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Research Article

Ex vivo: Anthelmintic Evaluation of *Bombax ceiba* Stem Extracts

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ABSTRACT

Bombax ceiba plant belongs to the Bombacaceae family and is commonly known as *Semal* (Hindi) and cotton tree (English). It has numerous effects like stimulant, astringent, haemostatic, diuretics, antidiarrhoeal, emetic, and antipyretic. Major pharmacological activities reported in this plant are hypoglycemic, anti-anxiety, antibacterial, anti-viral, and anti-inflammatory.

Numerous side effects and increased resistance to anthelmintic drugs prompted us to discover cost effective, safe formulation of conventional medicinal plants. In the present study, research work is focused on evaluating the possible anthelmintic effects of *B. ceiba*. To investigate *B. ceiba* plant's anthelmintic potential, different extracts from dried powder stem bark were tested on adult Indian earthworms (*Pheretima posthuma*) having physiological and anatomical resemblance with human intestinal roundworm parasites.

The drugs were divided in 14 groups consisting of test, standard, and control. Each group was consisting of six Indian earthworms of approximately the same size. Albendazole was used as a standard drug, while Tween 80 in normal saline (1:1) was used as control. The result data reveals that all extracts have significant anthelmintic activity as compared to albendazole taken as a standard drug. The methanolic extract at a dose of 50 mg/mL concentration showed paralysis time 30 ± 0.96 minutes and death 33.66 ± 0.71 minutes, which is equivalent to standard drug values at a dose of 20 mg/mL. Ethylacetate extract at a dose of 50 mg/mL showed paralysis at 28.66 ± 0.71 minutes and death at 34.16 ± 1.1 minutes, which proves that it is even better than standard drug in terms of paralysis time and equipotent in terms of time taken for causing death. Thus, among the extracts, ethylacetate and methanolic extracts showed maximum activity with least time taken for paralysis and death of earthworms and were found equally potent as albendazole at a dose of 50 mg/mL. Thus, *B. ceiba* traditional use in helminitic infestation was proved scientifically. On comparing phytochemical evaluation with anthelmintic activity, it is assumed that glycosides may be responsible for this activity. A further investigation may lead development of novel anthelmintic drug or formulation with anthelmintic activity.

INTRODUCTION

Helminthiasis is one of the most common infections caused by worms contaminant to various human body parts. Normally, the worm lives in the gastrointestinal tract, liver, and other organs.[1] Infection of parasitic worms or soil-transmitted helminths (STHs), *viz.*, roundworm (*Ascaris lumbricoides*), *Ancylostoma duodenale,* whipworm (*Trichuris trichiura*), and *Necator americanus* (hookworm), which affect more than two billion people worldwide called as helminths disease. These diseases have the utmost contamination rates in marginalized populations in the tropics and subtropics areas along with schistosomiasis as widely spread chronic infections in the world.[2,3] Chronic STH infection diminishes nutrition absorbing propensities, which in turn exacerbates malnutrition. Additionally, severe infections have been found to be linked with other abnormalities, like anemia, stunted growth, chronic abdominal pain, and malnutrition. The more severe but rare complications include bladder cancer, hepatosplenomegaly, and death.[4,5]

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The existing anthelmintic drugs have numerous side effects, such as, abdominal pain, loss of appetite, nausea, vomiting, and diarrhea. Prolonged use of anthelmintic drugs causes headaches, hyperthermia, alopecia, and neutropenia.[1] In order to avoid the harmful side-effects of available marketed anthelmintic drugs, it is essential to promote research on traditionally used anthelmintic herbal drugs, which may lead to the development of potent, safe anthelmintic substances with ease of availability and without any side-effects.[6] Even most pharmacopeia still contains almost 25% of drugs that are derived from plants. Many of the drugs are semi-synthetic in nature and are built on prototype compounds isolated from plants. Also, as per the World Health Organization (WHO), 75% world's population depends on traditional medicines to meet their primary health care needs, most types of which use remedies from plants.[7]

