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In vitro use of Organic extracts of *Tinospora cordifolia* as anthelmintic against *Fasciola gigantica* larvae

Dharmendra Kumar Yadav[1] and *Pradeep Kumar[2]

[1]Department of Zoology, S.G.N. Government P.G. College, Muhammadabad, GOHANA, MAU-276403 (U.P.), INDIA
[2]Department of Zoology, Government Degree College SUKRAULI, KUSHINAGAR-274207 (U.P.), INDIA
*Corresponding Author
E-mail : pkumar_gpu@yahoo.co.in

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ABSTRACT

This study aims to evaluate the *in vitro* anthelmintic larvicidal efficacy of *Tinospora cordifolia* against *Fasciola gigantica* larvae (sporocyst, redia, and cercaria). Fascioliasis caused high economic loss in cattle as well as in the human population. It can be controlled by the break of the life cycle of the *Fasciola* in the host snail *Lymnaea acuminata*. *In vitro,* exposure of dried stem powder (DSP), various organic extracts, and column purified extract (CPE) of *T. cordifolia* was observed upto 8h exposure at various concentrations against *F. gigantica* larva. The toxic effect of various preparations against larva was observed at 2, 4, 6, and 8h of the treatment. The column purified extract of dried stem powder of *T. cordifolia* against sporocyst, redia, and cercaria in 2h LC_{50} was 50.27, 55.26, and 53.11 mg/L and 8h LC_{50} was 48.20, 48.37, and 48.12 mg/mL, respectively. Maximum effects of column purified of *T. cordifolia* were observed against sporocyst larva (8h LC_{50} 45.20 mg/L) of *F. gigantica*.

Figure : 00 References : 40 Tables : 03
KEY WORDS : Cercaria, *Fasciola*, Redia, Snail, Sporocyst, *Tinospora cordifolia*.

Introduction

Fasciola gigantica caused a significant economic loss in ruminants (cattle, sheep, goats, pigs, horses, and rabbits) and in humans infect fascioliasis infection worldwide[21,22,33]. The impact of fascioliasis is very high due to its pathogenicity[20]. Human fascioliasis is caused by *F. hepatica* and *F. gigantica* began to show its importance over several decades, with the progressive description of many human endemic areas[19,21]. The prevalence of zoonotic disease in animals is as high as 30 to 90% in Africa, 25 to 90% in Indonesia, and 94% in the eastern part of Uttar Pradesh, India[1,2]. In India, human fascioliasis has been reported in the state of Assam, Bihar, Maharashtra, Uttar Pradesh, Arunachal Pradesh, and West Bengal[3,4,26,29,38]. Freshwater host snail *Lymnaea acuminata* is an intermediate host of *Fasciola* species[14-16,39]. In host snails the digenetic trematodes larval development is complex involving initial infection of the fresh-water snail by the free-swimming miracidium larva. These larval stages transform into a parasite sporocyst larval stage, followed by asexual reproduction and producing sporocyst or redia, and finally the eventual formation and release of cercaria the next free-swimming larval stage. Therefore, one of the strategic approaches to control the fascioliasis infection is to break their life cycle by the elimination of the larval stages.

Many synthetic chemicals are frequently used as larvicides for the control of trematodes larvae, but they develop a resistance and adverse effect on the aquatic ecosystem and other organisms[35, 36]. Plant-derived larvicides are biodegradable, easily available, and eco-friendly. The medicinal plant *Tinospora cordifolia* commonly known as “Giloe”, stem is softly wooded that is traditionally used as an anti-inflammatory, for diabetes, jaundice, urinary tract infections, seminal weakness, skin disease, fever, expectorant, carminative, aphrodisiae, and anti-stress[24]. The extract of *T. cordifolia* stem has also exhibited *in vitro* inactivating properties against Hepatitis-B, and Hepatitis-E[23]. There are several reports available for the various uses of *T. cordifolia* as an anti-inflammatory, anti-allergic, anti-cancerous, immunomodulatory, immunosuppressive, hypoglycemic, and anthelmintic activity[12]. The aqueous and ethanolic extracts of *T. cordifolia in vitro* have anthelmintic activities against *Eisenia foetida*[13]. The aim of the present study is to observe *in vitro* anthelmintic larvicidal activity of dried stem powder their various organic extracts and

TABLE-1: *In vitro* toxicity of dried stem powder, various organic extracts, and column purified fraction of *Tinospora cordifolia* was observed against sporocyst larva

