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Research Article
Molluscicidal Activity of Different Organic Root Extract of *Potentilla fulgens* Against Liver Fluke Vector Snail *Indoplanorbis exustus*

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Abstract

Background and Objective: Snail *Indoplanorbis exustus* is an intermediate host of liver fluke. The control of snail population below threshold levels is major tool in reducing the incidences of fasciolosis. The present research was designed for studying the effect of dried root powder of *Potentilla fulgens* and their different products use as molluscicides against host snail *I. exustus.* **Materials and Methods:** The molluscicidal studies of different organic extracts and column purified fraction of *P. fulgens* were continuously observed for 96 h at different concentration. Mortality was observed for 24, 48, 72 and 96 h exposure. Six aquariums were setup for each concentration. The control group animals were kept in the equal volume of water under similar conditions without treatment. **Results:** The dried root powder of *P. fulgens* at 24 h and 96 h LC_{50} against *I. exustus* was 170.33, 140.29 mg L^{-1}, respectively. Among different organic extracts, ethanol extract was more toxic than other organic extract. The ethanol extract of *P. fulgens* was more toxic (24 h LC_{50}-112.75 mg L^{-1}) against *I. exustus*. The 24 h and 96 h LC_{50} of column purified fraction of dried root powder of *P. fulgens* was 55.63 and 33.75 mg L^{-1}, respectively. **Conclusion:** The present study revealed that the different product of *P. fulgens* has potent molluscicidal activity and their product may be used as potent source of molluscicides.

Key words: *Potentilla fulgens*, *Indoplanorbis exustus*, molluscicides, fasciolosis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fasciolosis is a parasitic zoonosis disease of ruminants which caused by parasitic trematodes, *Fasciola hepatica* and *F. gigantica*[1]. *Fasciola hepatica* and *F. gigantica* is a major worldwide zoonotic disease of domestic ruminants animals and human[2-4]. The human fasciolosis are classified as a plant/food borne trematode infection, commonly acquired by eating metacercaria encysted on aquatic leaves that are eaten as vegetables[2]. The fluke *F. hepatica* is widely distributed in temperate zones, whereas *F. gigantica* is typically found in tropical zones around the world[1,5]. These parasitic diseases caused serious economic losses to animal husbandry in the Northern part of India[6]. They live in the liver of cattle, sheep, goats and buffaloes, which have a significant importance on growth rate, developments and productivity of ruminants and therefore, are considered economically significant[7,8]. Snail *Indoplanorbis exustus* is the intermediate host for the liver fluke *F. gigantica*, which caused endemic fasciolosis in the Northern part of Utter Pradesh, India[6,9-13]. Snail is considered to be one of the weakest links in Fasciola. Control of snail population below a threshold level is advocated for effective control of fasciolosis[14-16]. The control of fasciolosis includes strategic use of different antihelminthic drugs which reduce the fasciolosis. Heavy use of synthetic molluscicides for control of vector snail population has created serious problem for the aquatic organisms. However, it has been advocated that the use of synthetic molluscicides is not safe for environment[17].

Alternatively plant derived molluscicides are becoming increasingly popular because they are cheaper, more acceptable and safer than their synthetic molluscicides, as well as being potentially biodegradable and eco-friendly[9,18,19]. *Potentilla fulgens* is a medicinal plant which commonly called Himalayan Cinquefoil in English, Bajradanti in Hindi[20]. *Potentilla fulgens* are commonly found in north-east region of India and it used in Unani, Ayurvedic, Siddha, Chinese and Tibetan systems of medicine[21-25] due to high content of polyphenols, phenolic tannins in their aerial and underground parts. The pharmacological studies of *P. fulgens* possess hypoglycemic, anti-hyperglycemic, antitumor, anti-hyperlipidemic, antioxidant, antiulcerogenic and antiinflammatory properties[26] thus supporting its ethnotherapeutic use. The phytochemical compound of *P. fulgens* root contain epicatechin, potifulgens (epiafzelechin-6-O-8"epiafzelechin) and aerial parts are potentene A, potentene B, afzelechin-4α→8"-catechin, epiafzelechin and rutin[27]. The present study was to evaluate the molluscicidal activity of *P. fulgens* dried root powder, different organic extracts and column purified fractions against vector snail *I. exustus.*

MATERIAL AND METHODS

Collection of experimental animals: Adult *I. exustus* (0.85±0.20 cm in length) were collected in the year 2017-2018 from lakes and low lying submerged field in Gorakhpur (U.P.) India. The snails were acclimatized for 72 h in dechlorinated tap water at 25±3°C. The pH of water was 7.3-7.1 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.2-7.1, 5.2-6.3 and 104.0-106.0 mg L^{-1}, respectively.

Plants: The fresh dried root of *Potentilla fulgens* were procured from local market in Gorakhpur, (UP) India.

