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Research Article

IN VITRO EVALUATION OF THE ANTHELMINTIC ACTIVITY OF RHIZOME EXTRACTS OF *CURCUMA LONGA* (LINN.)

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ABSTRACT

Objective: The current study was carried out to evaluate the anthelmintic activity of the rhizome extract of *Curcuma longa* as an alternative source of effective remedies for nematodiasis.

Methods: The anthelmintic activity of the *C. longa* was assessed *in vitro* against *Haemonchus* spp., a gastrointestinal (abomasum) parasite of goats. Different concentrations of the extracts (1 mg/mL, 2.5 mg/mL, 5 mg/mL, and 10 mg/mL) in phosphate-buffered saline (PBS) were tested, and the results expressed in terms of time of paralysis (minute) and time of death (minute) of the worms. Albendazole (1 mg/mL) was used as a reference (positive control) and PBS as a control group (negative control).

Results: The qualitative phytochemical analysis of the methanolic extract (ME) of the plant disclosed the presence of alkaloids, glycosides, terpenoids, flavonoids, tannins, saponins, phenol, anthraquinone, and carbohydrates; whereas, the aqueous extract (AE) showed the presence of alkaloids, carbohydrate, flavonoids, and saponins. Both ME and AE of the *C. longa* (rhizome) expressed significant efficacy ($p \leq 0.05$) in causing paralysis as well as the death of the worms within 12 h of exposure at all tested concentrations, as compared to the negative control. The rhizome extracts of *C. longa* showed dose-dependent efficacy in causing paralysis of the worm motility and the final progression to death. The results showed that the ME at 10 mg/mL was significantly more potent ($p \leq 0.05$) over the AE.

Conclusions: This study concluded that the rhizome extract of *C. longa* exhibited potent anthelmintic efficacy against the nematode parasite, *Haemonchus* spp.

Keywords: Anthelmintic activity, *Curcuma longa*, Nematodiasis, *Haemonchus* spp., Albendazole.

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INTRODUCTION

Haemonchosis has been recognized as a serious constraint to the small ruminants throughout the world [1] and has also been reported from several states of India, namely Himachal Pradesh, Punjab, and Uttarakhand [2-4]. Due to the high prevalence rate, ubiquitous distribution and serious pathological consequences, these parasites have been responsible for massive economic losses to the rural farmers and livestock industry [5]. These parasites are associated with severe damage to the host in several ways including extensive damage to the intestinal wall and sucking of host blood and nutrients. Prolonged infections may result in hypoproteinemia, gauntness, anorexia, diarrhea, edema, and high mortality rate in affected animals [6].

Rid of the infections from grazing animals is complicated due to variations in the host propensity to the parasite and its cosmopolitan nature. To control haemonchosis, farmers, generally, use modern synthetic drugs as anthelmintic, in combination with proper grazing management practices. Synthetic anthelmintics, till date, are repeatedly used to evict parasitic helminths from the host body by killing [7], however, due to high cost and inadequate availability; these drugs are off limits to the resource-poor farmers of the developing countries [8]. Furthermore, unfortunately, due to imprudent and frequent use of these drugs, their effectiveness is continuously decreasing, and in the past few years, the parasites not only have developed resistance against the available anthelmintics, but their chemical residue and toxicity have also become a severe problem, the world over [9].

In view of the above, it was found imperative to look for alternatives that may be developed as sustainable, safer, and environmentally friendly anthelmintic [10], and for these reasons, there has been a renewed interest in the screening of traditionally used therapeutic plants in search of their anthelmintic properties.

Curcuma longa Linn. (turmeric) belongs to the family Zingiberaceae and is usually known as "Haldi." It is a perennial herb having tuberous roots and short stem with large leaves, being widely cultivated in India, Indonesia, Bangladesh, France, and other Asian countries. The turmeric plant shows medicinal properties and contains phytochemical constituents such as essential oil, alkaloid, starch grain, and curcumin (a polyphenol) [11]. Since ancient times, *C. longa* has been used as a herbal medicine for different therapeutic purposes [12] and some previous studies have reported the positive effects of *C. longa* in the treatment of cancers, heart disease, and soreness [13]. Extract of *C. longa* has also been established for antiparasitic activities against leishmaniasis, hydatidosis, giardiasis, trypanosomiasis, and schistosomiasis [14]. *C. longa* successfully worked as an anthelmintic against *Ascaridia galli* both *in vitro* and *in vivo* [15], *Haemonchus* spp. of sheep *in vitro* [16], and *Fasciola gigantica* [17]. Based on the traditional uses and scientific reports, the objective of the present study was to evaluate the anthelmintic activity of the rhizome extract of *C. longa* using the gastrointestinal (GI) nematode parasite of the goat, *Haemonchus* spp. as a model.

