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Pithecellobium dulce Used as Anthelmintic *in vitro* against Sporocyst, Redia, and Cercaria Larva of *Fasciola gigantica*

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Abstract

The aim of the present study is to evaluate *in vitro* anthelmintic larvicidal activities of the medicinal plant *Pithecellobium dulce* against *Fasciola gigantica* (sporocyst, redia, and cercaria) larvae. Zoonotic diseases are worldwide in cattle and human populations. It can be controlled by the break of the life cycle in the host snail *Lymnaea acuminata*. The exposure of leaf powder and, various extracts, of *P. dulce,* was observed for up to 8h at various concentrations against sporocyst, redia, and cercaria larvae. The anthelmintic larvicidal effect of various preparations was observed at 2, 4, 6, and 8h of the exposure. The exposure of dried leaf powder *in vitro* of the *P. dulce* was more effective against the redia (2h LC_{50} 82.76mg/L and 8h LC_{50} 75.88mg/L). The exposure of column extract of dried leave powder of *P. dulce* against sporocyst, redia, and cercaria in 2h LC_{50} was 52.09, 56.09, and 62.21 mg/L and 8h LC_{50} was 76.19, 64.71, and 72.25 mg/mL, respectively. The ethanolic extract and ethanolic column extract of the *P. dulce* leaves powder have better anthelmintic larvicidal activities than the other organic extract.

***Key words*:** Anthelmintic activity, *Pithecellobium dulce, Lymnaea acuminata,* Sporocyst, Redia, Cercaria

Zoonotic diseases are caused by *Fasciola hepatica* and *Fasciola gigantica* in ruminants, and in humans with complex life cycles in the fresh-water host snail *Lymnaea acuminata* [1-3]. This disease occurs on all inhabited continents and worldwide [2], [4]. Humans can be infected after the ingestion of contaminated water or aquatic wild vegetables [6]. This disease damages the liver of the animals and causes liver cirrhosis which decreases their growth, development, and body weight, higher mortality, and low production of milk, meat, and wool [3], [7]. The spreading of fascioliasis worldwide is concerned with global exchanges and the movement of infected animals [8-10]. Initially, host snails are infected through a free-swimming miracidium larva that develops sporocyst, redia, and cercaria in the snail body. These larval stages transform into a parasite stage, followed by asexual reproduction. Therefore, one of the strategic techniques to control fascioliasis by breaks their life cycle at various larval stages.

Anthelmintic drugs are frequently used as larvicides for the control of *Fasciola* larvae, but they develop resistance and caused adverse impacts on the aquatic ecosystem [11]. The plant-derived phytochemicals are easily available, biodegradable, and eco-friendly. Medicinal plants play the most important role in traditional medicine in various developing countries. The common Indian medicinal plant *Pithecellobium dulce* is commonly known as Indian jalebi or Indian sweet jalebi [12], Jungal jalebi [13], the leaves of the plant contain phytochemicals like cyclitol, dulcitol, α-spinasterol, octacosanol, kaempferol-3-rhamnoside, afzelin, and quercetin [14]. The leaves can be applied as plasters for pain and veneral sores [15]. Traditionally the leaves of *Pithecellobium dulce* are used for intestinal disorder, peptic ulcer, tooth ache, earache, emollient, leprosy, abortifacient, and larvicidal properties [16]. In the present study, the leaves extracts of the medicinal plant *Pithecellobium dulce* were subjected for the determination of *in vitro* anthelmintic larvicidal activity of dried leaves powder their various organic extracts, and column purified extract against sporocyst, redia, and cercaria larvae.

MATERIALS AND METHODS

Collection of infected host snails

Adult snail *Lymnaea acuminata* (2.5 ± 0.21 cm in length) were collected locally from ponds and low-lying areas. Infected snails have often followed the allocation of more resources to growth with the result the infected snails can grow large than uninfected snails [17], locomotion is very slow than uninfected ones, and it appeared yellowish spots, foots are swollen and shedding cercaria were appeared at the mouth of snails and shell morphology is changed in infected snails [18-20].