Preparation of crude plant products: Dried root of *P. fulgens* were pulverized separately in the electric grinder and crude powders thus obtained were then sieved with the help of fine mesh cloth. This fine powder was then used separately for toxicity experiments against vector snail *I. exustus*.

Organic solvent extracts: Two gram dried roots powder *P. fulgens* were extracted with 200 mL of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone and 95% ethanol at room temperature for 24 h. Each preparation was filtered separately through sterilized whatman No-1 filter paper[28] and the filtered extracts where subsequently evaporated under vacuum. The residues, thus obtained were used for the determination of molluscicidal activity. The root powder of *P. fulgens* yielded 250 mg ethanol, 320 mg chloroform, 360 mg ether and 410 mg acetone extracts.

Column purification: One hundred milliliters of ethanol extract fraction of dried root powder of *P. fulgens* were subjected to silica gel (60-120 mesh, Qualigens Glass, Precious Electrochemidus Private Limited, Bombay, India) chromatography through a 5×45 cm column. Five milliliter fractions eluted with ethanol (95%) were collected. Ethanol was evaporated under vacuum and the remaining solids obtained were used for the determination of molluscicidal activity of each fraction.

Concentration-response relationship for toxicity experiment: Toxicity experiment of different organic extracts and column purified fraction of *P. fulgens* was performed by the method of Kumar and Singh[9]. Ten experimental animals were kept in a glass aquarium containing 3 L of dechlorinated tap water. Snails were exposed continuously for 96 h to different concentrations and preparation of *P. fulgens* and mortality was observed for 24, 48, 72 and 96 h. Six aquariums

were setup for each concentration. The control animals were kept in the equal volume of water under similar conditions without treatment. Mortality of snails was recorded at interval of 24 h each up to 96 h. The mortality of snails was established by the contraction of snail body within the shell, no response to needle probe was taken as evidence of death. The mortality data were observed after every 24 h up to 96 h.

Statistical analysis: Lethal values (LC_{50}), lower and upper confidence limits (LCL and UCL), slope values, t- ratio, 'g' value and heterogeneity factor were calculated using POLO computer programme[29]. The regression coefficient between exposure time and different values of LC_{50} was determined by the method of Sokal and Rohlf[30].

RESULTS AND DISCUSSION

The toxicity of dried root powder of *P. fulgens* and their different fractions of organic extract against the *I. exustus* were time and concentration dependent. The LC_{50} of dried root powder of *P. fulgens* at 24 h were 170.33 mg L^{-1} and at 96 h 140.29 mg L^{-1} (Table 1). Among all the different organic solvent extract fractions, the ethanol extract of dried root powder of *P. fulgens* were more effective (Table 1). The 24 and 96 h LC_{50} of ethanol extract of dried root powder of *P. fulgens* against *I. exustus* were 112.75 and 91.48 mg L^{-1}, respectively. The column purified fractions of all the organic solvent extract fractions were highly toxic. The LC_{50} of the column purified fractions of dried root powder of *P. fulgens* at 24 h were 55.63 mg L^{-1}. The LC_{50} of column purified fraction of dried root powder of *P. fulgens* at 96 h were 33.75 mg L^{-1} (Table 1).

There was significant negative regression ($p<0.05$) between the exposure time and LC_{50} of the treatments (Table 1). The slope values given in Table were steep and the separate estimates of LC 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).

Table 1: Toxicity of *P. fulgens* their different organic extract and column purified against *I. exustus* at different time exposure

Exposure period (h)	Molluscicides	LC_{50}	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24	*P. fulgens* (DRP)	170.33	165.36	173.44	1.55±0.70	4.55	0.21	0.26
	Ether extract	138.11	135.66	142.75	1.42±0.28	3.27	0.28	0.20
	Chloroform extract	140.75	137.22	145.22	1.50±0.70	3.11	0.28	0.21
	Methanol extract	143.89	140.06	146.71	1.34±0.28	4.55	0.23	0.24
	Acetone extract	124.63	120.77	130.96	1.30±0.86	3.15	0.34	0.20
	Ethanol extract	112.75	107.44	118.52	1.58±0.36	4.50	0.33	0.19
	Column purified	55.63	48.96	58.19	1.48±0.27	4.33	0.25	0.22
48	*P. fulgens* (DRP)	168.37	164.26	171.69	1.95±0.33	3.68	0.24	0.26
	Ether extract	135.55	130.26	139.96	1.65±0.32	4.24	0.16	0.21
	Chloroform extract	136.92	131.75	142.69	2.45±0.38	4.26	0.17	0.25
	Methanol extract	138.12	134.02	141.77	1.48±0.32	4.16	0.22	0.27
	Acetone extract	120.66	117.85	126.96	1.67±0.50	4.66	0.26	0.21
	Ethanol extract	110.39	107.33	115.73	1.62±0.48	3.26	0.17	0.22
	Column purified	41.35	36.28	48.36	1.28±0.35	4.50	0.20	0.28
72	*P. fulgens* (DRP)	164.26	158.55	169.38	1.64±0.38	3.54	0.24	0.18
	Ether extract	133.66	129.61	138.48	1.38±0.26	4.26	0.25	0.31
	Chloroform extract	134.51	130.65	140.58	1.55±0.53	3.51	0.21	0.29
	Methanol extract	135.63	129.81	138.42	1.61±0.43	4.84	0.24	0.29
	Acetone extract	116.37	113.19	120.85	1.63±0.60	3.28	0.19	0.28
	Ethanol extract	102.75	98.67	108.66	1.79±0.35	4.45	0.20	0.26
	Column purified	36.93	32.75	40.96	1.75±0.65	4.50	0.22	0.22
96	*P. fulgens* (DRP)	140.29	137.26	145.39	1.82±0.30	4.85	0.25	0.31
	Ether extract	128.46	124.33	132.68	1.93±0.28	4.20	0.21	0.24
	Chloroform extract	125.36	122.69	130.36	1.78±0.36	3.28	0.22	0.30
	Methanol extract	127.92	124.71	129.85	1.54±0.75	4.5 5	0.26	0.25
	Acetone extract	110.75	106.55	115.63	1.80±0.32	4.33	0.20	0.21
	Ethanol extract	91.48	87.52	99.76	1.48±0.23	3.25	0.22	0.26
	Column purified	33.75	28.75	38.26	1.58±0.20	3.37	0.18	0.25