B. ceiba or silk cotton tree belongs to the family of Bombacaceae is a large deciduous tree found throughout India's tropical and subtropical region. The stem bark extract of *B. ceiba* plant is known for numerous pharmacological activities, such as, anti-inflammatory, antihypertensive, antimicrobial, analgesic, antiangiogenic, anti-viral, etc.[8,9] The stem bark of *B. ceiba* contains shamimicin, like flavonoids, lupeol, β-sitosterol, glycosides, sterols, and terpenoids.[10,11] Hossain *et al.* reported the use of *Bombax malabaricum* (*B. ceiba*) leaves as an anthelmintic in the Southern Punjab of Pakistan.[12]

Higher incidences of side effects and increased resistance to anthelmintic drugs prompted us to discover cost-effective, safe drugs from conventional medicinal plants. Since the *B. ceiba* plant consists of very useful phytoconstituents, which is reported as anthelmintic, we planned to evaluate its stem bark extracts for anthelmintic activity.

The research work is conducted to evaluate the possible anthelmintic effects of *B. ceiba*. To investigate the anthelmintic potential of *B. ceiba*, plant extracts from dried powder stem bark were tested on adult Indian earthworms (*P. posthuma*). An Indian earthworm (*P. posthuma*), which has anatomical and physiological resemblance with our intestinal roundworm parasites were used in experimental models.

Materials and Methods

Collection of Plants

The stem bark of *B. ceiba* (Bombacaceae) was collected from village Bilaspur, district Yamuna Nagar, Haryana, during February 2019 and got authenticated at Department of Botany, Kurukshetra University, Kurukshetra. The herbarium sheet was prepared and is placed in Department of Botany, Kurukshetra University, Kurukshetra, for reference with a specimen number KUK/Bot/IPS-42. Collected stem barks were washed with tap water to removed adhering impurities. The stem bark was cut into small pieces and then dried at room temperature. The dried stem bark was crushed to make coarse powder and stored in sealed containers prior to the extraction.

Extraction

The powdered stem bark was successively extracted using different solvents as per the polarity index (toluene, ethyl acetate, and methanol) in the Soxhlet apparatus. A sufficient quantity of drug, as per the volume of the extractor, was packed in a thimble (made of filter sheet). A cotton plug was placed at the bottom of the extractor, and a thimble packed drug was adjusted in the extractor by taking necessary precautions to avoid chocking. A volume of solvent sufficient to boiler capacity was added, and a hot continuous extraction process was initiated. During this process, more volume of solvent was added to prevent interruption of siphoning. The extraction was continued for about 36 to 48 hours or until extracting solvent in the siphoning tube became colorless. The solvent was concentrated by distilling excess of solvent under reduced pressure on rotatory vacuum evaporator (Heidolph Laborota 4011, digital) temperature below 40 to 45°C. Solid mass (extracts) were recovered from the flask and concentrated at room temperature, and the percentage yield of each extract was calculated. Each of the extracts was stored in an airtight glass container in a refrigerator (-10°C) for further studies. The toluene extract (TEBC), ethyl acetate extract (EAEBC), and methanolic extract (MEBC) so obtained were used for evaluation of anthelmintic activity after preparing suitable dilution.

Test Organisms

Indian earthworms (*P. posthuma*) were procured from an organic company near a village Mirzapur, district Kurukshetra, Haryana, during March 2019. Indian earthworms (*P. posthuma*) were placed in a sorting tray for anthelmintic activity and washed to remove adhering fecal matter. Earthworms 5 to 8 cm in length and 0.2 to 0.3 cm in width were selected for the experimental protocol.

Drugs

The control solution was prepared as a suspension of Tween 80 in normal saline (1:1). The standard drug (albendazole) and test drugs (*B. ceiba* extracts) were dissolved in the suspension of Tween 80 prepared in normal saline in a ratio of 1:1 to get final drug concentrations of 20, 5, 10, 25, and 50 mg/mL, respectively.

