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Assessment of anthelmintic potential (*In vitro*) of *Curcuma longa* on gastrointestinal strongyles of goats

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Abstract
The present study was carried out to assess the *In vitro* anthelmintic activity of *Curcuma Longa* (turmeric) gastrointestinal strongyles infecting goats. Aqueous, methanolic and hydroethanolic extracts of both the plants were prepared following standard protocol. *In vitro* efficacies of the different extracts were investigated by egg hatch assay (EHA), larval paralysis test (LPT) and adult mortality test (AMT). In egg hatch assay, the aqueous, methanolic and hydroethanolic extracts of *C. longa* at 100 mg/ml exhibited 98.02%, 97.48% and 98.04% efficacy, respectively, The ED_{50} values obtained for aqueous, methanolic and hydroethanolic extracts of *C. longa* were 1.262 mg/ml, 2.226 mg/ml and 1.535 mg/ml, respectively. In larval paralysis test, the aqueous, methanolic and hydroethanolic extracts of *C. longa* at 100 mg/ml concentration recorded an efficacy of 99.09%, 98.28% and 95.89%. The ED_{50} values derived for aqueous, methanolic and hydroethanolic extracts of *C. longa* were 1.297 mg/ml, 2.077 mg/ml and 5.391 mg/ml, respectively. In adult mortality test, for methanolic extract of *C. longa*, highest corrected average adult mortality %, 81.91 was observed, followed by 60.48% for aqueous extract of *C. longa* and 50.48% for hydroethanolic extract of *C. longa*. The differences among the various extracts of *C. longa* for corrected average adult mortality % were found to be significant (p<0.05).

Keywords: *Curcuma longa*, extracts, efficacy, gastrointestinal strongyles, goats

Introduction
Gastrointestinal strongyles affect the economy of goat farming in terms of reduced body weight, poor hair fiber growth, decreased production of milk and the most critically death of animal which can sometime damage the spine of poor farmers' livelihood. Some of the common and pathogenic strongyles affecting small ruminants are *Trichostrongylus colubriformis, Haemonchus contortus, Bunostomum trigonocephalum, Cooperia curticei, Oesophagostomum columbianum.* Conventionally chemotherapy through different therapeutic drugs particularly with benzimidazole group of drugs is widely used to overcome this situation but, nowadays increasing resistance in helminth parasites against various anthelmintic drugs is a challenge to which veterinarians have to deal with. To combat anthelmintic resistance specially benzimidazole resistance in goats, it is crucial to implement a thorough parasite control programme that includes routine faecal testing to monitor worm burden, appropriate management practices, such as rotational grazing and pasture management, and the use of alternative anthelmintic drugs and non-chemical approaches, such as herbal cures, vaccination, and genetic selection of resistant animals.
Alternative therapies such as use of herbal drugs in veterinary science bring together the veterinarians throughout the world to use these drugs in animal health practices. Lots of herbal drugs have been developed to cure animal diseases as these are least toxic, more effective, eco-friendlier and cheapest one, in terms of manufacturing cost. Although herbal treatments have been used for many years to treat a wide range of illnesses, but scientific evidences to support their efficacy in the treatment of gastrointestinal parasitism is less. Albeit studies have shown that several plants and herbs, like garlic, ginger, pepper and turmeric, may have antiparasitic effects, further study is required to completely comprehend their potential advantages and their pharmacokinetics and pharmacodynamics. We can directly administer the whole plant or part of the plant to the affected animal and quantifying the consequences of their ingestion or plant

extracts and concoctions derived from medicinal plants would be tested in *In vitro* and *in vivo*systems. Therefore the present study was designed to investigate the *In vitro* efficacy of different extracts of *Curcuma Longa* gastrointestinal strongyles of goats.

