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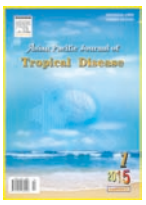

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Phytochemical screening and *in vitro* anthelmintic activity of methanol extract of *Terminalia citrina* leaves

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ABSTRACT

Objective: To evaluate anthelmintic activity of methanolic extract of leaves of *Terminalia citrina* (*T. citrina*) plant belonging to the Combretaceae family.

Methods: The tests of phytochemical screening included alkaloids, flavonoids, tannins, saponins, quinines, anthocyanins, glycosides, carbohydrates and reducing sugars. The anthelmintic activity of methanolic extract of leaves of *T. citrina* was evaluated against *Pheretima posthuma* at three different concentrations (25 mg/mL, 50 mg/mL and 100 mg/mL) of extracts which involved determination of time of paralysis and time of death of worms.

Results: The phytochemical screening of *T. citrina* leaves revealed the presence of flavonoids, tannins, alkaloids, carbohydrates and reducing sugars. The present study indicated that methanolic extract significantly exhibited paralysis and also caused death of worms especially at highest concentration of 100 mg/mL, as compared to standard reference Albendazole (10 mg/mL).

Conclusions: This study suggests that the leaves of *T. citrina* possess potent anthelmintic activity.

1. Introduction

Terminalia citrina (*T. citrina*) (Bengali name: Haritaki, Family: Combretaceae) is a deciduous tree wide spread throughout the forest of Gazipur, Tangail, Sylhet, Chittagong, Rangamati and Chittagong hill tracts of Bangladesh. It is an important medicinal plant having various ethno pharmacological uses. Different parts of the plant are used for various ailments. The fruit is used in long-term fever, loss of appetite and as sexual stimulant in Bangladesh[1]; diarrhea, helminthes and other digestive disorders in Iran[2]. Its bark is diuretic and cardio tonic[3]. Seed is used in stomach aches and intestinal diseases[4]. The plant is also used in asthma, diarrhea, boils, burns, constipation, migraine, dental disease, haemoptysis, dizziness, bleeding hemorrhoids, eye disease, gastric hyperacidity, anemia, arthritis, hoarse voice, dysentery, pyrexia, infections, traumatic cuts, cardiac diseases, cough, hepatomegaly, urolithiasis and for life longevity in Myanmar[5]. A detailed literature survey revealed that seed of plant was reported to possess antioxidant properties[6]and five tannins identified as corilagin (1) (3), punicalagin (2) (4), 1, 3, 6-tri-O-galloyl-β-D-glucopyranose (3) (5), chebulagic acid (4) (6), and 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucopyranose (5) (7) was isolated from methanol extract of fruit[7].

However, no detailed pharmacological study has been reported in the literature. Therefore in the present investigation, we aimed to investigate anthelmintic activity of *T. citrina* leaves.

2. Materials and methods

2.1. Collection of plant material

T. citrina leaves were collected from Rangamati District, Bangladesh during the month of May 2013. The plant was identified and authenticated by Sardar Nasir Uddin, Senior

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Scientific Officer, Bangladesh National Herbarium Mirpur, Dhaka and a voucher specimen (Accession No: DACB 38094) was deposited there for future reference.

2.2. Extraction preparation

The leaves of the plant were collected in fresh condition. The dried and coarse powder (1000 g) was extracted with methanol (4.0 L) in an air tight flat bottomed container for 15 days at room temperature with occasional stirring. The extract was then filtered through a cotton plug followed by a Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at low temperature and pressure to afford crude methanolic extract (50 g).

2.3. Selection of worm

The assay was performed on adult earthworm, *Pheretima posthuma* (*P. posthuma*) due to its anatomical and physiological resemblance to the human intestinal round worm parasite[8,9]. Because of easy availability, earthworms have been widely used for the initial evaluation of anthelmintic compounds *in vitro*. Adult earthworms (*P. posthuma*) were collected from moist soil of Savar area of Dhaka, Bangladesh and washed with normal saline to remove all fecal matters. Then the worms were used for anthelmintic study. The earthworms of 3-5 cm in lengths and 0.1-0.2 cm in width were used for all the experimental protocol.

2.4. Chemicals

Albendazole was collected from SK + F Pharmaceuticals Ltd., Bangladesh. Dimethyl formamide was purchased from Merck, Germany. All other chemicals were of analytical grade.