Phytochemical Screening

Preliminary phytochemical analysis of *B. ceiba* extracts was carried out to check the presence or absence of various phytoconstituents, like carbohydrates, glycosides, alkaloids, saponins, flavonoids, steroids, tannins, phenols, proteins, and amino acids, using suitable chemical tests procedures.[13]

Anthelmintic Activity

Anthelmintic activity of test extracts was carried out as per the method explained by Ajaiyeoba *et al.* and Ghosh *et al*.[14,15] The activity was performed on the mature Indian earthworm (*P. posthuma*) with physiological and anatomical similarity to the human intestinal roundworm parasite. The Petri dishes were number as control group (I), standard group (II), and test drug groups (III–XIV). A volume of 20 mL of control, standard, and test drugs solutions together were poured into each of the Petri dishes (I–XIV). Previously sorted six earthworms (n = 6) of similar size were place in each Petri plate at room temperature. Time for paralysis was recorded upto time when no movement was observed, except when the worms were shaken forcefully. The time of death for worms was counted after ascertaining that neither the worms moved and shaken vigorously or put in warm water (45 to 50°C).

Results

Preliminary Phytochemical Screening

The test extracts' phytochemical screening was carried out using various chemical test procedures,[16-26] as presented in Table 1. The results showed the presence and absence of phytoconstituents in *B. ceiba* plant extracts. Phytochemical screening of *B. ceiba* showed the presence of carbohydrates, alkaloids, glycosides, saponins, tannins, phenols, flavonoids, and steroids in the stem bark. Toluene extract was found to have the least number of phytoconstituents and showed the presence of carbohydrates, saponins, flavonoids, steroids, tannins, and phenols. Toluene extract did not show the presence of glycosides (legal test), alkaloids, and proteins. Ethylacetate extracts were found to have a moderate number of phytoconstituents and showed all the phytoconstituents except alkaloids, proteins, and

Table 1: Results of phytochemical evaluation of *B. ceiba* stem bark extracts

S. No.	*Chemical test*	*TEBC*	*EAEBC*	*MEBC*
% yield	*Successive extraction*	*15*	*10*	*20*
1.	Carbohydrates			
	Molisch test	+	+	+
	Benedict test	+	+	+
	Fehling test	+	+	+
2.	Glycosides			
	Legal test	-	+	+
3.	Alkaloid			
	Mayer's test	-	-	+
	Hager's test	-	-	+
	Wagner's test	-	-	+
4.	Saponins			
	Foam test	+	+	+
5.	Flavonoids			
	Shinoda test	+	+	+
	conc^n H_2SO_4 test	+	+	+
6.	Steroids			
	Salkowski test	+	+	+
	Libermann-Burchard test	+	+	+
7.	Tannins and phenol			
	Fe^{3+} chloride test	+	+	+
	Pb acetate test	+	+	+
8.	Proteins and amino acids			
	Biuret test	-	-	-
	Million test	-	-	-
	Ninhydrin test	-	-	-

"+" sign indicates presence of phytoconstituents; "-" sign indicates absence of phytoconstituents

Table 2: Results of anthelmintic activity of *B. ceiba* (stem bark) extracts

S. No.	*Drug/ extract*	*Dose*	*Paralysis time (min) (± SEM)*	*Death time (min) (± SEM)*
I.	Control (NS + Tween 80)	20 mL	-	-
II.	Standard drug (albendazole)	20 mg/mL	30.8 ± 0.79	34.66 ± 0.42
III.	MEBC	5 mg/mL	81.61 ± 0.94**	87.5 ± 0.95**
IV.	MEBC	10 mg/mL	61.66 ± 1.2**	66.16 ± 0.94**
V.	MEBC	25 mg/mL	39.83 ± 1.04**	44.16 ± 0.6**
VI.	MEBC	50 mg/mL	30 ± 0.96**	33.66 ± 0.71**
VII.	EAEBC	5 mg/mL	87.83 ± 1.01**	93.33 ± 0.95**
VIII.	EAEBC	10 mg/mL	71.51 ± 0.99**	78.16 ± 1.24**
IX.	EAEBC	25 mg/mL	44.83 ± 1.1**	56.5 ± 0.88**
X.	EAEBC	50 mg/mL	28.66 ± 0.71**	34.16 ± 1.1**
XI.	TEBC	5 mg/mL	112.83 ± 0.79**	118.16 ± 0.79**
XII.	TEBC	10 mg/mL	82.66 ± 1.45**	88.66 ± 0.98**
XIII.	TEBC	25 mg/mL	70.5 ± 1.25**	77.8 ± 1.42**
XIV.	TEBC	50 mg/mL	44.16 ± 0.6**	54.5 ± 1.33**

**p < 0.01; significant (p < 0.01); n = 6; data is expressed as mean ± SEM; MEBC = methanol extract of *B. ceiba*; EAEBC = ethylacetate extract of *B. ceiba*; TEBC = toluene extract of *B. ceiba* amino acids. The methanolic extract was the only extract that showed maximum tests positive for the presence of phytoconstituents except for proteins and amino acids. None of the extracts showed the presence of proteins and amino acids.