Materials and Methods

Collection of Plant material and preparation of extracts

The rhizomes of turmeric *(Curcuma longa)* were purchased from local market, Bada Fuhara, Jabalpur (M.P.). The rhizomes were washed thoroughly in distilled water to remove the dirt and cut into small pieces in order to activate the bioactive substances present in them. These were shade-dried at room temperature till they become brittle. Which were further dried by transferring them into an incubator at a precise temperature of 27 °C for two days. After that, these rhizomes were ground to powder from in electric blender as described by Karsha and Laxmi (2010) [6]. Powder of *C. longa* was stored in air-sealed containers till further use. Aqueous extract of *C. longa* was prepared as per the procedure described by Pundir and Jain (2010) [10] with slight modification. Twenty five grams of *C. longa* powdered plant material was dissolved in sterilized distilled water to make 100ml of aqueous extract (25% w/v). The mixture was kept undisturbed at room temperature of 27 °C for 24 h in a sterile flask covered with aluminum foil to avoid evaporation. Then the mixture subjected to filtration through sterilized Whatman no. 1 filter paper. After filtration the extract was evaporated in water bath until 25 ml extract left in container. The methanolic extract of *Curcuma Longa* was prepared as per the method described by Rajesh *et al.* (2013) [11]. The dried powder of turmeric (40g) was placed in the thimble of Soxhlet apparatus and150 ml of methanol was used as a solvent. The extraction was continued till clear solvent is seen. Then the extract was dried in a water bath to get the residue and will be refrigerated till use. The hydroethanolic extract of *C. longa* was prepared as per the method described by Nath *et al.* (2019) [8]. The powdered rhizomes were poured in thimble. Extraction was done by Soxhlet extractor with ethanol and distilled water in the ratio of 50:50.

Collection of faecal Sample

Faecal samples of goats was collected directly from rectum irrespective of age, sex or body weight and stored in aerobic and anaerobic conditions to be taken to laboratory for further investigation.

Egg hatch test

Egg concentration suspension of about 100-150 eggs/ 200 µl was prepared by using Mc-master's egg counting technique (Sloss *et al.*, 1994) [3]. A concentration of 400 mg plant extract per 1 ml of Phosphate Buffer Saline (PBS) was prepared as stock solution (A) and desired concentrations of plant extract were made by serial dilutions of stock solution as shown in table 01.

Flat based 24 multi-well plates (Make-Himedia) were used for this procedure. 0.5 ml of prepared dilutions of varying concentrations (1.562 mg/ml, 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 50 mg/ml and 100 mg/ml) were added to seven corresponding wells and only PBS was taken in the control well. An amount of 200 µl of fresh egg suspension with 100-150 fresh eggs were added in each well. Addition of 300 µl of distilled water was done, making the volume in each well up to 1 ml. The plates were incubated under sterile conditions at 28 °C for 48 h . After the specified time period, a drop of lugol's iodine solution was added in each well to prevent further hatching. The solution was then transferred into Eppendorf tubes and labelled, centrifuged at 2500 rpm for 3 minutes. Supernatant discarded carefully and the sediment was examined, presence of hatched larvae and unhatched eggs were counted under the 10x microscopic lens.

Larval Paralysis Test

The larval paralysis test was performed as per the guidelines with the procedure described by Kanojiya *et al.* (2015) [2]. Faecal culture was done to obtain the nematode larvae and the larval concentration suspension was adjusted to 100-150 larvae/100 µl. One hundred µl of various concentration dilutions (1.562 mg/ml, 3.125mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) of all the extracts were added to seven corresponding wells and distilled water was taken in control well. 100 µl of larval suspension was added to the corresponding wells. The plates were kept at room temp. (28 °C) for 24 h.

Adult Mortality Test

For adult mortality test adult strongyle worms were obtained from the gastrointestinal tract and eight small petri dishes each containing ten strongyle worms were subjected to 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml plant extract and control receiving 1 ml pbs. The plates were incubated at room temperature for 24 h. Corrected percent mortality was estimated for each plant extract.

Statistical analysis

The ED50 values of all the extracts were calculated by RESO calculator and log-probit analysis as per Finney (1971) [1] and Snedecor and Cochran (1994) [12]. One way ANOVA was done to test the significance of difference between various means of average % efficacy, average % corrected efficacy and average % corrected adult mortality by using statistical analysis software (IBM SPSS for Windows, Version 28.0. Armonk, NY: IBM Corp.)

Result and Discussion

Write Conclusion in 100-120 words following

In egg hatch assay against all extracts of the herb, the aqueous, methanolic and hydro-ethanolic extracts of *C. longa* at 100 mg/ml exhibited 98.02%, 97.48% and 98.04% efficacy, respectively, The ED_{50} values obtained for aqueous, methanolic and hydro ethanolic extracts of *C. longa* were 1.262 mg/ml, 2.226 mg/ml and 1.535 mg/ml, respectively in egg hatch assay. The differences among all the three extracts of *C. longa* for efficacy % and corrected efficacy % found to be non-significant, in EHA. In egg hatch assay, average % efficacies of aqueous, methanolic and hydroethanolic extracts of *C. longa* were found to be 86.40, 84.93 and 84.76, respectively, whereas the corrected average % efficacies were found to be 63.34, 49.95 and 48.84 respectively for aqueous, methanolic and hydroethanolic extracts of *C. longa.* The differences among various extracts of *C. longa* were found to be non-significant (p>0.05) for both, average % efficacy as well as corrected average % efficacy, as shown in table 01. For egg hatch assay, the minimum ED50 value, 1.262 was recorded for aqueous extract of *C. longa* in comparison to methanolic and hydroethanolic extracts. For methanolic and hydroethanolic extracts of *C. longa* the values of ED50 were 2.226 and 1.535, respectively (Table 02).

