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FASCIOLA LARVAE: ANTHELMINTIC ACTIVITY OF MEDICINAL PLANT *Potentilla fulgens* AGAINST SPOROCYST, REDIA AND CERCARIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author PK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KS and RNS managed the analyses of the study. Author DKS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Fascioliosis caused by trematode *Fasciola hepatica* and *F. gigantica* with complex life cycle, and primarily affect in both livestock and human. This disease occurs on all inhabited continents. Humans become infected after ingestion of contaminated food mostly aquatic wild vegetables or water. The life cycle of *Fasciola* can be interrupted by killing the vector snail or larva (sporocyst, redia and cercaria) in their habitats. *In vitro* toxicity of dried root powder of *Potentilla fulgens*, different organic extract and column purified fraction was performed in the Petridis. Infected snail *Lymnaea acuminata* was dissected in a glass Petridis which containing 10 ml of dechlorinated water at 23°C-25°C. These larvae were kept in dechlorinated tap water where they survive up to 48 h in laboratory condition. Mortality of sporocyst, redia and cercaria were observed after 2 h, 4 h, 6 h and 8 h of treatment. Counting of a larva was done with help of microscope. Per cent mortality of larvae at each concentration for 2 h, 4 h, 6 h and 8 h were used for determination of LC_{50}. In *in vitro* treatment of different organic extract and column purified fraction of *P. fulgens* was used. Highest toxicity was observed against sporocyst, redia and cercaria after treatment of column purified fraction (2 h LC_{50} was 62.42, 59.25 and 45.11 mg/L, respectively). After 8 h LC_{50} of column purified fraction against sporocyst, redia and cercaria were observed 54.20, 49.37 and 38.13 mg/L, respectively. The present study conclusively shows that medicinal plant *P. fulgens* have anthelmintic (sporocyst, redia and cercaria) larvicides activities against *F. gigantica*. It can use in *in vivo* treatment of infected host snails of *Fasciola* larvae within the body, which may be useful for control of fascioliasis without killing host snails.

Keywords: Fascioliasis; *Potentilla fulgens*; sporocyst; redia; cercaria.

1. INTRODUCTION

Fasciola, commonly known as the liver fluke, is a helminth parasite of mammals and a member of the Class Trematoda [1,2]. The parasite has a worldwide distribution and is considered an important disease of domestic livestock, especially in temperate climatic zones [3]. It infects in sheep, goat, cattle, horse, deer

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and humans as definitive hosts [4]. The adult worm infects the liver of various species of mammals, particularly livestock that consume fresh plant material contaminated with infective-stage metacercaria [5]. Two spices of *Fasciola hepatica* and *F. gigantica* is found thought the world with several outbreaks in humans from many countries [6]. Snail *Lymnaea acuminata* serves as intermediate host of *Fasciola* species [7]. The intermediate host of *F. gigantica* is a hermaphroditic snail *Lymnaea acuminata* inhabiting freshwater ponds. Incidence of endemic fascioliosis is very common in eastern part of the Uttar Pradesh in India [8-13]. The development of larval digenetic trematodes is complex process involving initial infection of the snail host by the free-swimming miracidium larvae, its sequent transformation to a parasite primary sporocyst stage, followed by asexual reproduction and release of secondary, sporocyst or redia and finally the eventual formation and release of cercaria the next free-swimming stage in the life cycle. Therefore, one of the possible approaches to control of the fascioliosis is too interrupted the life cycle of the parasitic trematodes by eliminating the larva (sporocyst, redia and cercaria) inside the snail body or killing the host snail. Now it is realized that if plant product are effective in *in vitro* treatment of *Fasciola* larvae (sporocyst, redia and cercaria) which are present in host snail, it may be useful in *in vivo* phytotherapy of infected host snail. Sunita and Singh, [10] have been reported that the plant derived active components are effective in *in vivo* phytotherapy of the snail. The active components of medicinal plant *Zingiber officinale* have larvicidal activity against *Fasciola* larvae sporocyst, redia and cercaria [10]. The pharmacological studies of *Potentilla fulgens* possesses antioxidant, hypoglycemic, anti-hyperglycemic, anti-hyperlipidemic, antitumor, antinflammatory, antiulcerogenic and molluscicidal properties [12,14], thus supporting its ethnotherapeutic use. The aim of the present study is to in *in vitro* larvicidal activity of *P. fulgens* against sporocyst, redia and cercaria larvae of *F. gigantica*.

