



Evaluating the anthelmintic potential of leaf gall extracts of *Terminalia chebula* (Gaertn.) Retz. (Combretaceae)

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ABSTRACT

Objective: *Terminalia chebula* gall extracts are widely used Asian folk and traditional medicine. The present study was carried out to evaluate the anthelmintic potential of different extracts of leaf galls. **Materials and Methods:** The anthelmintic activity was assessed by applying five different concentrations of the plant extracts on Indian adult earthworms, and the time of paralysis and death was recorded. **Results:** The ethanolic extract processed potent anthelmintic activity, when compared to the other extracts. The lowest time for paralysis and death of worms, for test sample at highest concentration (250 mg/ml), were found to be 7.30 ± 2.66 and 14 ± 0.58 min, respectively. Albendazole, which was used as standard, caused paralysis and death of worms at 07.00 ± 3.55 min and 12.60 ± 2.01 min, respectively; whereas no mortality of the worms was observed, when distilled water was used as control. The presences of phenolics, flavonoids, triterpens, saponins, glycosides, phytosterols, reducing sugars were identified in the extracts and the significant anthelmintic property of *T. chebula* might be due to the presence of alkaloids, phenolic compounds, and flavanoids. **Conclusion:** The results of this study establishes the anthelmintic activities of *T. chebula* leaf gall extracts and justify the ethnobotanical approach in the search for novel bioactive compounds. The anthelmintic potential of *T. chebula* extracts may be due to the presence of phyto-constituents like alkaloids, phenolic compounds, and flavanoids. Future work will be interesting to know the chemical composition and better understand the mechanism of action of the antioxidants present in the extract for development as drug for therapeutic application.

Key words: Albendazole, anthelmintic, drug, galls, plants, *Terminalia chebula*

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INTRODUCTION

The treatment of various diseases with plant-based medicines has remained as an integral part of many cultures across the globe. Helminthiasis, is one of the most serious public health problems in the world.¹ Day by day, the prevalence of anthelmintic resistance in

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nematode is increasing, and the synthetic drugs that are used in the treatment are shown to have potential side effects.² Therefore, the medicinal properties of plants have been investigated and explored for their potent anthelmintic activity that pose minimum environmental hazards, have less adverse effects, and are easily available with affordability.²⁻⁴

Terminalia chebula (Gaertn.) Retz. (Combretaceae) commonly known as black myrobalan and haritaki, is an important medicinal plant that is native to tropical regions of southern Asia. It is amply referred to as “king of medicines” as it has been a component of many formulations for the treatment of various diseases in all streams of Indian system of medicines.⁵ It consists of gall-like excrescences formed by plant-insect *Dixotbripsonerosus* (Thysanoptera).⁵ These galls are commonly known as *Karkatsbringi* and is an important ayurvedic drug used in preparations of Dasamularista, Cyavanaprasa and Shringyadi Curna, which are used in the treatment of asthma, tuberculosis, indigestion, heart diseases, fevers and liver disorders etc.⁵ *Karkatsbringi* also finds usage in the treatment of children’s ear infections, suppress hemorrhage from gums and also used to suppress bleeding from nose.⁵ Hakims consider galls useful in pulmonary infections, diarrhea and vomiting.⁶ The accepted source of *Karkatsbringi* are the galls of *Rhus succedanea* L., but *Pistacia integerrima* and *T. chebula* are also generally used in preparations.⁵ Gall extracts of *T. chebula* have been found to possess anti-inflammatory activity, anti-bacterial, anti-tyrosinase activity, anti-cancer activity, and anti-ageing activity.⁷ In this study, we have evaluated the anthelmintic potential of *T. chebula* leaf gall extracts and elucidate its phytochemical constituents for having knowledge on the principal components responsible for its biological activity.

MATERIALS AND METHODS

Materials

All the chemical reagents and solvents of analytical grade were purchased from SRL Chemicals, India. Albendazole (ZENTEL, Glaxosmithkline Pharmaceuticals Ltd, Karnataka, India) dissolved in 0.5% dimethyl sulfoxide in normal saline (v/v) were used as standard reference drugs and distilled water was used as control.

Plant material and extracts preparation

The gall induced leaves of *T. chebula* were purchased from the local market of Bangalore, India. The plant materials were authenticated by Dr. S. Sundara Rajan and the voucher specimen (JU-RUV-52) were deposited at Research Centre

of Vrikshayurveda, Jain University, Bangalore. The galls were cleaned with distilled water, dried and crushed into a fine powder. The coarsely powdered gall materials were sequentially extracted with ethanol, petroleum ether, chloroform and aqueous solvents in soxhlet apparatus for 24 h.

Animals

Indian adult earthworms belonging to species *Pheretimaposthuma* (Annelida) were collected from the Indo-American hybrid seeds, Bangalore. The earthworms were maintained under normal vermicomposting medium with an adequate supply of nourishment and water, for about 2 weeks. Adult earthworms of approximately 4 cm in length and 0.2-0.3 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites in human beings.⁸

Phytochemical analysis

Phytochemical analysis was carried out using the standard methods as describe earlier.⁴

Anthelmintic activity

The anthelmintic activity of leaf gall extracts (petroleum ether, chloroform, ethanol and aqueous) of *T. chebula* was evaluated as per the method reported by Dash *et al.*,⁸ The adult earthworms *Pheretimaposthuma* in a petri-dish were divided into five major groups (ethanol extract of *T. chebula*; aqueous extract of *T. chebula*; chloroform extract of *T. chebula*; petroleum ether extract of *T. chebula* and albendazole - (0-100 mg/ml) with each containing sub-groups of I, II, III, IV, and V of different concentrations of extracts administration (each containing five worms). The different extracts at different concentrations (0-250 mg/ml) were applied to the petri-dishes, and the time of paralysis and death was determined in minutes. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C) followed with fading away of their body colors.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean \pm standard deviation. Statistical analysis of all data was carried out using Microsoft Excel 2007 (MicroSoft®, Inc., USA) statistical software. Student’s t-test was used to determine a significant difference

between the experimental groups. Statistical significance was assumed at *P* values of 0.05.