The comparative study could not be done with efficacy % of *C. longa* mentioned by other investigators because available literature did not reveal any reference on *In vitro* efficacy % of *C. longa* in egg hatch assay. However, Nath *et al.* (2019) [8] studied the *in vivo* effect of *C. longa* in egg hatch assay through eggs per gram (EPG) and faecal egg count reduction test (FECRT). In their study, mean EPG in the group treated with hydroethanolic extract of *C. longa* was found to be reduced from 1683 at 0th day to 275 at 10th day and FECRT % was recorded to be 66.

In larval paralysis test, the aqueous, methanolic and hydroethanolic extracts of *C. longa* at 100 mg/ml concentration recorded an efficacy of 99.09%, 98.28% and 95.89%. The ED_{50} values derived for aqueous, methanolic and hydroethanolic extracts of *C. longa* were 1.297 mg/ml, 2.077 mg/ml and 5.391 mg/ml, respectively (Table03 and 04). In larval paralysis test, among all the extracts of *C. longa*, minimum ED50, 1.297 was recorded for aqueous extract of *C. longa* then followed by methanolic and hydroethanolic extracts, which were 2.077 and 5.391, respectively (Table 04). The differences among all the three extracts of *C. longa* efficacy % and corrected efficacy % found to be non-significant, in LPT.

The findings of Nasai *et al.* (2016) [7] for ethanolic extract of *C. longa* revealed that there was an increase in the number of dead *Haemonchus* larvae (L3) over time as turmeric concentrations exhibited the highest dose (200 mg/ml) of turmeric extract, which was in accordance to present findings, in way of dose dependent response. Similar dose dependent results to present findings were also observed by Pandey *et al.* (2018) [9], in their outcomes mean paralysis time of larvae of *Haemonchus* spp. seemed to be decreased with increasing concentration of aqueous as well as methanolic extracts of *C. longa.*

For aqueous extract of *C. longa* at concentration (mg/ml) 200, just after 1 h of incubation, 100% mortality was recorded, whereas at concentration (mg/ml) 100, after 4 h of incubation, 100 % mortality was recorded. However, for concentration (mg/ml), 50, 25, 12.5, 6.25 and 3.125, 100% mortality was achieved after 12 h of incubation. The corrected mortality % at concentration (mg/ml), 200, 100, 50, 25, 12.5, 6.25 and 3.125 were found to be 100.00, 96.67, 60.00, 53.33, 46.67, 36.67 and 30.00, respectively (Table05). For methanolic extract of *C. longa* at concentration (mg/ml) 200, 100, 50, 25 and 12.5 after 4 h of incubation 100% mortality was recorded, whereas at concentration (mg/ml) 6.25, after 12 h of incubation, 100 % mortality was recorded. However, for concentration (mg/ml) 3.125, 100% mortality is seen after 18 hrs. The corrected mortality % at concentration (mg/ml), 200, 100, 50, 25, 12.5, 6.25 and 3.125 were found to be 90.00, 90.00, 90.00, 86.67, 86.67, 73.33 and 56.67, respectively (Table06). For hydroethanolic extract of *C. longa* at concentration (mg/ml) 200, just after 6 h of incubation, 100% mortality was recorded, whereas at concentration (mg/ml) 100 and 50 after 12 h of incubation, 100 % mortality was recorded. However, for concentration (mg/ml), 25, 12.5 and 6.25, 100% mortality was achieved after 24 h of incubation, while at last concentration (mg/ml) 3.125, 93.33% mortality was recorded at 24 h of incubation. The corrected mortality % at concentration (mg/ml), 200, 100, 50, 25, 12.5, 6.25 and 3.125 were found to be 60.00, 56.67, 56.67, 50.00, 50.00, 43.33 and 36.67, respectively (Table07). However, for methanolic extract of *C. longa*, highest corrected average adult mortality %, 81.91 was observed, followed by 60.48% for aqueous extract of *C. longa* and 50.48% for hydroethanolic extract of *C. longa.* The differences among the various extracts of *C. longa* for corrected average adult mortality % were found to be significant ($p<0.05$) as shown in table 08.