2. MATERIALS AND METHODS

2.1 Collection of *Fasciola* Larvae

Adult snail *L. acuminata* (2.6±0.20 cm in length) were collected locally, cercaria shedding infected and uninfected snails were separated in two groups. The snails were allowed to acclimatize for 24 h in laboratory condition. Each infected snail was dissected in a glass Petridis containing 10 ml of dechlorinated water at 23°C-25°C. The pH of the water was 7.2-7.4 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.4-7.3 mg/L, 5.3- 6.5 mg/L and 103.0- 104.0 mg/L, respectively. After dissection sporocyst, redia and cercaria were separated in different Petridis containing 10 ml of dechlorinated water by the method of Sunita and Singh [10]. These larvae were kept in dechlorinated tap water where they survive up to 48h in laboratory condition.

2.2 Preparation of Crude Products

The fresh dried root of *Potentilla fulgens* were procured from local market in Gorakhpur, (UP) India. Dried root of *P. fulgens* were pulverized separately in the electric grinder and the crude powders thus obtained, were then sieved with the help of fine mesh cloth. This fine powder was then used separately for *in vitro* larvicidal activity of sporocyst, redia and cercaria.

2.3 Extraction of Crude Products

For each extraction two gram dried roots powder of plant *P. fulgens* were extracted with 200 ml of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone, and 95% ethanol at room temperature for 24h and chemicals purchased from Sigma Chemical Co. USA. Each preparation was filtered separately through sterilized Whatman No-1 filter paper (Whatman International Ltd, UK) and the filtered extracts where subsequently evaporated under vacuum [9]. The root powder of *P. fulgens* yielded 250 mg ethanol, 350 mg chloroform, 360 mg ether and 415 mg acetone extracts. The residues, thus obtained, were used in *in vitro* larvicidal activities for sporocyst, redia and cercaria larvae of *Fasciola*.

2.4 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 in *in vitro* larvicidal activities for sporocyst, redia and cercaria.

2.5 *In vitro* Toxicity Determination

In vitro toxicity of different organic root extract and column purified fraction *P. fulgens* was performed in the Petridis by the method of Sunita and Singh, [10]. Ten sporocyst, redia and cercaria larva of *Fasciola* were separated in different Petridis containing 10 ml dechlorinated tap water. Treatment of different

organic extract and column purified fraction was made directly in the Petridis containing 10 sporocyst/redia/cercaria larvae. Mortality of sporocyst, redia and cercaria were observed after 2h, 4h, 6h and 8h of treatment. Counting of larvae was done with help of binocular microscope. Per cent mortality of larvae at each concentration for 2h, 4h, 6h and 8h were used for determination of LC_{50}. Lethal value (LC_{50}), lower and upper confidence limits (LCL and UCL), slop-values, t-ratio, g value and heterogeneity factors were calculated with the help of POLO computer programme of Robertson et al. [15]. One way ANOVA and product moment correlation coefficient were done by the method of Sokal and Rohlf [16].

3. RESULTS

In *in vitro* larvicidal activity of different organic root extract and column purified fraction of *Potentilla fulgens* against sporocyst, redia and cercaria larva of *Fasciola gigantica* was time and concentration dependent from 2 h up to 8 h (Tables 1-3). In *in vitro* treatment, highest toxicity was noted against sporocyst, redia and cercaria 2 h LC_{50} of column purified fraction was 62.42, 59.25 and 45.11 mg/L, respectively and lowest toxicity was ethanol extract 66.28, 64.71 and 51.39 mg/L, respectively (Tables 1-3). Whereas, 8 h LC_{50} of column purified fraction, highest toxicity against sporocyst, redia and cercaria were observed 54.20, 49.37 and 38.13 mg/L, respectively and lowest toxicity was ethanol extract 58.53, 54.31 and 45.70 mg/L, respectively (Tables 1-3).

The slope values were steep and separate estimation of LC_{50} based on each six replicate were found to be within the 95% confidence limit of LC_{50} .The t-ratio was greater than 1.96 and the heterogeneity factor is less than 1.0. The g value was less than 0.5 at all probability levels (90, 95 and 99 respectively) (Tables 1-3).

