and *F. gigantica*) have complicated life cycles in the host snails and mammals. The cattle and human population get infected after ingestion of contaminated miracidium stages of *Fasciola* through aquatic plants or contaminated water. The developmental stages of liver flukes can be discontinued by terminating larval stages such as sporocyst, redia, and cercaria in vivo or by killing intermediate hosts. Synthetic anthelmintic compounds are highly effective but they cause adverse effects in the environment. Plant products are eco-friendly and safe which can be used in the control of parasitic helminths.

Methods: In vitro anthelmintic activities of different preparations of *Solanum surattense* such as pulverized leaf products, extracts (ether, chloroform, methanol, acetone, and ethanol), and column purified fractions were assessed against sporocyst, redia, and cercaria larvae of *F. gigantica*. Different preparations of the *S. surattense* were assessed separately against *Fasciola* larva after 2 to 8 hours of exposure. These larvae, having up to 48-hour survivability, were kept in tap water at laboratory conditions.

Results: Larval mortality was observed after 2, 4, 6, and 8 hours of exposure for the calculation of LC_{50} value. Among all the organic extracts, the maximum larvicidal activity was observed in ethanol after 2 hours of exposure, the LC_{50} value against sporocyst, redia, and cercaria was reported to be 63.21, 64.24, and 63.54 mg/mL, respectively. However, maximum activity was observed after 8 hours of exposure in column purified fractions, and the LC_{50} value against sporocyst, redia, and cercaria was reported to be 48.25, 47.61, and 44.15 mg/mL, respectively.

Conclusion: Conclusively, the present research study indicates that *S. surattense* is a potent source of anthelmintic compounds which can be used for the control of sporocyst, redia, and cercaria larvae of *F. gigantica*.

Keywords: Liver fluke, *Solanum surattense,* Sporocyst, Redia, Cercaria, Fascioliasis

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Introduction

Fascioliasis is a zoonotic disease among wild and household animals as well as humans, which is caused by trematode species of *Fasciola* (1-3). *Fasciola hepatica* and *Fasciola gigantica* are common liver flukes that cause fascioliasis (4,5). The adult fluke (*Fasciola* species) produces infection in the liver of mammals especially cattle which feed on aquatic plants that are contaminated with infective-stage of metacercaria (6-8). Fascioliasis is listed as a neglected tropical disease by the World Health Organization (WHO) (8). The liver fluke is an important parasite for domesticated animals which causes major economic losses (9). The prevalence of *Fasciola* spp. infection is approximately 17 million in the human population worldwide (8,10). The lymnaeid snails are secondary or intermediate hosts of *Fasciola* spp. which have an effective role in the distribution of liver flukes (11). The species of *F. gigantica* is restricted to Asia and Africa (1,12). The risk of fascioliasis and distributions is followed by the secondary host, which is the main factor that sustains in the endemic part of the world (13). The snail *Lymnaea acuminata* is a secondary host of *F. gigantica* (14,15). This species of snail is a hermaphrodite in nature that inhabits freshwater ponds, pools, lakes, rivers, and low-lying submerged fields. The endemic zoonotic disease is very common among the bovine population of eastern Uttar Pradesh, India (16-20). The lifecycle of the *Fasciola* is very complex in the intermediate host (snail) and primary host (mammals, including humans). The eggs of the *Fasciola* can be seen in the host stool which embryonated in the water and each egg generates a single miracidium stage. This miracidium stage punctures the tegument layer of the host animals and causes infection. In digenetic trematodes, the development of the larva is

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a very complex process. It begins to develop in the host body and changes into miracidium. The miracidium stage changes into the sporocysts which are followed by asexual reproduction and convert into sporocyst, redia, and finally cercaria. The cercaria is a free-swimming larval stage of *Fasciola* spp. Therefore, it may be a basic approach to control fascioliasis by breaking the life cycle of *Fasciola* spp. through killing the larval stages in the host body. Synthetic anthelmintic/larvicidal drugs are useful and effective in the control of *Fasciola* larva, but they cause adverse effects in the environment. Currently, plant products are used in *in vitro* treatment of *Fasciola* larvae (sporocyst, redia, and cercaria) which may be a new tool for *in vivo* phytotherapy of the infected intermediate host snail. Sunita and Singh, (18) reported that the plant-derived active components are effective in *in vivo* phytotherapy of the *F. gigantica* larva in host snail. The present study aims to assess in vitro anthelmintic and larvicidal properties of *S. surattense* in the control of sporocyst, redia, and cercaria.