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Dissection and separation of larvae

Each infected snail was dissected in a clean glass Petri dish containing 10 ml of dechlorinated tap water at 24 °C-26 °C under a dissecting light microscope. The larva of the sporocyst, redia, and cercaria were carefully separated through droppers in different Petri dishes containing 10 ml of dechlorinated tap water. These larvae were kept in dechlorinated tap water where they survive up to 48h in laboratory conditions.

Leaves crude powder

The leaves of *P. dulce* were collected from the college campus. The leaves were washed with water, and dried under shade after complete drying, the dried leaves materials were grinded in an electric grinder machine and the crude powder thus obtained was then sieved with the help of a fine mesh cloth. The collected crude powder was stored in a small glass container in laboratory condition.

Organic extracts of crude powder

Ten-gram dried leaves powder *P. dulce* was extracted with 200 mL of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone, and 95% ethanol at laboratory condition (26 °C) for 48 h (hours). Each preparation was filtered separately through sterilized Whatman No-1 filter paper and the filtered extracts were subsequently evaporated under a vacuum machine. The residues, thus obtained, were used for the determination of larvicidal activity. The stem powder of *P. dulce* yielded 112 mg ethanol, 116 mg chloroform, 169 mg ether, and 185 mg acetone extracts.

Column extracts

Five hundred milliliter of ethanol extract purification of dried leave powder of *P. dulce* was subjected to silica gel (60-120 mesh, Qualigens Glass, Precious Electrochemidus Private Limited, Bombay, India) chromatography through a 5×45 cm glass column. Five-milliliter fractions eluted with ethanol (95%) were collected in a small glass bottle. Organic solvent was evaporated under a vacuum machine and the remaining solids extracts obtained were used for the experiment.

Experimental assay

The anthelmintic larvicidal activity of the dried leaves powder, organic extract (ether, chloroform, methanol, acetone, and ethanol), and column extract was performed in the Petri dish by the method of Sunita and Singh [17]. Ten sporocysts, redia, and cercaria larvae were kept in the different Petri dishes containing 10 ml of tap water at laboratory conditions. The exposure of dried leave powder, organic extracts, and column extract were used directly in the Petri dish which contain 10 sporocyst/ redia/ cercaria. Each set-up of the experiment was replicated six times for statistical analysis. The mortality of sporocyst, redia, and cercaria in vitro was observed after 2h, 4h, 6h, and 8h of exposure. In the control group of the experiment, no treatments were given in the Petri dish. Usually, *in vitro* conditions, *Fasciola* larvae can survive up to 48h in tap water under laboratory conditions. Counting of dead and live larvae in treated and control groups was performed with the help of a light microscope.

The value of LC_{50}, lower and upper confidence limit (LCL and UCL), and t-ratio were calculated with the help of a POLO computer program [21].

RESULTS AND DISCUSSION

In this study, we have observed that *in vitro* larvicidal effect of dried leaves powder of *P. dulce*, their various organic extract, and column extract against sporocyst, redia, and cercaria larvae was time and concentration-dependent (Table 1). The exposure of dried leaf powder *in vitro* of the *P. dulce* was more effective against the redia (2h LC_{50} 82.76mg/L and 8h LC_{50} 75.88mg/L) (Table 1). Therefore, the 8h LC_{50} of ethanol extract against sporocyst, redia, and cercaria was 80.53, 83.17, and 70.26 mg/L, respectively (Table 1). However, a higher effect was observed against cercaria larvae. The exposure of column extract of dried leave powder of *P. dulce* against sporocyst, redia, and cercaria in 2h LC_{50} was 52.09, 56.09, and 62.21 mg/L and 8h LC_{50} was 76.19, 64.71, and 72.25 mg/mL, respectively (Table 1). The high effects of column extract were observed against redia larva (8h LC_{50} 64.72 mg/L) of *Fasciola*. A significant ($p<0.05$) negative regression was noted between the LC_{50} and exposure period of various preparations of the *P. dulce* against sporocyst, redia, and cercaria. Therefore, no mortality was observed in the control group.