Table 1. *In vitro* anthelmintic toxic effect of dried root powder, different organic extract and column purified fractions of *Potentilla fulgens* against the sporocyst larva

Exposure	Larvicidal (mg/ml)	LC_{50}	LCL	UCL	Slope -value	t-ratio	g-value	Heterogeneity
2 h	*Potentilla fulgens* dried root powder	81.23	78.90	90.80	0.15±0.31	2.28	0.26	0.11
	Ether extract	78.53	73.51	82.75	0.32±0.53	2.67	0.36	0.20
	Chloroform extract	75.12	70.15	80.23	0.96±0.50	2.48	0.43	0.13
	Methanol extract	70.94	69.21	76.38	0.29±0.02	2.33	0.68	0.15
	Acetone extract	68.02	58.35	73.23	0.22±0.36	4.46	0.40	0.18
	Ethanol extract	66.28	61.40	72.91	0.44±0.39	2.10	0.12	0.13
	Column purified	60.42	58.33	68.12	0.31±0.16	2.31	0.36	0.23
4 h	*Potentilla fulgens* dried root powder	79.25	74.60	82.30	0.66±0.40	3.25	0.39	0.21
	Ether extract	76.13	71.50	80.55	0.66±0.30	3.50	0.32	0.10
	Chloroform extract	72.33	69.71	78.45	0.32±0.35	2.70	0.41	0.14
	Methanol extract	68.13	64.61	73.70	0.98±0.42	2.51	0.23	0.18
	Acetone extract	64.39	61.62	70.51	0.79±0.20	2.31	0.48	0.40
	Ethanol extract	63.22	58.20	66.28	0.43±0.77	3.43	0.14	0.37
	Column purified	58.30	53.15	62.74	0.47±0.24	2.41	0.30	0.10
6 h	*Potentilla fulgens* dried root powder	78.01	72.20	85.25	0.12±0.31	3.20	0.42	0.25
	Ether extract	74.52	69.40	78.91	0.46±0.70	2.41	0.41	0.16
	Chloroform extract	70.38	66.37	78.62	0.80±0.95	3.70	0.57	0.19
	Methanol extract	65.38	59.22	70.90	0.31±0.32	3.20	0.30	0.16
	Acetone extract	62.42	58.27	70.61	0.32±0.19	2.58	0.41	0.18
	Ethanol extract	61.13	57.30	64.80	0.34±0.33	3.31	0.62	0.12
	Column purified	41.39	38.40	58.97	0.38±0.23	3.20	0.48	0.15
8 h	*Potentilla fulgens* dried root powder	57.15	49.24	68.60	0.83±0.52	2.15	0.42	0.17
	Ether extract	72.21	68.33	78.50	0.97±0.80	3.40	0.76	0.12
	Chloroform extract	68.11	60.40	74.38	0.41±0.72	3.95	0.20	0.10
	Methanol extract	62.30	57.51	68.40	0.43±0.18	3.11	0.66	0.15
	Acetone extract	59.81	55.34	66.51	0.38±0.03	2.85	0.48	0.18
	Ethanol extract	58.53	55.90	64.39	0.66±0.38	2.76	0.43	0.18
	Column purified	54.20	48.80	58.20	0.25±0.25	2.44	0.51	0.20

Abbreviation: LCL- Lower Confidence Limits, UCL-Upper Confidence Limits.

Six batches of 10 redia larva were exposed different concentration of the above molluscicides treatments. Mortality was recorded every 2 h. Concentration given are the final concentration (W/V) in the glass aquarium water. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. TS- testing significant of the regression coefficient

Table 2. *In vitro* anthelmintic toxic effect of dried root powder, different organic extract and column purified fractions of *Potentilla fulgens* against the redia larva

Exposure	Larvicidal (mg/ml)	LC_{50}	LCL	UCL	Slope - value	t-ratio	g-value	Heterogeneity
2 h	*Potentilla fulgens* dried root powder	80.60	74.34	90.73	0.16±0.32	2.20	0.31	0.41
	Ether extract	79.21	70.30	85.12	0.56±0.50	3.17	0.62	0.36
	Chloroform extract	77.70	69.93	85.99	0.70±0.33	2.48	0.64	0.40
	Methanol extract	72.11	67.90	76.92	0.55±0.52	2.38	0.45	0.41
	Acetone extract	68.50	60.55	73.21	0.41±0.36	3.46	0.45	0.50
	Ethanol extract	64.71	59.80	68.39	0.50±0.40	3.80	0.72	0.29
	Column purified	59.25	54.18	65.25	0.48±0.71	3.71	0.71	0.36
4 h	*Potentilla fulgens* dried root powder	78.20	70.40	88.41	0.37±0.32	2.25	0.23	0.31
	Ether extract	77.25	68.71	85.61	0.78±0.34	2.41	0.52	0.30
	Chloroform extract	74.45	65.80	79.90	0.55±0.70	3.63	0.30	0.33
	Methanol extract	69.55	60.23	77.51	0.51±0.32	3.21	0.61	0.82
	Acetone extract	65.23	58.50	74.30	0.72±0.52	2.41	0.44	0.40
	Ethanol extract	61.50	55.22	70.32	0.80±0.71	3.57	0.45	0.14
	Column purified	56.72	52.70	69.81	0.53±0.31	3.42	0.32	0.30
6 h	*Potentilla fulgens* dried root powder	76.30	67.70	68.99	0.20±0.36	3.30	0.40	0.56
	Ether extract	74.20	71.53	79.30	0.58±0.71	3.38	0.32	0.12
	Chloroform extract	71.33	68.50	76.31	0.72±0.28	2.70	0.70	0.60
	Methanol extract	66.31	61.35	70.66	0.71±0.20	3.24	0.20	0.34
	Acetone extract	61.33	58.40	65.20	0.45±0.15	3.51	0.54	0.25
	Ethanol extract	58.33	52.60	64.76	0.30±0.25	3.80	0.35	0.15
	Column purified	53.27	49.14	59.23	0.31±0.53	3.22	0.25	0.24
8 h	*Potentilla fulgens* dried root powder	74.61	69.21	79.21	0.90±0.32	2.23	0.71	0.53
	Ether extract	71.20	66.22	79.90	0.27±0.85	3.81	0.40	0.36
	Chloroform extract	69.33	61.17	78.80	0.41±0.78	3.74	0.91	0.38
	Methanol extract	61.40	58.90	66.90	0.52±0.87	3.83	0.68	0.19
	Acetone extract	68.31	61.35	75.34	0.30±0.73	2.18	0.33	0.45
	Ethanol extract	54.31	49.61	59.73	0.61±0.38	3.35	0.61	0.11
	Column purified	49.37	35.63	56.11	0.25±0.28	3.40	0.48	0.44

