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Phytochemical screening, Cytotoxic and Anthelmintic activities of the methanolic leaf extract of *Terminalia catappa* Linn

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Abstract

The present study is aimed to evaluate phytochemical screening, cytotoxic activity and anthelmintic activity of methanolic extract of *Terminalia catappa* leaves. Phytochemical properties of different extractives of plant materials were tested. Anthelmintic activity was assessed applying three different concentrations of the plant extract and recording the time of paralysis and death. While brine shrimp lethality test (BSLT) method was used to evaluate the cytotoxicity of the plant extract, where vincristine sulphate and DMSO was used as positive and negative control respectively. The phytochemical screening revealed the potent source of different phytochemical constituents on different extractives including alkaloid, glycosides, tannin, saponins, flavonoids, steroids and carbohydrate. In case of cytotoxicity measurement, the crude methanolic extract showed positive result (with LC₅₀ 1.4 µg/ml) compared to standard Vincristine sulphate (0.35 µg/ml); which indicated that the leaves of *T. catappa* possess mild cytotoxic principles. An elaborate study was carried out on the anthelmintic potential of methanolic extract of *T. catappa* where Albendazole as a reference standard.

The lowest time for paralysis and death of worms, for test sample at highest concentration (100mg/ml) and standard drug were found 34.09±0.44 and 50.04±0.09 min respectively, which gradually increased with the decrease of concentration. Results showed a dose dependent increase in the anthelmintic activity. Therefore, further studies are suggested to evaluate the possible mechanism of action and the active compounds responsible for the biological activities of the plant extract.

Keywords: *Terminalia catappa*, Phytochemical screening, Cytotoxic activity and Anthelmintic activity.

Introduction

Traditional medicine has been practiced for many centuries in many parts of the world, including India especially in rural areas due to availability and low cost. Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. About 80% of the world's population relies on traditional medicine for significant part of their primary health care needs [2]. Extraction of bioactive compounds from medicinal plants allows the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity [3, 4, 5, 6, 7]. In this study *Terminalia catappa* (Combretaceae) also known as Indian almond, a tall deciduous and erect tree reaching a height of 15-25m, all parts of which (leaves, bark, roots and fruits) are useful in traditional medicine for the treatment of wounds and ulcerations, boils and some bacterial infections with which the test organisms are associated is used [7, 8, 9]. It has been reported that extracts of *T. catappa* leaves show antioxidative, anti-inflammatory and hepatoprotective actions and contains hydrolysable tannins or triterpenoids [8, 10, 11, 12]. This study therefore seeks to examine the antibacterial potency of the ethanol and aqueous leaf extracts of *Terminalia catappa*, against some selected clinically important bacteria, the minimum effective concentration and determine the phytochemical components of the medicinal plant. Coupled with our continuous interest of pharmacological screening of Bangladeshi medicinal plants, in this study we aimed to investigate phytochemical screening, cytotoxic and anthelmintic activities of the methanolic extract of *Terminalia catappa*.

Materials and Methods**Plant material**

The leaves of *T. catappa* were collected from Dhaka on September, 2015. After collection leaves were thoroughly washed with water and identified by expert of Bangladesh National

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Chemicals and Reference drug

All the chemical reagents used in the analysis of phytochemicals were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA) and E. Merck (Germany). For the anthelmintic test, the methanolic extract of leaves of *T. catappa* was tested using different concentrations. Distilled water was used as control and Albendazole (Square Pharmaceuticals Ltd., Bangladesh) was used as the standard drug for evaluation of anthelmintic activity. For the assessment of cytotoxic activity, Vincristine Sulphate (VS) was used as standard drug and DMSO was used as control.

