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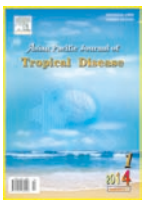

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In vitro anthelmintic efficacy of *Borassus flabellifer* Linn. (Palmae) against *Pheretima posthuma*

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PEER REVIEW

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Comments

This is valuable study in which the authors evaluated anthelmintic potency of *B. flabellifer* against *P. posthuma*. The results are significant and focuses effectiveness of *B. flabellifer* for managing parasite associated infections and complications.
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ABSTRACT

Objective: To investigate the anthelmintic activity of *Borassus flabellifer* (*B. flabellifer*) Linn. leaves against Indian adult earth worms (*Pheretima posthuma*).
Methods: The *B. flabellifer* leaves and Indian adult earth worms (*Pheretima posthuma*) were collected and authenticated. Earth worms were grouped and treated with extract at 10, 20 and 50 mg/mL concentration, albendazole (10 mg/mL) as a standard and normal saline. The paralysis time and mortality time was considered as indicator of anthelmintic efficacy.
Results: All the extracts showed concentration dependent activity but significant activity was observed at 50 mg/mL. At concentration 50 mg/mL extract pertained better activity with paralysis time (13.3 min) and death times (17.92 min) when compared to standard albendazole.
Conclusions: The study findings reveal that the methanolic extract of *B. flabellifer* leaves has effective anthelmintic activity against Indian adult earth worms.

KEYWORDS

Borassus flabellifer, Albendazole, Anthelmintic activity, *Pheretima posthuma*, Paralysis

1. Introduction

Booming widespread of the helminthic problems is highest in countries with warm, moist climates and poor sanitation. Numbers of cases are seen in Asia (72%), Africa (12%), and South America (8%). In typical area of Southeast Asia, prevalence rate is as 92% in children. Ascariasis represents third most common helminthic infection in the United States with approximately 4 million people affected; the majority of cases arise in immigrants from endemic regions. Around as many as 1.5 to 2 billion people worldwide have been infected with helminths and associated complications[1]. Hence, in the present study we are engrossing over the anthelmintic activity.

From the ancient era, the process of development and gathering of the knowledge about the plants and its traditional medicinal use has become the one of the basis for the cure of the general ailments. *Borassus flabellifer* Linn. (*B. flabellifer*) characterised as unbranched tall dioecious plant with a stout trunk. It is cultivated throughout the plains of India, Bangladesh, Burma, Sri lanka, Malaysia, and tropical Africa[2]. Palmyra palm (*B. flabellifer*) is a palm tree belonging to the family Palmae and the sub-family Boracidae generally found in the Asian continent. *Borassus aethiopum* Mart, *B. flabellifer* Linn and *Borassus sundaicus* Becc are the most economical and important species of

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genus *Borassus*[3]. *B. flabellifer* mainly contains gums, albuminoids, fats, steroidal glycosides, and carbohydrate like sucrose. It also contains spirostane type steroids like borassosides and dioscin[4]. Seed coat extract of the *B. flabellifer* shows antimicrobial activity[5]. Male inflorescence has significant anti-inflammatory activity[6]. Almost all parts of the palm are used medicinally. Traditionally root is used as diuretic and anthelmintic and fruits are useful in diverse situation like dyspepsia, flatulence, colic and skin disease[7,8].

2. Materials and methods

2.1. Plant material collection and authentication

B. flabellifer leaves were collected from Kurnapalli, Nizamabad district, in the state of Andhra pradesh, India and authenticated by Dr. B. D. Gachande associate professor of botany department, N. E. S. Science College, Nanded, Maharashtra, India.

2.2. Preparation of plant extract

The leaves were shade dried for 7 d and powder was prepared by using grinder (Coarse powder by sieve no.10, manual)[9]. The powdered dried leaves were extracted with methanol by using Soxhlet apparatus. After extraction the solvent was evaporated and concentrated extract was obtained[10,11].

2.3. Phytochemical screening

The phytochemical study of the extract was carried out using standard procedures[11-13]. Leaves of *B. flabellifer* plant were primarily intended for the phytochemical analysis and detection of major chemical constituents were carried out.

2.4. Worm collection and authentication

Indian adult earth worms [*Pheretima posthuma* (*P. posthuma*)] were used to study anthelmintic activity of the plant extracts. The adult earth worms were collected from moist soil of Vishnupuri, Nanded, Maharashtra, India. Worms with the length of 4-5 cm and width of 0.2-0.3 cm utilised for whole experiment which were authenticated at Department of Zoology, N.E.S. Science College, Nanded, India. The earth worms obtained resembled with intestinal roundworm parasites of human beings both anatomically and physiologically and hence were considered for anthelmintic activity[14].