Exposure period	Preparations of Larvicides (mg.L$^{-1)}$	LC_{50} -value	LCL	UCL	S-value	't' -ratio	g -value	HF -value
2h	*T. cordifolia* (DSP)	82.20	60.11	94.45	0.12±0.44	2.17	0.56	0.15
	Ether-Ext	78.44	55.50	83.12	0.30±0.55	2.24	0.66	0.18
	Chloroform-Ext	78.30	60.23	85.36	0.40±0.33	2.15	0.40	0.12
	Methanol-Ext	76.90	63.13	84.30	0.34±0.18	2.56	0.39	0.15
	Acetone-Ext	74.39	61.14	82.60	0.34±0.14	3.22	0.42	0.18
	Ethanol-Ext	65.28	55.31	74.24	0.24±0.38	2.45	0.38	0.19
	Column Purified-Ext (CPE)	50.27	41.30	56.12	0.22±0.23	2.55	0.46	0.11
4h	*T. cordifolia* (DSP)	80.21	72.23	96.53	0.35±0.18	2.65	0.32	0.18
	Ether-Ext	77.12	66.50	85.36	0.18±0.62	3.23	0.37	0.12
	Chloroform-Ext	75.02	69.23	86.25	0.30±0.17	2.23	0.43	0.16
	Methanol-Ext	75.66	70.18	82.45	0.77±0.32	3.45	0.23	0.13
	Acetone-Ext	73.27	66.23	80.50	0.71±0.35	2.16	0.48	0.18
	Ethanol-Ext	63.25	55.20	70.28	0.26±0.35	2.34	0.42	0.12
	Column Purified-Ext (CPE)	49.11	37.23	59.70	0.17±0.24	2.35	0.34	0.14
6h	*T. cordifolia* (DSP)	78.24	66.27	90.20	0.34±0.88	2.20	0.46	0.20
	Ether-Ext	75.11	58.42	85.34	0.16±0.64	2.16	0.41	0.12
	Chloroform-Ext	77.66	67.30	83.22	0.12±0.15	2.35	0.38	0.14
	Methanol-Ext	73.30	66.88	79.90	0.30±0.81	2.18	0.33	0.11
	Acetone-Ext	73.02	64.20	81.60	0.26±0.22	2.22	0.41	0.13
	Ethanol-Ext	60.99	52.38	75.82	0.22±0.60	3.18	0.26	0.12
	Column Purified-Ext (CPE)	47.30	39.45	59.90	0.45±0.15	2.20	0.45	0.14
8h	*T. cordifolia* (DSP)	77.40	65.23	88.60	0.54±0.23	2.18	0.38	0.18
	Ether-Ext	73.35	63.71	89.51	0.34±0.55	2.72	0.32	0.16
	Chloroform-Ext	75.16	64.40	86.31	0.24±0.66	2.36	0.36	0.19
	Methanol-Ext	71.66	60.33	84.12	0.23±0.44	2.16	0.52	0.16
	Acetone-Ext	70.04	59.44	81.18	0.38±0.35	2.22	0.43	0.15
	Ethanol-Ext	58.23	47.91	67.15	0.61±0.38	2.55	0.41	0.13
	Column Purified-Ext (CPE)	45.20	33.80	56.22	0.20±0.21	2.12	0.44	0.16

In each batch, 10 sporocyst larvae were treated in various concentrations of the above preparations.
The larval mortality was observed every 2hrs up to 8hrs.
DSP=Dried stem powder; Ext=Extract; CPF=Column purified fraction.

column purified extract of *T. cordifolia* studies against *F. gigantica* larva sporocyst, redia and cercaria.

Material and Methods

Collection and identification of infected snails and dissection

Adult host snail *L. acuminata* (2.6±0.20 cm in length) were collected locally from pond and low-lying areas of Muhammadabad Gohana, Mau, U.P. (India). Infected snails are often followed by the allocation of more resources to growth with the result the infected snails can grow larger than uninfected snails[7,25,28], locomotion is slow than uninfected ones, and it appeared yellowish, feet were more swollen and shedding cercaria appeared at the mouth of snails and shell morphology changed in infected snails[9, 17, 34]. Each infected host snail was dissected in a clean glass Petri dish containing 10 ml of dechlorinated tap water at 23ºC-25ºC under a dissecting microscope. The larva of the sporocyst, redia, and cercaria were separated in different Petri dishes containing 10 ml of dechlorinated tap water. These larvae were kept in dechlorinated tap water where they survived up to 48h in laboratory conditions.