Preparation, extraction and fractionation of plant material

Cold maceration technique was used for extraction. Powder of leaves (500 g) was soaked in 2500 ml of methanol for about 10 days at room temperature with occasional stirring. After 10 days the solutions were filtered using filter cloth and Whatman's filter paper which was concentrated by evaporating under ceiling fan and in a water-bath not exceeding 40 °C to have gummy concentrate of extract. The concentrated methanol extracts were separately partitioned by the modified Kupchan method using pet ether, carbon tetrachloride, and chloroform. The aqueous methanolic fraction was preserved as aqueous fraction^[13].

Preparation of fresh juice extract

Collected leaves were weighed (75g) and blended into liquification in 150ml of water. The mixture was then centrifuged at 150 rpm. The supernatant was filtered through sterile filter paper in to conical flask as the study extract 1ml of filtrate is expected to contain 0.5g i.e. 500mg/ml.

Collection of worms

The earthworms belonging to species *Pheretima posthuma* (Annelida), about 3-5 cm in length and 0.1-0.2 cm in width, & weighing about 0.8-3.04 g, were collected from the moist soil of of Savar area, of Dhaka in Bangladesh.

Phytochemical screening

Phytochemical properties of different extractives of plant materials were tested using the method of Trease and Evans^[14].

Evaluation of cytotoxicity

The cytotoxicity was conducted using brine shrimp lethality test following the method of Firdaus *et al.*^[15]. The brine shrimp eggs were placed in 1 L of sea water, aerated and

hatched for 48 hrs at 37 °C, to become nauplii. After 48 hr, ten brine shrimp nauplii were placed in a small container filled with seawater. Methanolic extract of *Terminalia catappa* leaves, serially diluted with DMSO (Dimethyl Sulfoxide), was then added to the container. The mortality of brine shrimp was observed after 24 hrs of treatment was given. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted and the values of LC₅₀ were calculated using Microsoft Excel® 2007. Vincristine sulphate was used as positive control.

Evaluation of anthelmintic activity

The assay for anthelmintic activity was carried out as per the method of Ajaiyeoba *et al.*^[16] with insignificant modifications. The test was conducted by using the adult earthworm, *Pheretima posthuma*, because of its anatomical and physiological similarity with the intestinal round-worm parasite of human beings^[17, 18, 19]. Earthworms have been used widely for the preliminary evaluation of anthelmintic activity in vitro because of easy availability and ease of use^[20, 21, 22]. All the worms were washed with normal saline water to remove all fecal matters. Extract was weighed and dissolved in 150 mL of distilled water to obtain the concentrations of 25, 50 and 100 mg/ml. Earthworms were divided into seven groups (each containing five worms) in petri-dish. The methanolic extract of *T. catappa* leaves was applied to the petri-dishes and the time of paralysis and death was determined. 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

Data were processed and analyzed using both Microsoft Office Excel® version 7 and SPSS (version 16.0). The LC₅₀ value of plant extracts, from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (Microsoft Office Excel® version 7) and finally the LC₅₀ was derived from the best-fit line obtained. The data of anthelmintic studies were reported as mean ± standard deviation.

Results

Phytochemical screening

The preliminary phytochemical investigation showed the presence of phytochemical constituents such as alkaloid, glycosides, tannin, saponins and carbohydrate but absence of saponins and flavonoids in different extractives (Table 1).

Table 1: Result of chemical group tests of the methanol extract of *T. catappa*

Extract	Carbohydrate	Tannin	Flavonoid	Saponin	Glycosides	Steroid	Alkaloid
Methanolic extract of <i>P. T. catappa</i>	+	+	-	-	+	+	+

(+) =Presence; (-) =Absence

Cytotoxic activity

The lethal concentration (LC₅₀) of the test samples after 24 hours was determined by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. The

lethality of the extracts to brine shrimps was determined and the results are given in Table 2. Vincristine sulphate (VS) was used as positive control and the LC₅₀ value was found as 0.35µg/ml. The LC₅₀ value of methanolic extract was found to be 1.4 µg/ml.