Plants produce a variety of phytochemicals such as dulcitol, cyclitol, octacosanol, α-spinasterol, afzelin, quercetin, and kaempferol-3-rhamnoside is a promising source of antimicrobial and larvicidal products. *In vitro* exposure of dried leaves powder and different organic extracts of *P. dulce* have toxic properties against sporocyst, redia, and cercaria larvae of the *F. gigantica*. The leaves of *P. dulce* are used as antibacterial and antifungal products [22]. The aqueous and ethanolic leave extract of *P. dulce* have free radical-scavenging, and antimycobacterial properties [23]. Therefore, the present study was undertaken to investigate of anthelmintic larvicidal activity of the leaf extract of *P. dulce*. The phytochemicals Kaempferol-3-O-α-L-rhamopyranoside (afzelin) were isolated from the leaves of *P. dulce* which has antimycobacterial properties [24]. The ethanolic leaf extract of *P. dulce* contains major phytochemicals like alkaloids, anthraquinones, cardiac glycosides, tannins, proteins, sugar, and terpenoids [25]. However, secondary metabolites like tannins and other compounds of phenolic nature have antimicrobial properties [25]. The alkaloids reported from leaves of *P. dulce* are belonging to different groups of the phytochemicals, such as aporphine and benzoquinazoline which have various medicinal values.

The phytochemicals of *P. dulce* may be diffused in the larval body either which progressively increases along with concentration and exposure period in the larvae. The presence of cyclitol, dulcitol, afzelin, alkaloids, cardic glycosides, anthraquinones, and tannins phytochemicals may be responsible for anthelmintic larvicidal effects on the sporocyst, redia, and cercaria larvae. The results indicate that *P. dulce* leaf extracts have a significant amount of anthelmintic phytochemicals which easily diffused in larvae and caused toxic effects. The maximum anthelmintic effect was observed in the ethanol extract among other organic extracts that denoted the active phytochemicals of leave extract of *P. dulce* are more soluble in ethanol. After the exposure of extracts in water, it may be possible that the phytochemicals of *P. dulce* cause an impact on a few enzyme actions in larvae and caused moiety.

The lower confidence limits (LCL) and upper confidence limits (UCL) values of LC_{50} are significantly variable in all the exposure periods (Table 1). The t-ratio value is greater than 1.96 which denoted that the regression is significant in all groups of the experiment along with various concentrations.

CONCLUSION

The results of the present study reveal that dried leaf powder of *Pithecellobium dulce* and their various organic

extract have anthelmintic larvicidal properties against sporocyst, redia, and cercaria larvae of *Fasciola gigantica*. The various formulations of *P. dulce* leaves can be used for the control of zoonotic disease. However, the ethanolic extract and ethanolic column extract of the *Pithecellobium dulce* leaves powder have better anthelmintic larvicidal activities than the other organic extract. Therefore, further investigation needs to be carried out that how the phytochemicals of this plant caused effects on the *Fasciola* larvae.

Conflict of interest

The authors have no conflict of interest.

Table 1 *In vitro* toxicity of dried stem powder, various organic extracts, and column extract of *Pithecellobium dulce* was observed against sporocyst, redia, and cercaria larva