Materials and Methods

Collection of Host Snails and Fasciola Larvae

The snail *L. acuminata* (2.7±0.26 cm in length) was collected from a submerged field of the Muhammadabad Gohna, Mau Uttar Pradesh, India. The host snails were acclimatized for 72 hours in lab condition at 26°C. Infected and non-infected snails were separated into two groups based on the morphological characteristics and cercarial shedding rate. The shells of infected snails were operated on and larvae of *Fasciola* were collected. After collection, sporocyst, redia, and cercaria larvae were poured into different Petri dishes containing 10 mL of tap water (18). The sporocyst, redia, and cercaria larvae, having up to 48-hour survivability, were kept in tap water in the lab conditions at 24-26°C.

Preparation of Plant Leaf Powder

The leaf of the plant *Solanum surattense* was identified and collected from the college campus. The collected leaves were washed by distilled water, sun-dried for 4 to 5 days, and pulverized in a grinder. The powder was sieved with sterilized fine cloth mesh and used against sporocyst, redia, and cercaria.

Organic Extraction of Leaf Powder

In each extraction, 5 g of dried leaf powder of *S. surattense* was added separately to 500 mL of 97.8% ether, 98.5% chloroform, 97.4% methanol, 97.6% acetone, and 94.6% ethanol at lab conditions. After 48 hours, all the extracts were filtered by sterilized filter paper and the obtained extracts were evaporated in a vacuum machine. The extract from leaf powder of *S. surattense* yielded 345 mg ethanol, 330 mg chloroform, 335 mg ether, and 325 mg acetone. Then, extract residues were collected and used in in vitro treatment.

Preparation of Column Purified Fractions

In this study, 1 L of organic solvent (ethanol 94.6%) was used for the preparations of column fractions of *S. surattense* leaf powder and it was subjected to silica gel (60-120 nm mesh size) column chromatography through a 5×45 cm column cylinder. The fractions were collected in the conical flask and evaporated using a vacuum machine. Then, the remaining residue of the column purified was collected and used against larvae.

In Vitro Anthelmintic Activity

The leaf powder, various extracts, and column purified fractions of *S. surattense* were separately assessed against *Fasciola* larva using the method used by Sunita and Singh, (18). In six batches, 10 sporocysts, redia, and cercaria larvae were separately kept in Petri dishes which contained 10 mL of tap water. Different preparations of dried leaf powder, organic extracts, and column purified fractions were separately made in the Petri dishes which contained 10 sporocyst, redia, and cercaria larvae. The larval (sporocyst, redia, and cercaria) mortality was calculated after 2, 4, 6, and 8 hours of exposure. In each experimental setup, larval counting and observation were done with the help of a binocular microscope. The larval mortality (lethal concentration=LC_{50} value) at each concentration was calculated after 2, 4, 6, and 8 hours of exposure. The LC_{50}, LCL (lower confidence limits), UCL (upper confidence limits), t-ratio, and slope values were calculated by POLO software (21).

