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Phytochemical Screening and *in vitro* Antihelmintic Properties of *Justicia betonica* L.

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Abstract
The study was intended to evaluate the preliminary phytochemical screening and antihelmintic property of *Justicia betonica* L. under *in vitro* conditions in comparision to standard antihelmintic drug. The phytochemical analysis of the extract confirmed the occurrence of many phytochemicals and maximum being observed in methanolic extract. The *in vitro* anthelmintic activity carried out on mature *Pheretima posthuma* revealed that among the different solvent extracts evaluated, methanolic extract showed significant anthelmintic property. The methanol extract of *J. betonica* required a minimum of 21 min and 27 min to paralyze and cause death to *P. posthuma* at 10 mg mL^{-1} concentration, respectively. Likewise, negative results were observed in petroleum ether extract and dimethylformamide, while piperazine citrate required a minimum of 10 and 13 min to cause paralysis and death of *P. posthuma* at 5 mg mL^{-1} concentration. In addition, studies on *J. betonica* are required to recognize the active principle responsible for anthelmintic activity.

Keywords
Justicia betonica; Phytochemical screening; *Pheretima posthuma*; Anthelmintic property

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INTRODUCTION
Humans and livestock have to dealt with severe gastrointestinal parasitic worms (helminths) as the infection by these worms have resulted in severe economic loss and also affecting the food security [1-3]. The World Health Organization (WHO) has pointed out that more than 2 billion people throughout the world are infected with parasitic worms and about quarter of the same have morbidity towards the infection and major chunk among the infected are children's [4]. Helminthiases infection leads to decrease in fertility rate, milk, and meat production in animals [5]. Infection with helminthiases results in loss of appetite and weight, vomiting, dermatological consequences, anaemia, etc. [1, 5-6[.

To date synthetic drugs have been used extensively against the helminthiases infection and these drugs also pose some serious side effects during their usage including and refugia but still some of the developing countries lack the drugs to treat the infection [7-8]. Nowadays, alternative drugs for the treatment against helminthiases infection is gaining importance due to side effects of the synthetic drugs and medicinal plants form the best alternative as they are known to possess many bioactive compounds with vast biological properties [9]. Several studies have been employed against helminthiases infection using various medicinal plant extracts and have been proved to effective and safe in controlling the infection [10-12]. The use of these herbal extracts with anthelmintic properties will help

the community to serve better and may also add considerable interest along with the existing local anthelmintics [13].

The species of *Justicia* are herbaceous plants which belong to the family Acanthaceae. The species possess diversified biologically active metabolites like phenolics, alkaloids, saponins, flavonoids, etc. and are known to possess antioxidant, antibacterial, anticancer, hepatoprotective, antiangiogenic, etc. [13]. The leaves of *Justicia* spp. are known to contain β-sitosterol, lupeol, friedelin and other aromatic amines which have several beneficial activities [13-14]. Among the species of *Justicia*, *Justicia betonica* L. is an traditional folk medicinal herb and used to cure malaria, orchitis, pain, constipation, diarrhea, snake bite, vomiting etc. Apart from these beneficial activities the plant also comprises of a unique compound jusbetonin in leaves which yield bluish purple dye [15]. Due to the plants medicinal importance, the study was aimed to investigate on the presence of phytochemicals and to evaluate the anthelmintic properties of different solvent extracts of leaf against *P. posthuma*.

MATERIALS AND METHODS

Collection of Plant Material

Healthy leaves of *Justicia betonica* L. were collected from Manasagangotri, Mysuru, Karnataka, India and authenticated at the Dept. of Studies in Botany, University of Mysore, Mysuru.

Preparation of Plant extract

The collected leaves were washed (running tap water), blotted and shade dried completely. The dried leaf samples were powdered using a wearing blender and about 50 g of the sample was filled in a thimble and subjected to Soxhlet extraction from polar to non-polar solvents. After extraction, each of the extract was concentrated before storage (4° C in airtight vials) until further use.

Phytochemical Screening

The collected different solvent extracts were subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents using the methods described by Harborne [16] and Trease and Evans [17].

Anthelmintic activity

The anthelmintic properties of different solvent extracts of *J. betonica* were evaluated by following the method of Ghosh et al. [18]. *P. posthuma* (adult earthworm) which resemble both anatomically and physiologically to the human intestinal parasitic roundworms were used to evaluate the anthelmintic activity. Each of the solvent extracts of *J. betonica* were dissolved to different concentrations (2.5, 5, 7.5 and 10 mg mL^{-1}) with dimethylformamide (DMF) and evaluated for its efficacy against *P. posthuma*. A total of six *P. posthuma* worms (3-5 cm length and 2-4 mm in width) were placed in each Petri dish containing 25 ml of each of the extracts, while piperazine citrate (5 mg mL^{-1}) and DMF served as positive and negative control, respectively. Each of the petri dishes with the earthworms was observed carefully for the time taken for paralysis (when no sort of movement observed even after vigorous shaking) and death (when dipped in water bath kept at 50 °C).