Abbreviation: LCL- Lower Confidence Limits, UCL-Upper Confidence Limits.

Six batches of 10 redia larva were exposed different concentration of the above molluscicides treatments. Mortality was recorded every 2 h. Concentration given are the final concentration (W/V) in the glass aquarium water. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. TS- testing significant of the regression coefficient

Table 3. *In vitro* anthelmintic toxic effect of dried root powder, different organic extract and column purified fractions of *Potentilla fulgens* against the cercaria larva

Exposure	Larvicidal (mg/ml)	LC_{50}	LCL	UCL	Slope - value	t-ratio	g-value	Heterogeneity
2 h	*Potentilla fulgens* dried root powder	65.28	60.41	71.41	0.59±0.31	3.20	0.41	0.31
	Ether extract	63.30	58.26	71.60	0.34±0.51	3.31	0.17	0.38
	Chloroform extract	59.90	56.20	64.02	0.58±0.42	3.26	0.16	0.27
	Methanol extract	57.60	54.31	62.16	0.70±0.42	2.40	0.39	0.61
	Acetone extract	55.20	49.90	61.30	0.91±0.40	3.12	0.37	0.40
	Ethanol extract	51.39	47.38	56.70	0.53±0.66	3.58	0.72	0.25
	Column purified	45.11	41.90	52.40	0.68±0.30	2.41	0.46	0.30
4 h	*Potentilla fulgens* dried root powder	64.75	60.35	69.31	0.61±0.46	3.14	0.22	0.41
	Ether extract	61.60	58.20	65.40	0.53±0.31	2.52	0.33	0.22
	Chloroform extract	57.36	51.28	62.24	0.90±0.30	3.61	0.71	0.41
	Methanol extract	56.61	49.36	61.33	0.41±0.55	2.71	0.63	0.13
	Acetone extract	54.90	47.50	58.61	0.70±0.33	3.43	0.46	0.25
	Ethanol extract	49.30	45.29	55.90	0.51±0.60	2.18	0.28	0.28
	Column purified	43.50	39.20	51.68	0.59±0.38	3.40	0.38	0.39

Exposure	Larvicidal (mg/ml)	LC_{50}	LCL	UCL	Slope - value	t-ratio	g-value	Heterogeneity
6 h	*Potentilla fulgens* dried root powder	60.11	56.33	66.40	0.71±0.38	3.21	0.30	0.75
	Ether extract	58.36	54.21	65.21	0.48±0.81	3.55	0.51	0.39
	Chloroform extract	55.60	50.38	62.20	0.72±0.50	2.36	0.36	0.40
	Methanol extract	54.29	48.37	61.21	0.35±0.40	2.30	0.20	0.71
	Acetone extract	53.57	47.22	55.41	0.38±0.66	3.48	0.26	0.50
	Ethanol extract	47.32	44.25	51.23	0.36±0.40	3.41	0.81	0.68
	Column purified	40.55	37.20	47.90	0.51±0.86	3.25	0.20	0.32
8 h	*Potentilla fulgens* dried root powder	58.36	55.31	64.92	0.71±0.63	2.13	0.35	0.64
	Ether extract	55.20	48.23	61.25	0.60±0.51	3.19	0.81	0.70
	Chloroform extract	52.62	46.50	60.15	0.42±0.53	2.80	0.37	0.22
	Methanol extract	53.60	45.29	57.90	0.56±0.45	3.44	0.60	0.44
	Acetone extract	50.30	42.81	51.39	0.31±0.63	3.63	0.41	0.48
	Ethanol extract	45.70	40.63	49.33	0.60±0.79	2.52	0.33	0.41
	Column purified	38.13	33.21	45.55	0.20±0.35	3.64	0.55	0.35

Abbreviation: LCL- Lower Confidence Limits, UCL-Upper Confidence Limits.