METHODS

In vitro analysis was conducted to evaluate the anthelmintic potential of aqueous extract (AE) and methanolic extract (ME) of rhizome of *C. longa* against *Haemonchus* spp. of the goat. Albendazole was used as reference drug for the assay. The study was carried out at the Parasitology and Silkworm Pathology Laboratory of the Department of Zoology (formerly the Department of Applied Animal Sciences)

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Collection of plant material and preparation of extracts

Rhizomes of *C. longa* were purchased from the local markets in Lucknow and authenticated in the Department of Applied Plant Sciences of the University. The rhizomes were washed carefully in running water, cut into small pieces, and shade dried for 2–3 weeks under room temperature. Finally, it was ground to a fine powder with the help of electric grinder before further processing: 100 g of fine powder of the rhizomes was subjected to extraction in Soxhlet apparatus using 500 mL each of water and methanol separately, as solvents, for 48 h at room temperature. The respective extracts were concentrated by evaporation in the water bath and then dried in open air and stored in air-tight containers at 4 ^{0}C until further use [18,19].

PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemical analysis was carried out on the *C. longa* rhizome extract, and the results revealed the existence of various biological active compounds such as tannins, saponins, alkaloids, flavonoids, glycosides, terpenoids, phenol, carbohydrates, and anthraquinone [8,20-24].

Test organism

Adult nematode parasitic worms of *Haemonchus* spp., collected from the GI tracts of goats, were used for all the trial protocols. Briefly, the method of collection of the parasites from the GI tract of goats is as follows: GI tracts of goats were collected from the slaughterhouses in the Lucknow region and immediately transported to the Parasitology Laboratory of the Department of Zoology (formerly the Department of Applied Animal Sciences) B.B.A.U., Lucknow. The GI tracts were dissected following standard procedure and examined for the presence of *Haemonchus* spp. worms. Adult worms were collected from the abomasum of the GI tract of the goat, washed with normal saline, and kept in phosphate-buffered saline (PBS, pH - 7.5–8.0) until further use in the anthelmintic activity assays.

***In vitro* anthelmintic activity**

The anthelmintic activity of the AE and ME of *C. longa* was carried out by following the standard protocol [16,25,26] with some minor modifications. All the working solutions were freshly prepared before the start of the experiment. 10 actively moving, equal sized worms were placed in Petri dishes at room temperature (25°C–30°C) containing 1 mg/mL, 2.5 mg/mL, 5 mg/mL, and 10 mg/mL of AE and ME of the *Curcuma* rhizome, respectively, in PBS. Positive and negative controls were simultaneously set up with Albendazole and PBS, respectively. Three replicates were set for each concentration and observations were made at 1, 2, 3, 4, 5, 6, 7, and 12 h of the time taken to get paralyzed and finally die for all the worms. After each interval of time, the paralyzed worms were placed in PBS for 30 min for possible recovery of the parasite motility. After 12 h, from each of the experimental groups, the respective extract concentration solution was discarded and the number of alive and dead worms in each concentration was counted under a dissecting microscope and recorded. The worms were counted, as dead when they lost their motility permanently and did not recover even after placing in PBS. The loss of motility or paralysis was supposed to occur when the worms were not able to move even after placing in the PBS. Paralysis was followed by fading away of the body color of the dead worm [25,27,28].

Statistical analysis

The results are expressed as mean ± S.E.M of 10 worms for each concentration level in the treatment regimen. The statistical analysis was done using one-way ANOVA (SPSS version 20.00) followed by Tukey *post hoc* test. The level of significance was set at $p<0.05$.

RESULTS

In the present study, the phytochemical analysis of the *C. longa* rhizome extracts, both ME and AE, was carried out, and the results are shown in Table 1. In the screening analysis, different types of results have been observed in the methanolic and aqueous solvents, i.e., ME showed the presence of glycosides, alkaloids, flavonoids, tannins, saponins, terpenoids, phenol, anthraquinone, and carbohydrates while the AE showed the presence of alkaloids, carbohydrate, flavonoids, terpenoids, and saponins.