Anthelmintic Activity

In this study, the anthelmintic activity of *B. ceiba* extracts was evaluated on Indian earthworms. All the extracts of *B. ceiba* were found to have good anthelmintic activity as shown in Table 2, compared with the standard drug (albendazole; 20 mg/mL). The standard drug showed paralysis and death time at 30.8 ± 0.79 and 34.66 ± 0.42 minutes, respectively. The control group did not show any paralysis and death. Test groups treated at different doses of extracts were found to have reduced paralysis and death time. The methanol extract at a dose of 50 mg/mL concentration showed paralysis and death time as 30 ± 0.96 and 33.66 ± 0.71 minutes, respectively, equivalent to standard drug values at a dose of 20 mg/mL. The lower doses of the methanolic extract also showed a good reduction in paralysis and death time (39 to 81 minutes and 44 to 87 minutes, respectively) compared to control values.

Fig. 1: Picture showing control group (I) and standard group (II) with Indian earthworms (*P. posthuma*)

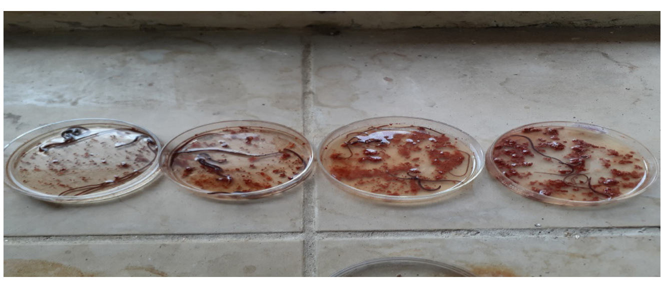

Fig. 2: Picture showing methanolic extracts group (III–VI) with Indian earthworms (*P. posthuma*)

Fig. 3: Picture showing ethylacetate extracts group (VII–X) with Indian earthworms (*P. posthuma*)

Ethyl acetate extract at a dose of 50 mg/mL showed paralysis at 28.66 ± 0.71 minutes and death at 34.16 ± 1.1 minutes, proving that it is even better than the standard drug in terms of paralysis time and equipotent in terms of time taken for causing death. The lower doses of the extract showed only a moderate reduction in paralysis and death time (44 to 87 minutes and 56 to 93 minutes, respectively) compared to control values.

The toluene extracts showed paralysis at 44.16 ± 16 minutes and death at 54.5 ± 1.33 minutes, which was the least effective values among the extracts as anthelmintic. On comparing the time taken for paralysis and death among the extracts, ethyl acetate and methanolic extract showed maximum anthelmintic activity and found equipotent as a standard drug at a dose of 50 mg/mL.

Picture of Indian earthworms (*P. posthuma*) showing control group (I) and standard group (II) is shown in Fig. 1 and various test groups (III-IV) are shown in Fig. 2 to 4. The paralysis time and death time of control, standard and tests (I–XIV) for anthelmintic activity is presented as bar diagram in Figs. 5 and 6.

Statistical Analysis

All anthelmintic results are expressed as mean ± SEM (n = 6) and analyzed for statistical significance by one-way analysis of variance (ANOVA) with Dunnett multiple comparisons test, using computerized GraphPad software InStat. Here, p < 0.01 is considered statistically significant.