Six batches of 10 redia larva were exposed different concentration of the above molluscicides treatments. Mortality was recorded every 2 h. Concentration given are the final concentration (W/V) in the glass aquarium water. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. TS- testing significant of the regression coefficient

4. DISCUSSION

The present study in *in vitro* toxicity of different organic root extract and column purified fraction of *Potentilla fulgens* against sporocyst, redia and cercaria larva of *Fasciola gigantica* was significantly ($p<0.05$) change with respect to time and concentration. In *in vitro* treatment, highest toxicity was noted against sporocyst, redia and cercaria 2 h LC_{50} of column purified fraction was 62.42, 59.25 and 45.11 mg/L, respectively. Whereas, 8h LC_{50} of column purified fraction, highest toxicity against sporocyst, redia and cercaria were observed 54.20, 49.37 and 38.13 mg/L, respectively. It may be possible that the different active component of *P. fulgens* become more soluble in water along with time and concentration and diffuses in the sporocyst, redia and cercaria larvae of *F. gigantica* which causing high mortality. Hemalatha et al. [17] has been demonstrated that the ethanolic root extract of *P. fulgens* preventing gastric ulcers in rats due to anthihistaminic and H^+ K^+ -ATPase inhibitory activities. It may be possible that the different active component of *P. fulgens* in larval body could change the enzyme activity. Roy et al. [18] has been studies that alcoholic extract of dried root powder of *P. fulgens* reduced significantly vital tegumental enzyme activity of acid phosphatase, alkaline phosphatase and adenosine triphosphatase (ATPase) in cestodes parasite *Raillietina echinobothrida* and trematodes *Gastrothylax crumenifer*, respectively. Previously different experimental studies of *P. fulgens* have revealed that its root extract possesses antitumor [19], antioxidant [20], anthelmintic [18] and gastroprotective [17] activities. *P. fulgens* root extract is rich in polyphenolic components [17] with the maximum quantity of phenolic tannins. Jaitak, et al. [20] reported that the root extract of *P. fulgens* contain high amount of tannin and flavonoid. *In vivo* and *in vitro* studies have been evidences to support the anthelmintic effect which feed tannins and other polyphenols against abomasal and intestinal parasitic nematodes [21,22]. Athanasiadou et al. [23] has been reported that tannin shows anthelmintic activities against infected sheep with *Trichostrongylus colubriformis* and causing larval death. In *in vitro* tannin inactivates the enzyme activities of *Trichostrongylus colubriformis* larvae which are responsible for hatching and development [24]. Plant (*Onobrychis viciifolia*) derived essential bioactive component flavonoids have *in vitro* anthelmintic activity against larvae of *Haemonchus contortus* [25]. The root extract of *P. fulgens* in *in vito* inhibit enzymes such as amylase, α-glucosidase, β-glucosidase, and lipase in the liver, kidney and eye lens of diabetic mice [26]. Many numbers of medicinal plants have been used to treat parasitic infection in animal and man [27]. Present study clearly demonstrated that active component of *P. fulgens* can be use for the control of the different stages of the *Fasciola* larvae in the host snail.

The steep slope value indicates that a small increase in the concentration of plant derived larvicides caused higher larval mortality. Heterogeneity factor value less than 1.0 denote that in the replicate test of random sample the concentration response limits and thus the model fits the data adequately. A t- ratio value greater than 1.96 indicates that the regression is significant. The index of significance of the potency estimation g indicates that the value of mean is within

the limit at all probability level (90, 95, and 99, respectively) since it is less than 0.5.

5. CONCLUSION

It can be concluded from the present study that medicinal plant *Potentilla fulgens* and their different organic extract in *in vitro* significantly killed the sporocyst, redia and cercaria larva of *F. gigantica*. Plant products are easily available, safer for non target organisms, biodegradable and eco-friendly. It may be one of the new approaches in *in vivo* treatment of *Fasciola* larval infected snail without killing of host snail for the control of epidemic fascioliasis programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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