Results

The in vitro anthelmintic and larvicidal activities of dried leaf powder, organic extracts (ether, chloroform, methanol, acetone, and ethanol), and column purified fractions of *S. surattense* against sporocyst, redia, and cercaria stages of *F. gigantica* were concentration and time-dependent from 2 hours up to 8 hours (Table 1). In vitro larvicidal activity of leaf powder was assessed against sporocyst, redia, and cercaria after 2 hours of exposure, and the LC_{50} value was found to be 70.23, 68.35, and 66.11 mg/mL, respectively. Likewise, among all the organic extracts (ether, chloroform, methanol, acetone, and ethanol), the maximum activity was observed in ethanol extract after 2 hours of exposure, and the LC_{50} value against sporocyst, redia, and cercaria was reported to be 63.21, 64.24, and, 63.54 mg/mL, respectively (Table 1). The highest activity was observed after 8 hours of exposure in column purified fraction, and the LC_{50} value against sporocyst, redia, and cercaria was found to be 48.25, 47.61, and 44.15 mg/mL, respectively. However, the minimum activity was observed in methanol extract after 2 hours of exposure, and the LC_{50} value against sporocyst, redia, and cercaria was found to be 69.45, 67.65, and 65.96

Table 1. *In Vitro* Anthelmintic and Larvicidal Activities of Leaf Powder, Organic Extracts, and Column Purified Fractions of *S. surattense* against Sporocyst, Redia, and Cercaria Larvae of *F. gigantica*