The anthelmintic activity of the ME and AE of the rhizome of *C. longa* and evaluated *in vitro* against *Haemonchus* spp., the abomasal nematode parasite of goat, and the results are shown in Table 2, Figs. 1 and 2. The results revealed that both the AE and ME of the plant exhibited significant ($p\leq0.05$) efficacy in causing paralysis as well as the death of the worms at all the tested concentrations (Table 2, Figs. 1 and 2). At the concentration of 1 mg/mL, ME of *C. longa* caused paralysis followed by the death of the worm's in 139.0±0.6 min and 175.3±2.0 min, respectively; while, at 2.5 mg/mL concentration, paralysis and death occurred at 120.0±1.1 min and 160.7±1.8 min, respectively. However, at the 5 mg/mL of concentration, the time taken for paralysis of the worms was 100.3±0.9 min while death occurred at 147.7±3.5 min. The most proficient anthelmintic activity was exhibited at the concentration of 10 mg/mL at least time was taken for paralysis and finally death of the worms at 66.0±1.5 min and 96.0±2.1 min, respectively. Similar

Table 1: Preliminary phytochemical screening of aqueous and methanolic rhizome extracts of *C. longa*

Test	Procedure	Observation	AE	ME
Alkaloids	Mayer's test	Cream/off-white color Ppt	+	+
Flavonoids	Alkaline reagent test	Yellow color	+	+
	$ZnCl_2$ test	Red color		
Glycosides	Extract+dil. H_2SO_4 ⇨Boiled then Cooled+solution of NaOH+Fehling's solution A and B, heat on the water bath	Brick red Ppt	-	+
Terpenoids	Salkowski's test	Yellow color	+	++
Tannins	$FeCl_3$ test	Dark green color	-	+
Saponins	Foam test	Formation of honeycomb-like structure (Forth)	+	++
Phenol	$FeCl_3$ test	Blue-green color	-	+
Carbohydrates	Benedict's test	Red Ppt	+	++
	Fehling's test	Red Ppt	+	++
Anthraquinone	Extract+NH_3 solution⇨ shake well	Bright pink color	-	+

-Not detected, +low concentration, ++high concentration, Ppt - precipitate. *C. longa: Curcuma longa*, AE: Aqueous extract, ME: Methanolic extract

Table 2: *In vitro* anthelmintic assay of AE and ME of rhizome of *C. longa* against *Haemonchus* spp.

Treatment	Concentration (mg/mL)	Paralysis time (min) (mean±SEM)	Death time (min) (mean±SEM)
Control	-	0.0	0.0
Albendazole	1	32±2.3*	57.3±5.5*
C. longa (AE)	1	159.7±2.0*	198.0±2.5*
	2.5	140.0±1.1*	178.7±1.9*
	5	109.3±1.8*	154.3±1.2*
	10	83.3±0.9*	119.0.±2.6*
C. longa (ME)	1	139.0±0.6*	175.3±2.0*
	2.5	120.0±1.1*	160.7±1.8*
	5	100.3±0.9*	147.7±3.5*
	10	66.0±1.5*	96.0±2.1*

Values are mean±SEM, (n=10), *p≤0.05 as compared to control group (one-way ANOVA followed by Tukey *post hoc* test), (p>0.05). AE: Aqueous extract, ME: Methanolic extract, *C. longa: Curcuma longa*

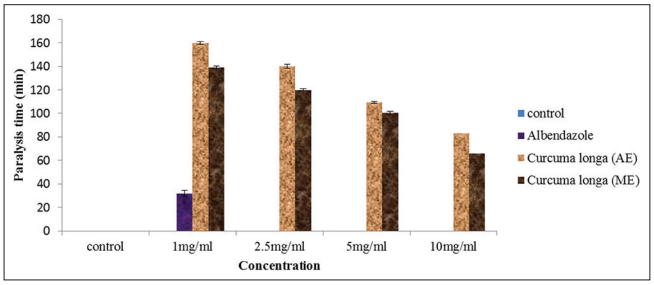

Fig. 1: Anthelmintic assay (worm paralysis) of aqueous extract and methanolic extract of *Curcuma longa* rhizome as compared to standard drug (albendazole)

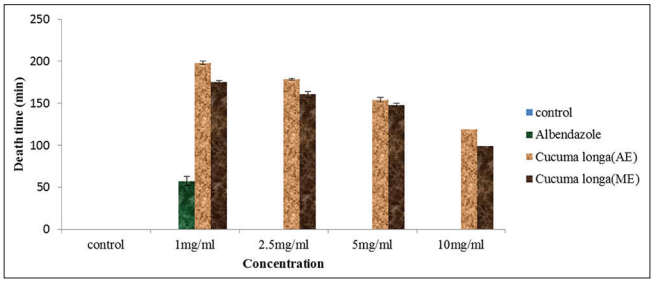

Fig. 2: Anthelmintic assay (worm death) of aqueous extract and methanolic extract of *Curcuma longa* as compared to standard drug (albendazole)

dose-dependent anthelmintic efficacy was shown by the AE of *C. longa,* wherein it took 159.7±2.0 min for paralysis and 198.0±2.5 min for the death of the parasite in the concentration of 1 mg/mL. At 2.5 mg/mL concentration of AE, the time of paralysis and death was 140.0±1.1 min and 178.7±1.9 min, respectively. For the concentration of 5 mg/mL, worms got paralyzed at 109.3±1.8 min and died at 154.3±1.2 min. Similar to the efficacy of ME, the AE at 10 mg/mL concentration proved to be most effective, causing paralysis at 83.3±0.9 min and death at 119.0±2.6 min. In the negative control (PBS) group, the worms were found to be efficiently motile and showed no paralysis or death within the 12 h study period. The positive control (albendazole) at the concentration of 1 mg/mL was very effective, causing paralysis at 32.0±2.3 min and death at 57.3±5.5 min.