2.5. Drugs and chemicals

Albendazole and saline water were prepared as the drugs and chemicals in the experiment.

2.6. Preparation of test drug and reference drug

Extracts for *in vitro* study were prepared as, having concentration 10mg/mL, 20mg/mL, 50mg/mL. Samples of methanolic extract were prepared by dissolving 100 mg, 200 mg, and 500 mg crude extract of each in 1 mL dimethylsulfoxide and made the volume up to 10 mL with normal saline solution and final concentration of samples achieved were 10 mg/mL, 20 mg/mL, and 50mg/mL respectively. Normal saline solution was used as control and albendazole was used as the standard drug for this study[15,16].

2.7. Anthelmintic activity

Anthelmintic study of extract was carried out at concentrations 10, 20, 50 mg/mL against the Indian earth worms (*P. posthuma*) by affirming the method of Hussain *et al*. Five groups of Indian earth worms, each containing five earth worms approximately of equal size were used for the study. Three groups of earth worms were tested with extract of different concentrations (10 mg/mL, 20 mg/mL, and 50 mg/mL) and one group was treated with 10 mg/mL with reference standard as albendazole and one group was used as control which is treated with normal saline[14,15]. The anthelmintic activity on earth worm was observed and time required for paralysis and death recorded.

2.8. Statistical analysis

All data were expressed as the mean±SEM. Data was subjected to one way ANOVA followed by Dunnett test. The statistical analysis was conducted with Graph pad Instat Software (Version 3, USA). The values of $P<0.05$ were considered as statistically significant.

3. Results

Preliminary phytochemical investigation of extract revealed

the presence of tannins, steroidal saponins, carbohydrates and fats. The effect of different concentrations of methanolic extract of *B. flabellifer* leaves and albendazole on pheretima posthuma is depicted in Table 1. The dose dependent onset of paralysis and mortality were observed in the earth worms treated with the extract which was compared with albendazole as reference drug. The methanolic extract at 10, 20, 50 mg/mL concentrations showed paralysis time as 15.93, 14.96, 13.23 min and death time as 24.89, 21.90, 17.92 min respectively (Figure 1). At highest concentration it produces paralysis and death in short time which is comparable with albendazole (Figure 1c). The albendazole treated group at concentration 10 mg/mL showed the paralysis time 14.19 min and death time as 17.36 min (Figure 2). The normal saline solution treated earth worms have not showed any change in physical activity and remained active with whole body movements (Figure 3). The paralysed earth worms has not shown any movement and remains scattered (Figure 1) in the media whereas earth worms which did not showed paralysis remained mobilized and aggregates many times (Figure 3).

Table 1

In vitro effect of different concentration of methanolic extract *B. flabellifer* leaves and albendazole on survival of Indian earth warms.

Control/ Albendazole/ Extract	Concentration of albendazole/extract (mg/mL)	Time taken in minutes	
		Paralysis	Death
Control	Saline	–	–
Albendazole	10	14.19±0.184	17.36 ±0.107
Extract	10	15.93±0.229**	24.89±0.157**
	20	14.96±0.124**	21.90±0.168**
	50	13.23±0.089**	17.92±0.311**

$P>0.05$, *$P<0.05$, **$P<0.01$ values are in mean±SEM, $n=5$, when compared with albendazole and results were analyzed by one way ANOVA followed by Dunnttes multiple comparision test. Values of $P<0.05$ were considered as statistically significant.

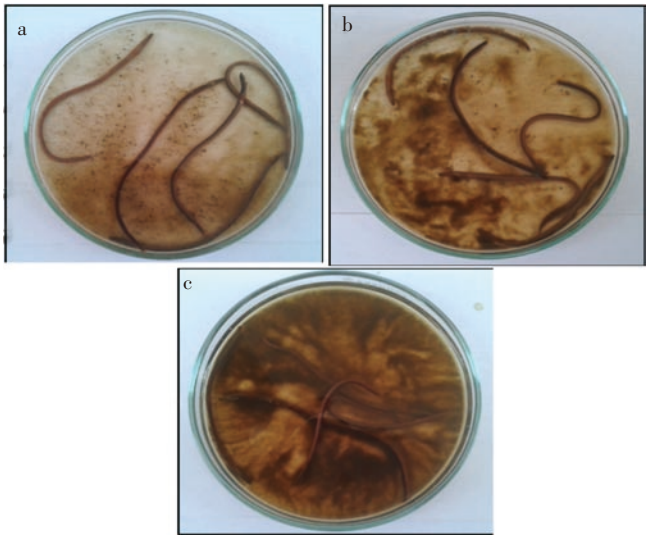

Figure 1. Effect of methanolic extract of *B. flabellifer* leaves on paralysis and mortality time for 10 mg/mL (a), 20 mg/mL (b) and 50 mg/mL (c) concentration.