In-vitro effects of curcumin methanolic extract on *Ascaridia galli*, reported by Bazh and El-Bahy (2013) [13] revealed that the mean % of dead worms were 65.8±1.5 after 24 h at concentration 100 mg/ml, which was in contrast to present findings. In present findings, 100% mortality was achieved at 25 mg/ml concentration, just after 4 h of incubation. The outcomes of El-Bahy and Bazh (2013) [5] were also in contrast to present findings. In their investigation, for a dose 1000 mg of curcumin, the mean % of dead adult *Raillietina cesticillus* was found to be 60±0, after 24 h of incubation. However organism experimented in their study was different.

In conclusion, a dose dependent effect of the extract was observed in EHA, LPT and AMT. Hydroethanolic extract of *C. longa* demonstrated better result in EHA. The aqueous extract of *C. longa* showed better results in larval paralysis test, with minimum ED_{50} among all the plant extract. On the basis of average % corrected adult mortality, methanolic extract of *C. longa* was significantly ($p<0.05$) effective than aqueous and hydro-ethanolic extracts. *Curcuma Longa* contains curcumin one of the active ingredient has been reported to block thioredoxin-reductases (TrxRs) of nematehelminthes, which are necessary for cell development and survival and important in alternative medicine (Hudson *et al.*, 2010) [4].

Table 1: Efficacy of aqueous, methanolic and hydroethanolic extracts of *Curcuma Longa* in EHA

S. No.	Extract		Concentration of the extracts (mg/ml)						
			100	50	25	12.5	6.25	3.125	1.562
1	*C. longa* aqueous	% Efficacy	98.02	95.71	93.67	90.11	85.94	81.17	60.17
		% Corrected efficacy	80.17	76.38	72.67	67.03	61.33	52.91	32.9
2	*C. longa* methanolic	% Efficacy	97.48	96.24	93.97	87.83	85.47	77.87	55.62
		% Corrected efficacy	81.13	65.88	58.68	49.08	46.9	31.27	16.68
3	*C. longa* hydroethanolic	% Efficacy	98.04	96.05	94.25	86.75	83.76	79.17	55.33
		% Corrected efficacy	79.8	64.84	56.88	48.04	42.14	31.81	18.4

Table 2: ED_{50}, Lower limits and Upper limits of aqueous, methanolic and hydroethanolic extracts of *C. longa* in EHA

S. No.	Extract of *C. longa*	ED_{50} (mg/ml)		
		ED_{50}	Lower limit	Upper limit
1	aqueous	1.262	0.539	2.145
2	methanolic	2.226	1.112	3.505
3	hydroethanolic	1.535	0.796	2.353

Table 3: Efficacy of aqueous, methanolic and hydroethanolic extracts of *C. longa* in larval paralysis test

S. No.	Extracts		Concentration of extracts (mg/ml)						
			100	50	25	12.5	6.25	3.125	1.562
1	*C. longa* aqueous	% Efficacy	99.09	98.72	91.36	84.28	78.52	70.98	58.76
		% Corrected efficacy	91.82	91.03	83.95	76.73	70.07	62.59	50.00
2	*C. longa* methanolic	% Efficacy	98.28	97.04	94.44	83.74	79.12	63.67	50.91
		% Corrected efficacy	88.79	86.18	82.22	72.32	68.01	52.25	38.91
3	*C. longa* hydroethanolic	% Efficacy	95.89	88.74	82.51	68.09	64.31	51.61	39.34
		% Corrected efficacy	82.19	75.00	67.93	50.35	48.23	33.69	20.96

Table 4: ED_{50}, Lower limits and Upper limits of aqueous, methanolic and hydroethanolic extracts of *C. longa* in larval paralysis test

S. No.	Extract of *C. longa*	ED_{50} (mg/ml)		
		ED_{50}	Lower limit	Upper limit
1	aqueous	1.297	0.752	1.900
2	methanolic	2.077	1.395	2.797
3	hydroethanolic	5.391	3.925	6.976

Table 5: *In vitro* anthelmintic effect of *Curcuma Longa* aqueous extract against gastrointestinal strongyles