2.5. Phytochemical screening

The preliminary phytochemical group test was carried out by following standard procedure[10,11].

2.6. Anthelmintic activity

The anthelmintic assay was carried out as reported earlier[12] with minor modifications. The methanolic extract was dissolved in minimum amount of dimethyl formamide and the volume was adjusted to 50 mL with normal saline. All drugs and extract solutions were freshly prepared before starting the experiment. 50 mL formulations containing three different concentrations (25, 50 and 100 mg/mL in normal saline) were prepared and six worms were placed in it. Observations were made for the time taken for paralysis (paralysis was said to occur when no movement of any sort could be observed except when worm were shaken vigorously) and death (time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 ℃), followed with their body colors fading away). Albendazole (10 mg/mL) was used as reference standard while normal saline as the control.

2.7. Statistical analysis

All values were expressed as the mean ± SEM and the results were analyzed statistically by One-way ANOVA followed by Dunnett's *t*-test by using SPSS version 16. $P < 0.05$ compared to standard was considered to be statistically significant.

3. Results

3.1. Phytochemical screening

The phytochemical screening test showed the presence of various compounds in the leaves of *T. citrina* (Table 1).

Table 1
Phytochemical composition of methanol extract of *T. citrina* leaves.

Phytochemical	Interference
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	-
Quinones	-
Anthocyanins	-
Glycosides	-
Carbohydrates	+
Reducing sugars	+

(+): Presence; (-): Absence.

3.2. Anthelmintic activity

The effect of methanolic extract of *T. citrina* leaves at different concentration (mg/mL) to paralyze and cause death to earthworm to evaluate *in vitro* anthelmintic activity were observed as follows as shown in (Table 2).

Table 2
Effects of *in vitro* anthelmintic activity of methanolic extract of *T. citrina* leaves in *P. posthuma*.

Test sample	Cconcentration (mg/mL)	Time taken for paralysis (min)	Time taken for death (min)
Methanolic extract	25	3.21 ± 0.08[a]	3.78 ± 0.14[a]
	50	2.42 ± 0.02[a]	2.86 ± 0.10[a]
	100	1.44 ± 0.02[a]	1.59 ± 0.08[a]
Albendazole	10	3.76 ± 0.12	5.92 ± 0.18
Normal saline	-	-	-

Values are expressed as (mean ± SEM, $n = 6$). [a]$P < 0.001$, significant compared to standard (albendazole). Worms in control group were alive up to 24 h of observation.

It is evident that methanol extracts of *T. citrina* exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with 100 mg/mL concentration. The methanol extract caused paralysis at 1.44 min ($P < 0.001$) and time of death at 1.59 min ($P < 0.001$) with 100 mg/mL against the earthworm *P. posthuma*. The standard drug Albendazole at 10 mg/mL concentration showed the same at 3.76 and 5.92 min respectively which is comparable with extract at 25 mg/mL.

4. Discussion

Anthelmintic drugs are losing their efficacy due to resistance of

these drugs especially as a single dose regimen. Widespread efforts have been made to find out more effective and safe anthelmintic drugs. Plant extracts are potential sources of anthelmintic drugs and have been extensively studied for anthelmintic activities[13]. This study has been carried out *in vitro* because it is cost effective method[14]. Albendazole is known to cause paralysis of worms so that they are expelled in faeces of man and animals. Experimental plant extract not only demonstrated this property, but it also caused early death of worms at all concentrations compared to drug. Thus findings from the current study revealed that extract have shown promising *in vitro* anthelmintic activity.

The phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, carbohydrates and reducing sugars. The presence of some of these secondary metabolites suggests that the plant might be of medicinal importance.

The literature review reveals that tannins which are chemically polyphenolic compounds are responsible to produce anthelmintic activity. The reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death[15,17]. The presence of alkaloids[18] and flavonoids[19] were also responsible for the observed anthelmintic activity.

As phytochemical analysis of the methanolic leaf extract of *T. citrina* revealed the presence of the tannin, flavonoids and alkaloids among other chemical constituents, it is possible that these constituent contained in the extracts produced similar effects. According to the above mentioned results it can be concluded that the methanolic leaf extract of *T. citrina* possess potent anthelmenthic activity, which is reported first time by our study. This study also suggests further investigation is required to isolate bioactive compounds responsible for the observed effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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