Statistical Analysis

The experiment was carried out in triplicates and the significant differences between the treatment mean values were determined by Honestly Significant Difference (HSD) obtained by Tukey's test at $p \leq 0.05$ levels using SPSS, version 17 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Phytochemical Screening

The collected plants were extracted sequentially from polar to non-polar solvents viz., petroleum ether, chloroform, ethyl acetate, methanol and water and the results are depicted in Table 1. The leaf extract confirmed the presence of flavonoids, carbohydrates, alkaloids, proteins, resins, sterols, saponins and triterpene in one or the other solvent extract, while glycosides and tannins were absent irrespective of the solvent extract evaluated. It was noticed that, methanol extract was found positive for maximum number of phytochemicals evaluated. It has been well documented that, secondary metabolites in plants are of immense value has they possess various biological properties and also exhibit protective and therapeutic effects [9,19-20]. The secondary metabolites such as like alkaloids, triterpenes, saponins, flavonoids, sterols, etc., are produced/ synthesized within the plants for its defense purposes which may be toxic or useful to man [21]. Likewise, these phytochemicals possess diverse protective and therapeutic effects [22]. It has been well documented that about 80% of the world's population directly or indirectly primarily depend on herbal medicines for their primary healthcare as they are readily available and are also less toxic [23-25]. From the results of the qualitative phytochemical analysis it was observed that the plant *J. betonica* comprises of many secondary metabolites which may be beneficial to humankind and methanol extract confirmed the occurrence of maximum number of phytochemicals.

Table 1: Phytochemical screening of *J. betonica* leaf extracts

Tests		Solvent Extracts				
		H	CHL	EA	M	AQ
Alkaloids	Mayer's Test	+	+	-	+	-
	Dragendorff's Test	+	+	-	+	-
	Wagner's Test	+	+	-	+	-
	Hager's Test	+	+	-	+	-
Carbohydrates	Molisch's Test	+	-	-	+	+
	Fehling's Test	+	-	-	+	+
	Benedict's Test	+	-	-	+	+
Flavonoids	Shinado Test	+	-	+	+	-
	$FeCl_3$ Test	+	-	+	+	+
	Lead Acetate Test	+	-	+	+	-
Glycosides	Keller-Kiliani Test	-	-	-	-	-
Proteins	Biuret Test	-	-	-	+	-
	Ninhydrin Test	-	-	-	-	-
Resins	Turbidity Test	-	-	-	+	-
	Acetic anhydride Test	-	-	-	+	-
Saponins	Foam Test	-	-	-	+	-
Sterols	Salkowski Test	-	+	+	+	-
	Liebermann-Burchard Test	-	+	+	+	-
Tannins	$FeCl_3$Test	-	-	-	-	-
	Gelatin Test	-	-	-	-	-
Triterpenes	Salkowski Test	-	-	-	+	-
	Liebermann-Burchard Test	-	-	-	+	-

H = Hexane extract; CHL = Chloroform extract; EA = Ethyl acetate extract; M = Methanol extract; AQ = Aqueous extract; + indicates the presence of the phytochemical and – indicates absence

Anthelmintic activity

The anthelmintic properties of different solvent extracts of *J. betonica* were evaluated against *P. posthuma*. It has been well documented that, Indian earthworm *P. posthuma* and *Eisema fetida* have been used extensively as an parasitic model to evaluate the efficacy of drugs with anthelmintic properties due to their similarity in gastrointestinal worms infecting humans and livestock [12, 26-27]. Among the different solvent extracts evaluated, methanol extract offered significant anthelmintic property compared to other solvent extract irrespective of the concentration used. The methanol extract was to paralyze the worm at 32 min and the time decreased to 21 min with increase in the concentration of extract from 2.5 to 10 mg mL^{-1} (Fig. 1), in addition the time required to cause death to the worm decreased from 39 min to 27 min with increase in the concentration of the extract (Fig. 2). Apart from methanol extract, chloroform and ethyl acetate extract were able to paralyze and caused the death of *P. posthuma* at 10 mg mL^{-1}, while no other concentration was effective in inducing death even after 60 min of treatment. The positive control piperazine citrate treatment was able to cause paralysis and death to the worm at 10 min and 13 min, respectively at 5 mg mL^{-1} concentration. Likewise, Lone et al. [5] and Majeed et al. [28] have reported that the methanolic extract *Euphorbia helioscopia* and *Euphorbia heterophylla* offered better anthemnintic properties compared to other solvent extract against *P. posthuma* upon treatment under *in vitro* conditions. of The results of the study are in corroboration with the findings of many other researchers, wherein plant extracts were able to cause paralysis and eventually leading to the death of *P. posthuma* upon treatment there by confirming the anthemintic properties of the plant extracts [29-30].