S. No.	Conc. (mg/ml)	Exposed	No. of parasites dead at various hours of exposure							% Mortality	% Corrected mortality
			1	2	4	6	12	18	24		
1	200.000	10	10.00	-	-	-	-	-	-	100.00	100.00
2	100.000	10	09.33	09.67	10.00	-	-	-	-	100.00	096.67
3	050.000	10	07.00	08.67	08.67	08.33	10.00	-	-	100.00	060.00
4	025.000	10	05.33	07.33	08.00	07.67	10.00	-	-	100.00	053.33
5	012.500	10	04.33	06.67	06.67	07.00	10.00	-	-	100.00	046.67
6	006.250	10	02.33	05.67	05.00	06.00	10.00	-	-	100.00	036.67
7	003.125	10	01.33	04.67	04.67	05.33	10.00	-	-	100.00	030.00
8	Control	10	00.00	00.00	01.33	02.33	04.67	05.67	05.67	056.67	

Table 6: *In vitro* anthelmintic effect of *Curcuma Longa* methanolic extract against gastrointestinal strongyles

S. No.	Conc. (mg/ml)	Exposed	No. of parasites dead at various hours of exposure							% Mortality	% Corrected mortality
			1	2	4	6	12	18	24		
1	200.000	10	09.33	09.67	10.00	-	-	-	-	100.00	90.00
2	100.000	10	08.67	09.67	10.00	-	-	-	-	100.00	90.00
3	050.000	10	08.67	09.67	10.00	-	-	-	-	100.00	90.00
4	025.000	10	08.33	09.33	10.00	-	-	-	-	100.00	86.67
5	012.500	10	08.00	09.33	10.00	-	-	-	-	100.00	86.67
6	006.250	10	07.33	08.67	09.33	09.67	10.00	-	-	100.00	73.33
7	003.125	10	07.00	07.33	09.00	09.33	09.33	10.00	-	100.00	56.67
8	Control	10	00.00	00.67	00.67	02.33	03.67	04.33	05.00	050.00	-

Table 7: *In vitro* anthelmintic effect of *Curcuma Longa* hydroethanolic extract against gastrointestinal strongyles

S. No.	Conc. (mg/ml)	Exposed	No. of parasites dead at various hours of exposure							% Mortality	% Corrected mortality
			1	2	4	6	12	18	24		
1	200.000	10	07.67	08.67	09.67	10.00	-	-	-	100.00	60.00
2	100.000	10	07.00	08.00	09.33	09.67	10.00	-	-	100.00	56.67
3	050.000	10	05.33	06.33	08.33	09.67	10.00	-	-	100.00	56.67
4	025.000	10	05.00	06.00	07.33	09.00	09.67	09.67	10.00	100.00	50.00
5	012.500	10	04.33	06.00	07.33	09.00	09.67	09.67	10.00	100.00	50.00
6	006.250	10	03.67	05.33	06.67	08.00	09.00	09.00	10.00	100.00	43.33
7	003.125	10	02.33	04.33	05.67	06.33	08.00	08.67	09.33	093.33	36.67
8	Control	10	01.67	02.67	03.67	04.00	04.33	04.67	05.67	056.67	-

Table 8: Average percent corrected mortality of adult gastrointestinal strongyles against aqueous, methanolic and hydroethanolic extracts of *Curcuma longa*

S. No.	Extract of *C. longa*	Average % corrected mortality at different concentrations (mg/ml)							Average % corrected mortality
		3.125	6.25	12.5	25.0	50.0	100	200	
1	aqueous	30.00	36.67	46.67	53.33	60.00	96.67	100	60.48^{b}
2	methanolic	56.67	73.33	86.67	86.67	90.00	90.00	090	81.91^{a}
3	hydroethanolic	36.67	43.33	50.00	50.00	56.67	56.67	060	50.48^{b}

Values with different superscript within the same column differ significantly ($p<0.05$)

Conclusion

This study demonstrated the potent anthelmintic properties of Curcuma longa extracts, with significant efficacy observed in egg hatch assays (EHA), larval paralysis tests (LPT), and adult mortality tests (AMT). Aqueous, methanolic, and hydro-ethanolic extracts of C. longa showed high efficacy at 100 mg/ml concentration, with dose-dependent effects noted across tests. The aqueous extract exhibited the lowest effective dose (ED50) values, indicating its superior potency in inhibiting egg hatch and paralyzing larvae. Methanolic extract, however, showed the highest corrected adult mortality rates, suggesting its significant impact on adult nematodes. These findings underscore Curcuma longa's potential in anthelmintic therapy, attributed to curcumin's ability to inhibit vital nematode enzymes. This research supports the incorporation of Curcuma longa as an effective, natural anthelmintic agent in alternative medicine, highlighting its significant role in combating parasitic infections.

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