DISCUSSION

Ethnoveterinary plants are the natural source for various types of the bioactive compound having the plentiful biological characteristic as medicine or for nourishment [29]. The present study demonstrated that the both AE and ME of *C. longa* have significant anthelmintic activity against *Haemonchus* spp. *in vitro*. The principal effect of the synthetic anthelmintic drug on the worm, as reported by other authors also, was to cause a drooping paralysis that results in the eviction of the worm by peristalsis. The high efficacy of standard drug albendazole is attributed to the action that it probably blocks the glucose uptake (by binding itself to free protein in the intestinal tract or on the body surface) which leads to reduced glycogen level in the parasite and finally resulting in occurrence of the death of the worms [30].

The qualitative phytochemical screening of the plant revealed the presence of secondary metabolites having great medicinal value as well as biological significance [31]. Several earlier studies have revealed that the anthelmintic activity of the plant is due to their phytochemical constituents', namely tannins, saponins, alkaloids, and phenols [22,32]. Tannins could bind to the cuticle of the worm body surface, causing paralysis and leading to death [9]. Alkaloids have antioxidating properties, hence, reduce nitrate generation which is used for protein synthesis, obstruct the transfer of sucrose from the stomach to the small intestine, and fail to support glucose uptake to the helminths that effects on CNS causing paralysis [33]. Saponin leads to vocalization and breakdown of helminths cuticular surface [34]. A number of allopathic phenolic anthelmintics such as niclosamide and bithionol are shown to hinder the path of energy generated in helminth parasites as a result of uncoupling oxidative phosphorylation, and this process probably supported the anthelmintic potency of tannins which is responsible for blocking ATP synthesis in these parasites [35].

The current study revealed that the ME showed the presence of glycosides, alkaloids, flavonoids, tannins, saponins, terpenoids, phenol, anthraquinone, and carbohydrates similar results were reported by other researchers [24,36], while the AE showed the presence of alkaloids, carbohydrate, flavonoids, terpenoids, and saponins which was similarly observed by other workers [37]. The results also disclosed that both AE and ME showed dose-dependent efficacy toward the paralysis of worms, which finally progressed to death as compared to the reference drug albendazole (positive control). Similar results were observed by studying other medicinal plants [38,39]. In the present study, the results shown that the ME of the plant rhizome at 10 mg/mL showed significantly higher potency (p≤0.05) toward the anthelmintic activity in comparison to the AE at the same concentration. The reason for this difference might be due to the presence of higher concentrations of biologically active compounds, i.e., tannins, saponins, phenols, and alkaloids in the ME of *C. longa* as compared to the AE. Similar findings have also been reported by other researchers with different ethnoveterinary plants [40-42]. Eventually, the outcomes of this study must be authenticated with *in vivo* analysis to evaluate the actual anthelmintic potency of this plant and could provide cheap and safe anthelmintic herbal drug.

CONCLUSION

The present study revealed that although both the AE and ME of *C. longa* showed dose-dependent anthelmintic activity *in vitro* against the *Haemonchus* spp. of GI parasite of goat, the ME possessed better anthelmintic efficacy. Both extracts of the plant rhizome (ME and AE) were screened for preliminary (qualitative) phytochemical analysis

and found to possess various types of secondary metabolites, especially tannins, saponins, phenols, and alkaloids which are reported to be responsible for the antiparasitic activity [43]. Thus, it is concluded from the present study that the extracts of *C. longa* could be explored further in the development of anthelmintic lead to treat parasitic diseases such as nematodiasis. The plant needs to be extensively studied in depth further for isolating the active component showing antiparasitic activity and for evaluating its potential for providing an alternative economical remedy resource so that common people can obtain the actual benefit of this important indigenous medicinal plant.

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AUTHOR'S CONTRIBUTIONS

Mrs. Jyoti Pandey contributed in carrying out the research, data compilation, and wrote the article. Dr. Kamal Jaiswal and Dr. Suman Mishra both were involved in corrections in the article and experimental set up of the study.

CONFLICTS OF INTEREST

The authors declare that this article has no conflicts of interest.

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