Figure 2. Effect of albendazole on paralysis and mortality time for 10 mg/mL concentration.

Figure 3. Normal saline treated earth warms remained active. Paralysis or mortality was not observed.

4. Discussion

P. posthuma exposed to different concentrations of methanolic extract of *B. flabellifer* leaves revealed a dose-dependent alteration in physical activity, paralysis and death. The results were subjected to one way ANOVA followed by Dunnett test and mean±SEM were calculated for all concentrations of extract. The anthelmintic efficacy on earth worms pheretima posthuma summerized in Table 1 reveals that methanolic extracts at different concentration has shown paralysis and death of earth worms and it was compared with albendazole as reference drug. Albendazole produces metabolic disruption at a number of different sites, most of which are involved in energy production in the parasite leading to death of earth worms[17].

All the concentrations of leaves extract of *B. flabellifer*

showed change in movement of earth worm which eventually progressed to death but the significant effect was observed at 50 mg/mL concentration. The present anthelmintic efficacy of extract may be because of presence of various phytoconstituents. Active anthelmintic components of *B. flabellifer* are not known; however, phytochemical screening and literature are reported to contain tannins, steroidal saponins, carbohydrates and fats[7,8]. Several phytochemicals have potential to alter metabolic pathways of earthworms and thereby produces mortality in earth worms. Tannins are one of such potential chemical constituent responsible for the anthelmintic activity which has ability to bind with the free proteins present in the gastrointestinal tract of earth worm and cause death[11,18]. Several mechanism contribute in anthelmintic effect of tannins like inducing formation of protein complexes by increasing supply of digestible proteins, imposing larval starvation by engaging free proteins available in the tubes, reducing energy production by uncoupling oxidative phosphorylation and interfering with nematodes cuticle that leads to paralysis[14,19]. Plants rich in tannin as main chemical constituents have potent anthelmintic activity and may therefore estimate as a strong choice to control nematodes[20]. Also steroids are known to have an effect on membrane permeability and pore formation of parasites thereby leads to mortality of parasites[14,19]. Steroidal saponin also produces disruption of monogenea teguments[14,19], microvilli that acts as an absorptive surface in the earth worms[21]. Alkaloids in parasite reduces nitrate generation thereby decreases ribosomal and mitochondrial protein synthesis[22,23] and interferes with the synthesis and activities of DNA and RNA[19,22], inhibits glucose supply[14,19] and causes paralysis of worms by acting on central nervous system[24].

The significant effectiveness of methanolic extract of *B. flabellifer* leaves as anthelmintic activity might be due to presence of phytochemicals like tannins that further needs to screen for precise anthelmintic mechanism. Further *in vivo* study is required for evaluation of *B. flabellifer* for its effectiveness and pharmacological rationale as anthelmintic agent. Although further study is needed but still the plant exhibits significant anthelmintic potential which is useful for the management of diverse diseases caused by worms.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The helminth infections are the most common infections worldwide and affect majority of the population of world. The infection is common in children. The major hindrance in the effective management of helminthiasis is rapid resistance to synthetic antheminthic drugs. Various other factors like safe water and sanitation, hygiene, poor health facilities also contribute in the wide spread of disease. Use of plants like *B. flabellifer* that are referred in traditional medication might be in helpful in helminth infections as they do not possess any side effects.

Research frontiers

The present research work depicts anthelmintic activity of *B. flabellifer* Linn. leaves (Palmae) against *P. posthuma* and assessed by comparing paralysis time and mortality time with standard.

Related reports

Infection of intestinal parasitic worms like *P. posthuma* leads to helminthiasis associated complications. Traditional system of herbal medicine has been found to be effective in managing such parasitic infections.

Innovations & breakthroughs

B. flabellifer commonly known as palmyra palm is a medicinal plant used in various ayurvedic formulations and almost all parts of the palm are used medicinally. This study has shown that extract of *B. flabellifer* leaves has effective anthelmintic activity against Indian adult earth worms.

Applications

B. flabellifer is commonly found and medicinally useful

tree. The outcomes of the present study suggest that *B. flabellifer* has potential against parasitic earth worms and might be significant to manage helminthiasis associated complications.

Peer review

This is valuable study in which the authors evaluated anthelmintic potency of *B. flabellifer* aganist *P. posthuma*. The results are significant and focus effectiveness of *B. flabellifer* for managing parasite associated infections and complications.

References

[1] Pockros PJ, Capozza TA. Helminthic infections of the liver. *Curr Infect Dis Rep* 2005; **7**: 61-70.

[2] Awal A, Haq QN, Quader MA, Ahmed M. Structural study of a polysaccharide from the seeds of *Borassus flabellifer* Linn. *Carbohydr Res* 1995; **277**: 189-195.