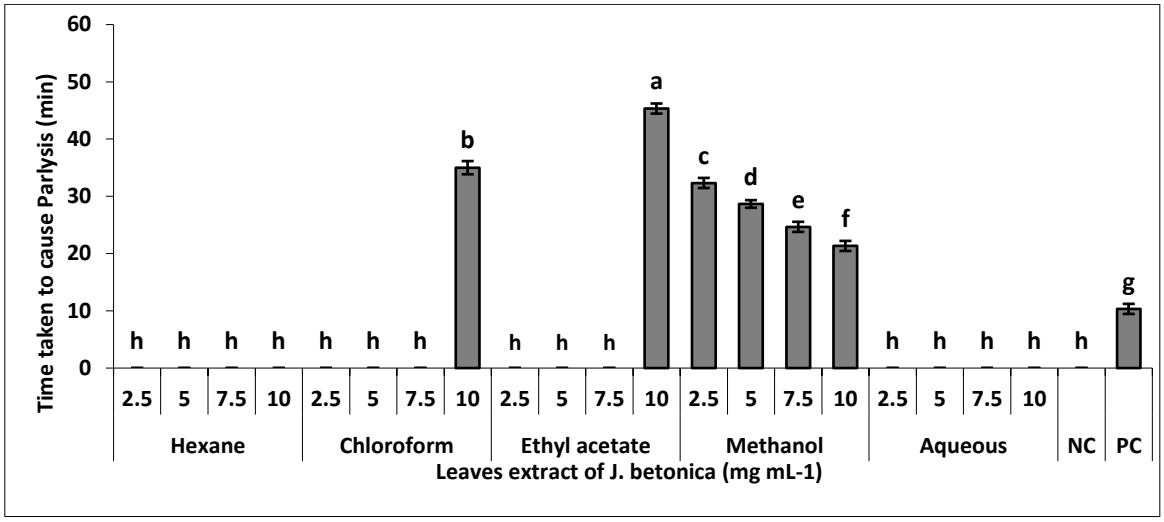

Figure 1: Paralyzing effect of *J. betonica* leaf extracts against *P. posthuma*

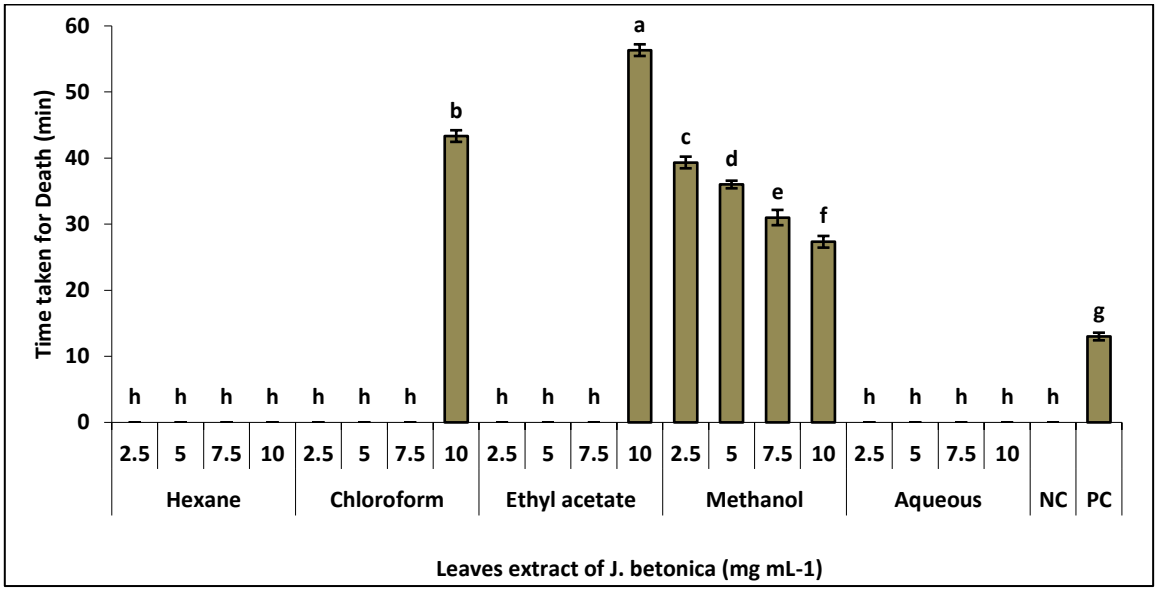

Figure 2: Anthelmintic activity *J. betonica* leaf extracts against *P. posthuma*

CONCLUSION

The study is the first report on the anthelmintic properties of *J. betonica*. The different solvent extracts of the leaf sample showed the presence of secondary metabolites which authenticates its beneficial properties to mankind. From the study, it was observed that, among the different solvent extracts of the leaf sample only methanolic extract was able to offer significant anthelmintic properties at a lower concentration compared to other extracts. The results showed that, the methanolic extract offered a dose-dependent anthelmintic property wherein the time required for paralysis and death of *P. posthuma* decreased with increase in the concentration of the extract. Despite ample evidence of on many secondary metabolites, further studies are needed to confirm the active metabolite which is responsible for its significant anthelmintic property.

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