Preparation of crude powder

The stems of *T. cordifolia* were collected from the college campus. The stems were washed with water, and dried under shade after complete drying, the dried plant materials were cut into small pieces and then ground in an electric grinder machine and the crude powder thus obtained was then sieved with the help of a fine mesh cloth. Finally, the powder was stored in a suitable glass container in laboratory condition.

Preparation of organic extracts

Twenty gram dried stem powder *T. cordifolia* was extracted with 400 mL of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone, and 95% ethanol at laboratory condition ($25C^0$) 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 *T. cordifolia* yielded 220 mg ethanol, 230 mg chloroform, 355 mg ether, and 380 mg acetone extracts.

Extraction of column purified

One liter of ethanol extract purification of dried root powder of *T. cordifolia* 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. Ethanol was evaporated under a vacuum machine and the remaining solids part of the extracts obtained were used for the experimental studies.

Determination of anthelmintic activity

In vitro determination of the anthelmintic activity of the organic extract (ether, chloroform, methanol, acetone, and ethanol), and column-purified extract was performed in the Petri dish[35]. Ten sporocysts, redia, and cercaria larvae were separated in different Petri dishes containing 10 ml of dechlorinated tap water. Treatments of dried stem powder, different organic extracts, and column purified were made directly in the Petri dish under laboratory conditions which containined 10 sporocyst/ redia/cercaria. Each experiment was replicated six times for statistical calculation. The mortality of sporocyst, redia, and cercaria was observed after 2h, 4h, 6h, and 8h of treatment. In the control group, no treatments were given in the Petri dish. Usually *in vitro* conditions (control group) larvae survived upto 48h in dechlorinated water. Counting of larvae in treated and control groups was performed with the help of a light microscope.

Lethal concentration (LC_{50}) value, lower and upper confidence limit (LCL and UCL), Slop-values, t-ratio, g value, and heterogeneity factor were calculated with the help of a POLO computer programme[30].

Results

Larvicidal properties *in vitro* dried stem powder of *T. cordifolia*, different organic extract (ether, chloroform, methanol, acetone, and ethanol), and column purified against *F. gigantica* larvae (sporocyst, redia, and cercaria) were concentration and time dependent (Tables 1-3). *In vitro* exposure of dried stem powder of *T. cordifolia* was more effective against the cercaria (2h LC_{50} 80.22mg/L and 8h LC_{50} 75.12mg/L) (Table-3). The 8h LC_{50} of ethanol extract against sporocyst, redia, and cercaria was 58.23, 65.19, and 62.12 mg/L, respectively (Tables 1-3). Maximum effect was observed against sporocyst larva. The column purified extract of dried stem powder of *T. cordifolia* against sporocyst, redia, and cercaria in 2h LC_{50} was 50.27, 55.26, and 53.11 mg/L and 8h LC_{50} was 48.20, 48.37, and 48.12 mg/mL, respectively (Tables 1-3). Maximum effects of column purified of *T. cordifolia* were observed against sporocyst larva (8h LC_{50} 45.20 mg/L) of *F. gigantica*. Significant ($p<0.05$) negative regression was observed between the exposure period and LC_{50} of dried stem powder, different organic extracts, and column purified extract of the *T. cordifolia* against larva of *Fasciola*. There was no mortality observed in the control group of the experiment.

Discussion

The result section indicates that *in vitro* treatment

TABLE-2: *In vitro* toxicity of dried stem powder, various organic extracts, and column purified fraction of *Tinospora cordifolia* was observed against redia larva