Exposure time	Treatments mg/mL	Sporocyst					Redia					Cercaria				
		LC_{50} mg/mL	LCL	UCL	Slope-value	t-ratio	LC_{50} mg/mL	LCL	UCL	Slope-value	t-ratio	LC_{50} mg/mL	LCL	UCL	Slope-value	t-ratio
2 hours	*S. surattense* (DLP)	70.23	63.12	75.56	0.46±0.35	4.25	68.35	64.92	72.15	0.76±0.21	2.26	66.11	61.11	69.82	0.63±0.15	3.27
	Ethe. Ext.	68.45	65.56	71.95	0.51±0.45	3.65	67.44	61.58	71.59	0.65±0.27	3.20	65.87	60.18	69.25	0.61±0.27	3.37
	Chlo. Ext.	69.14	63.75	73.32	0.65±0.66	3.22	67.25	60.56	72.56	0.58±0.86	4.34	64.23	61.28	70.56	0.35±0.71	4.20
	Meth. Ext.	69.45	64.36	74.58	0.59±0.33	3.27	67.65	61.21	71.65	0.46±0.35	3.27	65.96	60.20	69.48	0.55±0.30	4.22
	Acet. Ext.	67.11	68.10	72.65	0.25±0.61	4.26	66.45	62.86	70.58	0.24±0.81	3.38	64.11	59.14	70.65	0.71±0.88	3.74
	Etha. Ext.	65.21	60.86	68.35	0.86±0.30	3.68	64.24	60.16	69.45	0.69±0.35	4.29	63.54	59.48	66.47	0.52±0.37	3.31
	Colu. Pur.	55.48	51.69	59.78	0.79±0.61	4.25	53.45	50.24	57.34	0.28±0.64	3.55	51.66	47.65	57.34	0.44±0.39	4.29
4 hours	*S. surattense* (DLP)	65.34	63.11	68.45	0.68±0.31	3.24	65.11	60.15	70.68	0.85±0.39	3.20	62.78	58.61	66.58	0.58±0.37	3.54
	Ethe. Ext.	66.48	62.94	69.75	0.79±0.56	4.23	66.12	61.45	71.65	0.48±0.36	4.21	61.54	57.45	65.84	0.44±0.58	4.26
	Chlo. Ext.	65.91	61.34	68.46	0.55±0.44	4.11	66.13	62.62	70.29	0.70±0.56	4.35	63.25	58.24	66.47	0.55±0.71	3.20
	Meth. Ext.	67.63	63.58	71.31	0.35±0.30	4.27	67.16	62.15	71.36	0.59±0.31	3.26	60.45	55.61	65.86	0.38±0.66	4.77
	Acet. Ext.	65.83	61.45	70.65	0.56±0.34	3.20	61.95	59.67	63.52	0.85±0.69	3.11	62.18	58.44	66.49	0.25±0.44	3.21
	Etha. Ext.	62.57	59.33	65.84	0.25±0.67	3.56	60.16	57.64	65.94	0.49±0.27	3.35	59.46	55.49	65.47	0.50±0.86	3.65
	Colu. Pur.	53.21	50.48	65.85	0.45±0.85	4.65	51.28	48.65	55.21	0.57±0.82	4.29	49.16	44.88	54.61	0.51±0.91	4.23
6 hours	*S. surattense* (DLP)	63.58	60.25	66.45	0.85±0.33	3.20	62.56	58.61	65.88	0.34±0.39	3.85	61.88	57.66	65.81	0.77±0.31	4.29
	Ethe. Ext.	64.16	60.12	68.75	0.66±0.45	4.21	65.10	61.55	60.13	0.75±0.34	3.27	59.64	55.92	66.47	0.45±0.38	3.28
	Chlo. Ext.	63.84	59.24	66.88	0.54±0.65	3.85	62.65	59.45	66.51	0.55±0.38	3.55	61.42	57.61	65.42	0.52±0.35	3.33
	Meth. Ext.	65.14	61.67	71.84	0.85±0.36	3.29	62.45	59.11	65.46	0.71±0.28	4.25	59.15	55.34	64.64	0.34±0.31	4.55
	Acet. Ext.	63.84	59.48	68.64	0.77±0.65	4.56	60.15	57.64	64.54	0.94±0.30	3.20	60.45	54.32	65.18	0.79±0.32	3.37
	Etha. Ext.	60.52	57.86	64.95	0.44±0.31	3.29	59.18	55.96	63.48	0.51±0.37	4.35	57.29	52.34	63.64	0.88±0.39	3.64
	Colu. Pur.	51.10	48.65	54.86	0.50±0.37	4.33	49.66	45.95	55.42	0.58±0.33	3.38	47.18	44.31	51.67	0.59±0.31	4.35
8 hours	*S. surattense* (DLP)	61.58	57.66	64.86	0.51±0.35	3.65	60.53	57.65	65.48	0.88±0.36	3.72	59.65	55.61	64.84	0.56±0.30	3.11
	Ethe. Ext.	62.94	59.15	65.48	0.57±0.27	3.25	60.11	56.95	64.67	0.66±0.64	3.62	59.10	54.16	65.94	0.67±0.38	4.66
	Chlo. Ext.	62.25	58.92	66.16	0.51±0.65	4.22	60.45	55.94	64.12	0.27±0.15	4.25	59.13	53.94	64.91	0.34±0.94	3.21
	Meth. Ext.	63.46	59.45	65.84	0.86±0.31	4.21	61.45	57.63	65.15	0.56±0.38	3.86	58.14	51.64	63.84	0.50±0.55	3.29
	Acet. Ext.	62.42	59.34	66.56	0.50±0.75	3.20	59.15	55.88	65.34	0.12±0.31	3.21	58.25	50.64	64.34	0.58±0.41	3.66
	Etha. Ext.	58.64	54.91	65.18	0.65±0.30	3.29	57.46	53.42	61.61	0.64±0.30	4.35	55.64	50.15	61.67	0.37±0.30	4.25
	Colu. Pur.	48.25	44.32	51.61	0.37±0.28	4.27	47.61	43.61	52.65	0.50±0.35	3.20	44.15	40.25	50.19	0.75±0.41	4.64

Abbreviation: DLP: Dried Leaf Powder, Ethe: Ether, Ext: Extract, Chlo: Chloroform, Meth: Methanol, Acet: Acetone, Etha: Ethanol, Clou: Column, Pur: Purified, LCL: Lower Confidence Limits, UCL: Upper Confidence Limits

Six batches of 10 sporocyst/redia/cercaria larvae were separately exposed to different concentrations of the above-mentioned treatments. Larval mortality was recorded every 2 hours. Concentrations are the given final concentration (w/v) in the Petri dish water. Significant negative regression ($P<0.05$) was observed between exposure time and lethal concentration (LC_{50}) of treatments.

mg/mL, respectively.