Fig. 4: Picture showing toluene extracts group (XI–XIV) with Indian earthworms (*P. posthuma*)

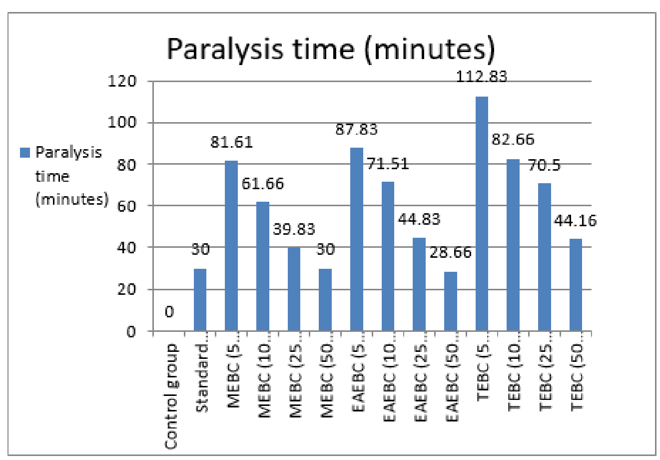

Fig. 5: Results of anthelmintic activity as paralysis time (I–XIV)

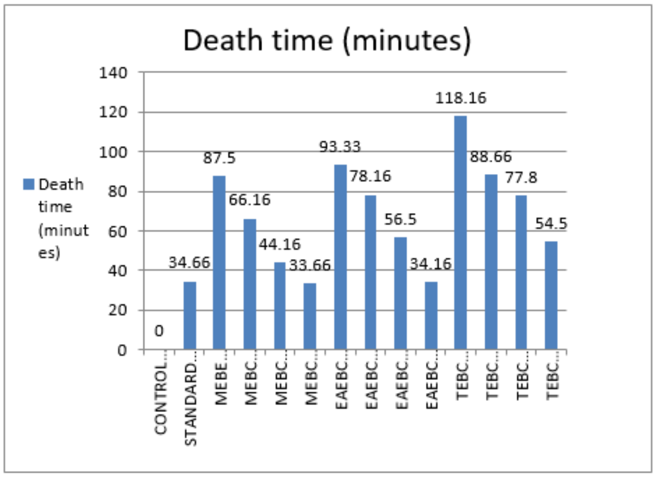

Fig. 6: Results of anthelmintic activity as death time (I–XIV)

Discussion

B. ceiba is a common, traditionally useful medicinal plant with many medicinal uses. It is a rich source of many phytochemicals, including triterpenoids, flavonoids, anthocyanins shamimicin, vicenin, scopolin, mangiferin vitexin, and simalin A and B. Most of its parts are used to cure various ailments.[8-11] Various plants of this family with similar phytochemicals have shown the anthelmintic effect.[12] The study confirmed the anthelmintic activity of various extracts (toluene, ethyl acetate, and methanolic extracts) of *B. ceiba* stem bark. Effectiveness was tested based on a decrease in spontaneous movement and time taken for the death of parasites.

Ethyl acetate and methanolic extract at a dose of 50 mg/mL concentration showed the most significant results. Ethylacetate and methanolic extracts are found to cause paralysis and death of the earthworms within a short period of less than 35 minutes. The methanolic extract that showed the presence of carbohydrates, alkaloids, glycosides, saponins, tannins, phenols, flavonoids, and steroids was the most effective anthelmintic. The ethyl acetate extract, which lacks alkaloids compared to methanolic extract, also showed almost the same effectiveness as methanolic extract. So, glycosides, saponins, tannins, phenols, flavonoids, and steroids present in the extract may be considered responsible for activity instead of alkaloids. However, toluene extract, which lacks glycosides in-addition to alkaloids, was found to lose anthelmintic effectiveness to a great extent. So, finally, it may be concluded that glycosides or aglycone part of any glycoside present in this plant may be responsible for anthelmintic activity.

The result obtained in this study clearly showed that ethyl acetate and methanolic extracts of *B. ceiba* stem bark possess significant anthelmintic activity against earthworms. Thus, the traditional use of *B. ceiba* in helminitic infestation was established scientifically.

Conclusion

In the present investigation, it can be concluded that out of different extracts of *B. ceiba* stem bark, the methanolic and ethylacetate extracts showed maximum anthelmintic activity comparable to standard drug (albendazole). It would be interesting to explore it further and identify the phytoconstituent(s) responsible for anthelmintic activity.

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