RESULTS AND DISCUSSION

The anthelmintic activity of leaf gall extracts (petroleum ether, chloroform, ethanol and aqueous) of *T. chebula* was evaluated by testing on the adult earthworm *Pheretima posthuma*. The results revealed that, the extracts possess varying degree of anthelmintic activity (Table 1) and the ethanolic extract was much more potent, when compared with the other extracts (Table 1). Further, the potential anthelmintic property of ethanolic extract was comparable to that of standard drug, i.e., albendazole used (07.00 ± 3.55 and 12.60 ± 2.01 , respectively) at 100 mg/ml (Table 1). It was found that the concentration of the extract had an inversely proportional relationship with the time of paralysis and death of the worms.

In the preliminary phytochemical studies, the qualitative presence of phenolics, flavonoids, triterpens, saponins, glycosides, phytosterols, reducing sugars were identified in different extracts (Table 2). The leaf gall of *T. chebula* are reported to be very rich in tannins, triterpenoids, flavonoids,

essential oils, and others phenolic constituents.^[5,7] It was reported that, phenolics or tannins may interfere with energy generation of worms by uncoupling oxidative phosphorylation or they binds to the free protein of the gastrointestinal tract of the worms and lead to death.⁹ Alkaloids were reported to cause paralysis of the worms by acting on its central nervous system.⁹ It is possible that phenolics, alkaloids and tannins present in the extracts of *T. chebula* gall produced similar effects and bring about significant anthelmintic property. Albendazole acts to increase chloride ion conductance of worm muscle membrane which produces hyper polarization and excitability reduction that leads to muscle relaxation and flaccid paralysis of worms.¹⁰ In the present study, the extracts not only demonstrated this property, they also caused the death of the worms. It is expected that the phytochemicals present in the extract of *T. chebula* may have produced similar effects, causing death of the worms.

CONCLUSION

The results of this study establishes the antihelminthic activities of *T. chebula* leaf gall extracts and justify the

Table 1: Antihelminthic activity of *T. chebula* leaf gall extract

Test samples	Group	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
ETC	I	50	19.00±1.44*	35.50±5.21*
	II	100	14.00±2.89*	26.67±0.33**
	III	150	11.50±3.84*	22.67±1.76**
	IV	200	9.33±0.33**	16.33±0.88**
	V	250	7.30±2.66*	14±0.58**
ATC	I	50	21.00±0.12**	39.00±5.85*
	II	100	17.00±2.77**	30.50±2.42**
	III	150	12.50±4.50**	25.50±1.74**
	IV	200	10.50±1.84**	18.50±3.17*
	V	250	09±0.58**	15.00±4.12*
CTC	I	50	35.33±1.76**	51±1.53**
	II	100	20.07±1.02**	39.00±1.04**
	III	150	13.67±0.88**	29.80±2.14*
	IV	200	10.00±1.30**	23.00±1.27**
	V	250	10.50±2.86	17.00±3.12
PTC	I	50	101.67±1.78**	180.44±0.85*
	II	100	75.33±0.45**	149.33±0.88**
	III	150	51.67±1.00**	130.67±0.76**
	IV	200	37.67±0.86**	119.33±0.48**
	V	250	23.67±1.76**	104.44±0.88**
Standard drug (Albendazole)	I	20	18.60±0.33	60.00±2.14
	II	40	14.90±2.68	49.33±0.04
	III	60	13.10±1.33	42.26±1.02
	IV	80	10.60±2.70	37.40±3.60
	V	100	07.00±3.55	12.60±2.01

Values are the mean±SE of three earthworms. **P*<0.05, ***P*<0.01 as compared to standard. ETC: Ethanolic extract of *T. chebula*, ATC: Aqueous extract of *T. chebula*, CTC: Chloroform extract of *T. chebula*, PTC: Pet ether extract of *T. chebula*, *T. chebula*: *Terminalia chebula*

Table 2: Preliminary phytochemical analysis of leaf gall extracts of *T. chebula*

Chemical constituents	Petroleum ether	Chloroform	Aqueous	Ethanol
Flavonoids	-	-	+	+
Steroids	+	+	-	+
Triterpenes	+	+	+	+
Tannins	-	-	+	+
Saponins	-	-	+	+
Alkaloids	-	+	-	+
Glycosides	-	-	-	+
Carbohydrates	-	-	-	-

(+) Indicates present, (-) Indicates absent. *T. chebula*: *Terminalia chebula*

ethnobotanical approach in the search for novel bioactive compounds. The anthelmintic potential of *T. chebula* extracts may be due to the presence of phyto-constituents like alkaloids, phenolic compounds, and flavonoids. Future work will be interesting to know the principal component involved and better understood the mechanism of action present for development as drug for therapeutic application.

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