[3] Naguleswaran S, Vasanthan T, Hoover R, Liu Q. Structure and physicochemical properties of palmyrah (*Borassus flabellifer* L.) seed-shoot starch grown in Sri Lanka. *Food Chem* 2010; **118**: 634-640.

[4] Sandhya S, Sudhakar K, Banji D, Rao KNV. Pharmacognostical standardization of *Borassus flabellifer* root. *Ann Biol Res* 2010; **1**(4): 85-94.

[5] Duddukuri GR, Yarla NS, Kaladhar DSVGK. Preliminary studies on *in vitro* antimicrobial activity and phytochemical analysis of tender seed coat aqueous crude extract of *Borassus flabellifer* Linn. *Asian J Biochem Pharm Res* 2011; **3**(1):517-523.

[6] Paschapur MS, Patil MB, Kumar R, Patil SR. Evaluation of anti-inflammatory activity of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) in experimental animals. *J Med Plants Res* 2009; **3**(2): 49-54.

[7] Pattanaik C, Reddy CS, Dhal NK. Phytomedicinal study of coastal sand dune species of Orissa. *Indian J Tradit knowl* 2008; **7**(2): 263-268.

[8] Pramod HJ, Yadav AV, Raje VN, Mohite M, Wadker G. Antioxidant activity of *Borassus flabellifer* (Linn.) fruits. *Asian J Pharm Tech* 2013; **3**(1): 16-19.

[9] *Test Seiving Manual. A guide to the use, terminology and general information relative to test sieves and equipment for practicle analysis.* London, UK: Endecotts Ltd.; 1989.

[10] Bart HJ. Extraction of natural products from plants-an introduction. In: Bart HJ, Pilz S, editors. *Industrial scale natural product extraction*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA Publisher; 2011.

[11] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *J Int Pharm Sci* 2011; **1**(1): 98-106.

[12] Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. Pune, India: Nirali Prakashan; 2007.

[13] Khandelwal KR. *Practical pharmacognocy*. 6th ed. Pune, India: Nirali Prakashan; 2006.

[14] Hussain A, Sonkar AK, Ahmad MP, Wahab S. *In-vitro* anthelmintic activity of *Coleus aromaticus* root in Indian adult earthworm. *Asian Pac J Trop Dis* 2012; **2**(Suppl 1): S425-S427.

[15] Hapse SA, Pagar HJ, Suruse SD, Ugale SS. *In-vitro* anthelmintic activity of *Croton bonplandianum* Baill. *J Appl Pharm Sci* 2012; **2**(4): 191-193.

[16] Agarwal G, Pant AK, Hore SK. *In vitro* evaluation of anthelmintic efficacy of *Trichilia* and *Ajuga* species on *Ascaridia galli*. *Hygeia J Drugs Med* 2010; **2**(2): 43-53.

[17] Horton J. Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology* 2000; **121**: S113-S132.

[18] Rubini D, Sudhahar D, Anandaragopal K. Phytochemical investigation and anthelmintic activity of *Celosia cristata* leaf extract. *Int Res J Pharm* 2012; **3**(5): 335-336.

[19] Tiwari P, Kumar B, Kumar M, Kaur M, Debnath J, Sharma P. Comparative anthelmintic activity of aqueous and ethanolic stem extract of *Tinospora cordifolia*. *Int J Drug Dev Res* 2011; **3**(1): 70-83.

[20] Hoste H, Brunet S, Paolini V, Bahuaud D, Chauveau S, Fouraste I, et al. Compared *in vitro* anthelmintic effects of eight tannin-rich plants browsed by goats in the southern part of France. In: Papachristou TG, Parissi ZM, Ben Salem H, Morand-Fehr P, editors. *Nutritional and foraging ecology of sheep and goats*. Zaragoza, Spain: CIHEAM/FAO/NAGREF; 2009, p. 431-436.

[21] Pappas PW. The relative roles of the intestines and external surfaces in the nutrition of monogeneans, digeneans and nematodes. *Parasitology* 1988; **96**: S105-S121.

[22] Akinboye ES, Bakare O. Biological activities of emetine. *Open Nat Prod J* 2011; **4**: 8-15.

[23] Gupta RS, Krepinsky JJ, Siminovitch L. Structural determinants responsible for the biological activity of (-)-emetine, (-)-cryptopleurine, and (-)-tylocrebrine: structure-activity relationship among related compounds. Mol Pharmacol 1980; **18**(1): 136-143.

[24] Ikechukwuogu G. *In vitro* anthelmintic potentials of *Bambusa vulgaris* (L.) leaf extracts using adult African earthworm (*Eudrilus eugeniae*) from Southern Nigeria. *Indian J Novel Drug Deliv* 2012; **4**(4): 306-310.