Exposure period	Preparations of Larvicides (mg.L^{-1})	LC_{50} -value	LCL	UCL	S-value	't' -ratio	g -value	HF -value
2h	*T. cordifolia* (DSP)	81.14	73.31	98.15	0.36±0.34	2.23	0.33	0.41
	Ether-Ext	78.18	69.22	94.40	0.42±0.51	3.17	0.17	0.33
	Chloroform-Ext	77.70	65.88	92.25	0.40±0.30	3.42	0.28	0.41
	Methanol-Ext	78.12	63.24	89.93	0.84±0.51	2.26	0.31	0.28
	Acetone-Ext	76.37	65.45	87.64	0.40±0.22	2.43	0.44	0.44
	Ethanol-Ext	71.93	63.55	88.25	0.51±0.44	3.49	0.72	0.26
	Column Purified-Ext (CPE)	55.26	41.31	65.23	0.42±0.32	3.50	0.34	0.31
4h	*T. cordifolia* (DSP)	79.29	62.23	88.16	0.17±0.32	2.26	0.20	0.24
	Ether-Ext	77.29	61.32	89.60	0.74±0.31	3.74	0.22	0.28
	Chloroform-Ext	76.11	65.19	87.56	0.41±0.70	3.61	0.34	0.40
	Methanol-Ext	75.70	61.33	89.67	0.53±0.41	2.51	0.13	0.23
	Acetone-Ext	75.99	62.24	88.30	0.75±0.44	2.43	0.42	0.41
	Ethanol-Ext	71.02	60.73	89.51	0.81±0.73	3.48	0.15	0.54
	Column Purified-Ext (CPE)	53.11	42.22	66.80	0.52±0.30	2.52	0.36	0.34
6h	*T. cordifolia* (DSP)	79.01	61.45	89.12	0.21±0.32	3.61	0.18	0.21
	Ether-Ext	75.88	60.13	86.30	0.43±0.74	3.47	0.75	0.49
	Chloroform-Ext	75.22	59.13	85.30	0.75±0.26	2.15	0.39	0.17
	Methanol-Ext	74.24	61.65	87.81	0.77±0.29	2.23	0.22	0.31
	Acetone-Ext	73.46	60.82	88.20	0.42±0.13	3.48	0.19	0.49
	Ethanol-Ext	68.12	55.22	82.33	0.45±0.25	3.85	0.81	0.60
	Column Purified-Ext (CPE)	50.88	41.90	66.21	0.67±0.27	2.24	0.23	0.30
8h	*T. cordifolia* (DSP)	77.60	62.20	89.25	0.83±0.49	2.13	0.22	0.18
	Ether-Ext	73.18	60.33	87.65	0.17±0.42	3.26	0.16	0.22
	Chloroform-Ext	73.88	59.16	88.80	0.43±0.74	3.34	0.90	0.35
	Methanol-Ext	72.86	55.17	89.11	0.55±0.16	3.14	0.35	0.70
	Acetone-Ext	70.22	54.23	87.35	0.37±0.78	2.27	0.30	0.33
	Ethanol-Ext	65.19	49.65	79.22	0.69±0.31	3.24	0.62	0.28
	Column Purified-Ext (CPE)	48.37	37.83	59.66	0.22±0.23	2.41	0.45	0.14

In each batch, 10 redia larvae were treated in various concentrations of the above preparations.
The larval mortality was observed every 2hrs up to 8hrs.
DSP=Dried stem powder; Ext=Extract; CPF=Column purified fraction.

TABLE-3: *In vitro* toxicity of dried stem powder, various organic extracts, and column purified fraction of *Tinospora cordifolia* was observed against cercaria larva

Exposure period	Preparations of Larvicides (mg.L^{-1})	LC_{50} -value	LCL	UCL	S-value	't' -ratio	g -value	HF -value
2h	*T. cordifolia* (DSP)	80.23	70.12	99.47	0.19±0.34	2.21	0.42	0.30
	Ether-Ext	77.40	65.20	90.65	0.20±0.26	2.30	0.51	0.13
	Chloroform-Ext	75.12	66.21	93.38	0.55±0.41	2.41	0.28	0.67
	Methanol-Ext	77.22	61.40	91.25	0.70±0.38	2.36	0.36	0.34
	Acetone-Ext	75.55	63.32	93.22	0.92±0.52	3.40	0.30	0.23
	Ethanol-Ext	70.88	62.64	93.88	0.50±0.62	3.21	0.40	0.50
	Column Purified-Ext (CPE)	53.11	41.24	66.15	0.31±0.30	2.42	0.61	0.26
4h	*T. cordifolia* (DSP)	80.01	69.30	97.30	0.26±0.41	2.14	0.24	0.40
	Ether-Ext	76.22	60.54	89.40	0.50±0.36	2.38	0.36	0.15
	Chloroform-Ext	73.74	55.38	88.36	0.42±0.31	3.24	0.40	0.22
	Methanol-Ext	75.34	51.34	89.20	0.42±0.44	2.77	0.31	0.60
	Acetone-Ext	73.34	60.50	88.11	0.70±0.10	3.28	0.46	0.21
	Ethanol-Ext	68.30	55.72	87.90	0.52±0.23	2.34	0.20	0.27
	Column Purified-Ext (CPE)	52.16	39.54	66.61	0.21±0.30	2.46	0.41	0.50
6h	*T. cordifolia* (DSP)	78.02	60.34	89.44	0.71±0.24	2.25	0.31	0.56
	Ether-Ext	74.87	61.55	88.20	0.20±0.81	3.34	0.62	0.47
	Chloroform-Ext	71.60	55.28	89.99	0.26±0.40	2.64	0.35	0.41
	Methanol-Ext	73.88	48.32	88.20	0.62±0.26	2.18	0.20	0.36
	Acetone-Ext	71.19	49.38	90.41	0.34±0.12	3.22	0.28	0.13
	Ethanol-Ext	65.23	44.26	80.21	0.31±0.20	2.44	0.63	0.37
	Column Purified-Ext (CPE)	50.34	43.28	76.12	0.27±0.21	2.21	0.25	0.30
8h	*T. cordifolia* (DSP)	75.12	58.30	86.32	0.74±0.51	2.33	0.18	0.24
	Ether-Ext	72.33	56.21	87.25	0.65±0.53	3.12	0.83	0.70
	Chloroform-Ext	68.99	48.51	90.11	0.31±0.52	2.16	0.22	0.27
	Methanol-Ext	70.33	50.34	88.90	0.41±0.18	3.49	0.61	0.13
	Acetone-Ext	68.45	53.22	89.31	0.32±0.29	2.11	0.64	0.19
	Ethanol-Ext	62.12	48.63	87.34	0.47±0.30	2.30	0.13	0.18
	Column Purified-Ext (CPE)	48.12	32.45	58.38	0.21±0.85	3.34	0.23	0.12