Six batches of ten *I. exustus* were exposed different concentration of the above molluscicides. Mortality was determined after every 24 h. Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments. Ts: Testing significant of the regression coefficient-*P. fulgens* (dried root powder)- 8.16++; ether extract- 12.11+; chloroform extract-13.52++; methanol-13.30++; acetone extract-10.21++; ethanol extract-13.23++; column purified-12.20+. LCL: Lower confidence limits, UCL: Upper confidence limits, DRP: Dried root powder, +: Linear regression between x and y, ++: Non-linear regression between log x and log y

The present study of result section clearly demonstrates that the dried root powder of *P. fulgens* is potent molluscicides. Toxicity study revealed that toxic components of *P. fulgens* are soluble in water and enter in the snail body fluids which caused motility of intermediate host snail *I. exustus*. Their toxic effects are concentration as well as time dependant as evident from negative regression between exposure time and LC_{50} of different treatments. The time dependent toxic effect of *P. fulgens* plant products may be either due to the uptake of the active moiety which progressively increases the amount of toxic active components in the snail body with increase in exposure period or it might be possible that the active compound could change into more toxic forms in the aquarium water or in the snail body fluids due to the action of various enzymes activities. Higher toxicity of ethanol extract among other organic extracts indicates that molluscicidal components present in plant *P. fulgens* are more soluble in ethanol.

The toxicity of *P. fulgens* plant products is time-dependent. It may be due to the uptake of the active moiety which progressively increases in snail body with increase in exposure period. Laloo *et al.*[25] has been reported the ethanolic root extract of *P. fulgens* preventing gastric ulcers in rats due to antihistaminic and H^+ K^+-ATPase inhibitory activities. The reduction in granular endoplasmic reticulum, swelling of nuclear membrane, disruption of chromatin material[31] and vacuolization in nucleus, as observed in the plant *P. fulgens* treated cestode, are indicative of protein synthesis inhibition. It may be possible that the different active component of *P. fulgens* in snail body could change the different enzyme activity. Ray *et al.*[32] has been studies that the alcoholic extract of dried root powder of *P. fulgens* reduced significantly vital tegumental enzyme activity of alkaline phosphatase, acid phosphatase and adenosine triphosphatase (ATPase) in cestodes parasite *Raillietina echinobothrida* and trematodes *Gastrothylax crumenifer*, respectively. The acid phosphatase is a lysosomal enzyme[33] plays an important role in catabolism pathological necrosis autolysis and phagocytosis[34]. The enzyme alkaline phosphatase plays a critical role in protein synthesis[35], shell formation[36], other secretary activities[37] and transport of metabolites[38] in gastropods. The root extract of *P. fulgens* is rich in polyphenolic components[25] with maximum quantity of phenolic tannins. The condensed tannins with proven has anthelmintic activity which have been reported in several anthelmintic plants[39,40] and are known to inhibit[41] endogenous enzyme activities. Jaitak *et al.*[27,] reported the root extract of *P. fulgens* contain high amount of tannin and flavonoid. Several tannin bearing different families of plants have molluscicidal properties[42].

It is evident from the steep slope values indicate that a small increase in the concentration of different treatment in Table 1 caused mortality 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 are within the limit at all probability level (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

This study discovered that the different products of the *P. fulgens* plant can be used as potent molluscicide as it is easily and ecologically more acceptable by livestock keepers that can be beneficial for the fascioliasis control program. This study providing valuable support for the isolation and identify of the active components and ingredients of this plant to understand its precise the mode of action as a molluscicidal component in snail body at molecular level.

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