Table 2: Brine shrimp lethality bioassay of methanolic extract of *T. catappa*

Sample	LC ₅₀ (µg/ml)	Regression Equation	R ²
Vincristine Sulfate (Positive control)	0.35	y = 22.14x + 79.18	0.926
<i>T. catappa</i> extract	1.4	y = 12.18x + 31.93	0.698

Anthelmintic activity

The results of anthelmintic activity of the methanolic extract of leaves of *T. catappa* revealed that, it possesses varying degree of anthelmintic activity; i.e. the extract exhibited not

only paralysis but also death of worms at all the tested concentrations. From the above study, it was observed that the methanolic extract of *T. catappa* leaves was a potent anthelmintic, when compared to the standard drug in a dose dependent manner (Table 3). The highest activity (shortest time for paralysis and death of worms) of the plant extract was found at the concentration of 100 mg/ml, as compared to the standard drug albendazole at 50 mg/ml, which gradually decreased with the decrease of concentration of the plant extract. Thus it was also clear from the study that, the concentration of the extract has an inversely proportional relationship with the time of paralysis and death of worms.

Table 3: *In-vitro* Anthelmintic activity of methanolic extract of *T. catappa*

Drug (Treatment)	Conc. mg/ml	Time taken for paralysis (minutes)	Time taken for death (minutes)
Control (0.9% Normal Saline)	--	--	--
Methanolic extract of <i>T. catappa</i> Linn.	25	50.18 ± 0.16	55.28 ± 0.40
	50	45.34 ± 0.47*	58.91 ± 0.30*
	100	34.09 ± 0.44*	50.04 ± 0.09*
Standard drug (Albendazole)	25	45.63 ± 0.47*	61.97 ± 0.47*
	50	40.33 ± 0.47*	60.32 ± 0.44*
	100	35.53 ± 0.33*	52.18 ± 0.16*

Each value is presented as the Mean ± SEM (n=5). Values were found out by using ONE way ANOVA followed by Dunnett's test. Significance level *P = ≤0.05.

Discussion

Preliminary phytochemical studies on *T. catappa* exposed the presence of glycosides, carbohydrates, tannins, steroids and alkaloids. To assess the toxicity of the crude extracts towards the brine shrimp, the brine shrimp lethality bioassay (BSLA) is a routinely and widely used method. This method also indicates the possible toxicity of the extracts on the test materials and several antitumor and pesticidal natural products have been identified using this method [23]. Again, we know that plant extracts contains a higher concentration of bioactive compounds and also several compounds which show cytotoxic activity [24].

It was reported that several active compounds such as anthocyanins, saponins, tannins, flavones, and polyphenols etc. are known to be free radical scavenger, reactive species quencher, hydrogen donor, antioxidant enzymes activator, detoxification inducer, normal cell differentiation promoter, tumor production and proliferation cell inhibitor, and apoptosis inducer [25]. So, the bioactive compounds may be accountable for the possible cytotoxicity of the methanolic extract of *T. catappa* leaves, although the exact mechanism of action is yet to be discovered.

Some of these phyto-constituents like alkaloids, tannins, phenols etc. may be accountable to have a significant anthelmintic activity [26]. It was reported that, tannins may to 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 [27]. In another study, alkaloids were reported to cause paralysis of the worms by acting on its central nervous system [28]. The prime effect of albendazole is to cause a flaccid paralysis of the worm which results in expulsion of the worm by peristalsis. Albendazole acts to increase chloride ion conductance of worm muscle membrane which produces hyperpolarization and excitability reduction that leads to muscle relaxation and flaccid paralysis of worms [29].

It is expected that the phytochemicals present in the extract of *T. catappa* may have produced similar effects, causing death of the worms. Therefore, the usual claim of leaves of *T.*

catappa as an anthelmintic has been confirmed as the extracts shown significant activity against *Pheretima posthuma*.

Conclusion

The present study deduces that the plant *T. catappa* can be a good source of novel anthelmintic and cytotoxic agent. So, studies are suggested for the isolation, purification, characterization, and testing of individual compound in vivo.

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