In each batch, 10 cercaria larvae were treated in various concentrations of the above preparations.
The larval mortality was observed every 2hrs up to 8hrs.
DSP=Dried stem powder; Ext=Extract; CPF=Column purified fraction.

of dried stem powder; different organic extract and column purified extract of *T. cordifolia* have anthelmintic larvicidal properties against sporocyst, redia, and cercaria of the *F. gigantica*. The stem of *T. cordifolia* uses were for anti-inflammatory, anti-allergic, antioxidant activities[18], antipyretic, hepatoprotective, immune-modulatory[27], antidiabetic[32], anti-hyperlipidemic properties[8], and urinary diseases syphilis[37]. Traditionally the stems of *T. cordifolia* are used as an anthelmintic[40]. The methanolic extracts of *T. cordifolia* have carbohydrates, tannins, saponins, proteins, terpenoids, alkaloids, and glycosides[5,6]. The stem extract of *T. cordifolia* shows antibacterial activity against different types of bacteria like *Enterobacter faecalis*, *Staphylococcus sp*, *Salmonella typhi*, and *Escherichia coli*[10].

The toxic effect of *T. cordifolia* plant products may be diffused in the larval body which progressively increases the amount of active compounds in the larval with the increase in the exposure period. The phytochemicals screening of *T. cordifolia* revealed the presence of alkaloids, saponins, glycosides, and flavnoids could be responsible for toxic effects on the *F. gigantica* larva. *In vitro* anthelmintic larvicidal study of stem powder, organic extracts, and column purified revealed that various phytochemicals of *T. cordifolia* are easily diffused in sporocyst, redia, and cercaria that caused toxic effects. It might be possible that the phytochemicals of *T. cordifolia* cause an impact on the various enzyme actions in the sporocyst, redia, and cercaria that caused moiety. The higher toxicity of ethanol extract among other organic extracts indicates that the active phytochemicals of *T. cordifolia* are more soluble in ethanol. The aqueous extract of *T. cordifolia* had a radioprotective activity that enhanced the survival of mice against a sub-lethal dose of gamma radiation[31, 11].

The steep value of the result section indicates that a small increase in the concentration of all the treatments caused a toxic effect (Tables 1-3) in *F. gigantica* larva. The t-ratio value in the result section is greater than 1.96 which denoted that the regression is significant. The heterogeneity factor is less than 1.0 which indicates the all-replicate test of the random sample of the concentration response.

Conclusion

The results obtained in this study indicate that *in vitro* anthelmintic larvicidal activity of stem powder, organic extract, and column purified of *T. cordifolia* against sporocyst, redia, and cercaria larva of *F. gigantica* can be used for the control of fascioliasis. All the exposure against *F. gigantica* larvae were analyzed on concentration and time-dependent, where the ethanolic extract showed a significant anthelmintic larvicidal activity which might be due to the presence of various phytochemicals in the extract. The result of the present study is encouraging for more studies at the molecular level in *Fasciola* larva that how the phytochemicals of *T. cordifolia* caused an impact in the sporocyst, redia, and cercaria.

Conflict of Interest: The authors have no conflict of interest.

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