The separate estimation the LC_{50} and slope-values was based on six replicates of the experiment which is found within the 95% confidence limit of the lethal concentration of the various preparations. The value of t-ratio was greater than 1.96, which verifies significant anthelmintic and larvicidal activities (Table 1).

Discussion

The results demonstrate that the leaf powder of *S. surattense* is a vigorous source of anthelmintic compounds. The results of the study revealed that anthelmintic and larvicidal compounds of *S. surattense* are present in the leaf, which is soluble in organic solvents and causes mortality in sporocyst, redia, and cercaria larvae of *F. gigantica*. The efficacy (LC_{50}) of the various preparations of the *S. surattense* was concentration and time-dependent against sporocyst, redia, and cercaria. The efficacy of different preparations of *S. surattense* may possibly be due to the fact that various active phytochemicals are accumulated in the larval body which gradually increases with the increase of exposure time. It bound to the active site of the enzymes and caused larval mortality. It was indicated that the cuticle layers of the larvae permitted the penetration of the active components into the body that causes mortality. Mahesh Kumar et al (22) reported that leaf extract of *S. surattense* is more effective against *Culex quinquefasciatus* larvae. The ethanolic and aqueous extracts of the fruit powder of the *S. surattense* have anthelmintic activity against earthworms *Pheretima posthuma* (23). The maximum efficacy was observed in ethanolic extract among other extracts which indicates that the active larvicidal compounds of *S. surattense* are soluble in the ethanolic solvent (Table 1).

In vitro larvicidal efficacy of *S. surattense* is time and concentration-dependent which may be due to the fact that active components are gradually dissolved in tap water and then diffuse in the larval body which causes larval mortality with the increase in exposure time. Barik et al (24) indicated that the aqueous extract of the fruit of the *S. surattense* is a source of anthelmintic compounds. The leaf extract of *S. surattense* contains phytochemicals such as flavonoids and their glycosides (25), saponin (26), sterols (27), several alkaloids (28), tannins, and gums (29). The aqueous, ethanolic, and hydroethanolic extracts of the *S. surattense* have anthelmintic activity (30). The methanolic extract of the *S. surattense* has antibacterial properties against Gram-positive bacteria *Streptococcus aureus* and *Bacillus subtilis* at 50, 75, and 100 µg/mL concentrations (31). Bahuguna et al (32) investigated antiulcer activity of *S. surattense* using aqueous alcohol, petroleum ether, and chloroform extracts based on parameters such as total acidity, pH, free acidity, and ulcer index. Various products of *S. surattense* are also used in the treatment of the cold, insomnia, worms (33), and enlargement of the liver for their laxative, aphrodisiac (34,35), anti-nociceptive, anti-fungal, and molluscicidal properties (36,37). Hussein Ayoub and Yankov (38) reported that tannin is a potent molluscicidal component. The extract of *S. surattense* has anti-cancerous activities which may be due to the presence of flavonoids such as quercetin, apigenin, luteolin, and fisetin which are known to be vigorous inhibitors of cancer cell growth (39). The ethanolic extracts of *S. surattense* remarkably reduced parasitemia in infected mice (40). The slope-values clearly show that the minimum concentration of active components increases with the increase of exposure period which causes mortality in *Fasciola* larvae (Table 1). The value of t-ratio was greater than 1.96 which shows significant regressions.

Conclusion

This study established that medicinal plant *S. surattense* and its dried leaf powder, different organic extracts (ether, chloroform, methanol, acetone, and ethanol), and column purified fractions killed the sporocyst, redia, and cercaria larvae of *F. gigantica*. This study indicates that the active component of the *S. surattense* can be used in in vivo phytotherapy of the infected host snail. The plant-derived active components are easily available, biodegradable, safe, and eco-friendly for other non-target aquatic organisms. It may be one of the new approaches and tools for in vivo treatment of sporocyst, redia, and cercaria larvae without killing the intermediate host snail for the control of fascioliasis.

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Issues

In this study, ethical considerations have been fully observed.

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