Prepared larvicides (mg. L^{-1})	Larvae	2h Exposure				4h Exposure				6h Exposure				8h Exposure			
		LC_{50} value	LCL	UCL	't'-ratio	LC_{50} value	LCL	UCL	't'-ratio	LC_{50} value	LCL	UCL	't'-ratio	LC_{50} value	LCL	UCL	't'-ratio
P. dulce	Spo	85.15	65.12	96.18	2.37	85.01	68.32	91.34	2.60	83.25	70.22	97.59	2.17	82.24	63.20	95.41	2.73
(DLP)	Red	82.76	62.13	93.11	2.11	81.04	61.53	94.21	2.48	79.34	62.42	99.22	2.35	75.88	54.38	88.40	3.13
	Cir	82.88	71.22	91.90	2.12	80.73	68.71	93.50	2.16	78.34	65.31	82.21	2.16	77.54	60.13	85.12	2.53
Et-Ext	Spo	79.24	55.41	99.30	2.80	77.33	61.23	89.26	2.07	75.88	58.38	95.32	2.58	73.21	58.10	91.20	2.53
	Red	78.45	61.34	91.40	2.34	77.74	58.34	98.14	2.19	75.36	64.30	89.55	2.15	73.28	51.18	87.19	3.22
	Cir	78.45	69.15	88.34	2.43	76.63	55.60	89.21	2.27	75.53	61.24	86.13	2.42	73.34	62.38	87.16	2.27
Ch-Ext	Spo	78.41	57.11	94.20	2.13	77.34	60.18	92.18	2.50	75.88	59.28	98.08	3.22	73.29	52.35	94.51	2.38
	Red	77.90	60.12	92.31	2.41	75.22	57.30	88.25	2.24	73.88	62.76	86.39	2.40	71.22	54.22	86.32	2.16
	Cir	77.70	66.12	89.21	2.32	75.28	57.31	88.64	3.18	73.19	59.42	88.36	3.18	72.16	60.21	88.22	3.11
Me-Ext	Spo	78.56	57.32	92.33	2.32	75.74	64.35	94.50	2.21	73.56	56.22	96.32	2.70	71.76	51.02	95.23	2.51
	Red	78.14	63.14	93.30	3.44	75.39	59.21	89.35	2.06	73.34	65.60	87.51	2.37	70.84	53.88	94.21	2.24
	Cir	78.40	64.24	91.34	3.62	75.38	55.80	82.30	3.19	73.05	58.16	85.90	3.22	70.25	51.14	89.71	2.31
Ac-Ext	Spo	77.32	55.22	94.51	2.41	76.94	58.93	83.24	3.36	74.11	52.14	98.22	2.83	72.18	53.08	97.32	2.11
	Red	75.76	61.22	92.15	3.16	74.56	57.23	88.41	3.02	72.34	64.37	88.80	2.34	70.10	51.89	81.02	2.11
	Cir	75.88	63.11	90.25	3.28	73.02	49.35	81.24	2.41	70.60	53.22	81.16	3.17	68.29	49.53	78.40	2.60
Eth-Ext	Spo	73.53	54.13	89.14	2.50	70.37	48.11	87.31	2.31	68.35	48.70	85.09	2.70	64.45	46.24	80.53	2.35
	Red	72.56	60.50	91.36	2.29	70.45	55.22	85.88	2.63	68.35	56.45	87.30	2.18	65.12	50.29	83.17	2.35
	Cir	70.22	61.44	89.16	2.52	69.70	51.20	80.60	2.40	67.45	48.21	75.40	3.43	65.56	48.14	70.26	3.14
CE	Spo	52.03	48.63	84.35	2.21	51.13	42.52	72.32	2.73	49.65	31.49	67.11	2.47	47.88	38.55	76.19	2.38
	Red	56.09	42.64	82.40	2.51	53.20	45.12	76.13	2.13	51.11	43.61	73.15	3.16	48.36	34.22	64.71	2.13
	Cir	65.21	55.70	76.30	2.19	62.74	48.32	73.21	2.24	60.13	47.41	77.23	2.19	56.37	39.81	72.25	2.14

In each batch, 10 sporocyst, redia, and cercaria larvae separately were treated in various concentrations in six replicates of the above preparations

The larval mortality was observed every 2hrs up to 8hrs exposure.

Abbreviations: DSP=Dried stem powder; Et-Ext=Ether extract; Ch-Ext=Chloroform extract; Me-Ext=Methanol extract; Ac-Ext=Acetone extract; Eth-Ext= Ethanol extract; CE=Column extract; Spo=Sporocyst; Red=Redia; Cir=Cercaria; LC=Lethal concentration; LCL=Lower confidence limits; UCL=Upper confidence limits

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