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Original Article

Anthelmintic and cytotoxic activity of the ethanolic extract of *Phoenix sylvestris* root

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ABSTRACT

The present study examined the anthelmintic and cytotoxic properties of the ethanolic crude root extract of *Phoenix sylvestris* Roxb (family: arecaceae). In anthelmintic test, the extract showed significant and dose dependent (25, 50, 100, 200 mg/mL) decrease in paralysis time and death time of *Haemonchus contortus* (Nematode). However, the crude extract showed low toxicity (IC_{50} 58.745 µg/ml) against brine shrimp nauplii in comparion with vincristine sulphate (IC_{50} 0.6449 µg/ml). The results support the traditional medicinal uses of *P. sylvestris* agains worm infections.

Keywords: *Phoenix sylvestris,* Anthelmintics, Cytotoxic activity.

INTRODUCTION

Helminth infections are one of the most widespread infections in humans, animals and fishes around the world. A number of medicinal plants have already been proved to be effective against worm infections and could be useful for the discovery of effective anthelmintics against the treatment of parasite infections [1]. Medicinal plants constitute a precious natural wealth of a country and contribute a great deal to its health economy. They play a significant role in providing primary health care services to rural people and serve as important therapeutic agents for the manufacture of traditional medicines. *Phoenix sylvestris* is locally known as '*Khejur'* in Bangladesh and possesses a variety of medicinal uses. Sap of the plant is nutritious, cooling and laxative whereas central tender part is useful to treat gonorrhea [2]. Root is used in toothache [3] and in nervous debility and helminthiasis. Gum is useful in diarrhoea and genito-urinary diseases. Fruit is tonic and restorative. Being a sedative and nervine tonic the fruit is usefully employed for relieving backache and pain in the buttocks. It is also prescribed in cough, fever, nervous debility and gonorrhoea. Seed kernel, made into a paste with roots of *Achyranthesa spera* is taken with betel leaf for the remedy of ague. Marma tribes use leaves to expel thread worms. For this purpose crushed fresh leaves are soaked in water overnight, then water is taken next morning in empty stomach [4]. The methanolic root extract of *P. sylvestris* is reported to have analgesic and diuretic activity [5].

Traditionally the root of *Phoenix sylvestris* are used in toothache and few local traditional healers in Bangladesh use tender root of this plant to expel round worm in human and to get relief from nervous debility. However, these traditional uses of roots for years together lack any scientific basis behind the use of the root extract of *Phoenix sylvestris*.

This study was done to provide a preliminary scientific basis of the use of root extract of *Phoenix sylvestris* as a folk medicine. For this purpose we evaluated the anthelmintic and cytotoxic activities of the ethanolic extract of the root of *P. sylvestris*.

MATERIALS AND METHODS

Plant material and extraction: The roots of *Phoenix sylvestris* Roxb were collected from Khulna city of Bangladesh in between September

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to November, 2012. The plant was identified by Bangladesh National Harberium (Accession No. DACB 39536) and a specimen herbarium sheet was preserved on phytochemistry laboratory of Khulna University, Bangladesh. The collected roots were washed with water and then shed-dried. The dried plant materials were grinded into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The dried root-parts were then soaked in ethanol for a period of ten days, filtered through Whatmann filter paper and obtained the extract. The yield was 2.23% of plant material.

Phytochemical evaluation: The ethanol extract of root of *P. sylvestris* was evaluated for the presence of various phytoconstituents such as steroids, alkaloids, terpenoids, flavonoids and others as per the protocol of Harborne [6].

Anthelmintic activity test: *Haemonchus contortus* inhabit the abomasums of ruminent animals. *H. contortus* has been found in humans in Brazil and Australia [7]. Alive parasites *Haemonchus contortus* (Nematode) were collected from freshly slaughtered cattle at local abattoirs and identified by Dr Md. Royhan Gofur, Lecturer, Department of Animal Husbandry and Vaterinary Science, Rajshahi University, Rajshahi. After proper cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) at pH 7.4 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na_2HPO_4 and 0.27 g KH_2PO_4 in 1 L of distilled water at 37±1 ºC. Extract at the concentrations of 25, 50, 100 and 200 mg/mL and reference standard albendazole at the concentrations of 15 mg/mL and 10 mg/mL; for each concentration 10 mL in PBS were prepared and transferred to Petri dishes. Control group was treated with 0.1% tween-80 in PBS and also was transferred in a petridish. Six parasites were placed in each Petri dish and observed. The time of paralysis was recorded when no movement was observed unless shaken vigorously. The death time was recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50 ºC) or subjected to unresponsive to external stimuli. Anthelmintic activity was expressed as the time required for paralysis and death of parasites as compared to control.

Cytotoxic activity Test: The brain shrimp lethality bioassay was proposed by Michael AS [8] It is based on the ability to kill laboratory-cultured *Artemia* nauplii brine shrimp. The assay is considered a useful tool for preliminary assessment of cytotoxicity [9]. In this test, 10 alive brine shrimp nauplii were added to the plant extracts at different concentrations 5, 10, 20, 40, 80, 160, 320 µg/ml in 7 test tubes and to the standard at concentrations of 0.312, 0.625, 1.25, 2.5, 5 µg/ml in 5 test tubes. After 24 hours the alive nauplii were counted and the percentage of mortality was calculated. Alive nauplii were counted and the percentage of mortality was calculated by the formula:

% Mortality = [Avg. no. of alive shrimp of control - Avg. no. of alive shrimp of sample)/ Avg. no. of alive shrimp of control] × 100.

RESULTS AND DISCUSSION

The World Health Organization (WHO) estimates around approximately two billion people affected by parasitic worm infections and parasitic diseases continue to be a considerable burden in developing countries [10]. It is also a major cause for reduced productivity in livestock. This scenario will be more worsen due to the continuous resistance of pathogens to antiparasitic agents It has been a long history to use phytomedicine by farmers and traditional healers to treat parasitism. Recent surveys in developing countries have identified many plants that are intended and have potential to be used as anthelmintics [11]. However, regardless of their ethnomedicinal use there is very limited scientific evidence on the anti-parasitic efficacy of the plant products [12]. Therefore, anthelmintics from natural sources could play a key role in the treatment of parasite infections.

In the present study, ethanol extract of *P. sylvestris* root was used to evaluate anthelmitic activity against the worm *H. contortus*. Each extract of *P. sylvestris* root at concentrations of 25, 50, 100 and 200 mg/ml showed paralysis at 6.39, 3.33,2.16 and 1.30 min and death time at 8.23, 6.28, 4.41 and 2.45 minutes respectively (Table 1). Standard albendazole also showed paralysis time 5.34 and 4.27 min and death time at 6.23 and 5.45 min at concentration of 10 and 15 mg/ml, respectively (Table 1). The results demonstrated that ethanolic extract of *P. sylvestris* root showed a dose dependent paralysis ranging from loss of motility to loss of response to external stimuli and ultimately progressed to death (Figure 1).

A lot of chemical constituents isolated from *P. sylvestris* have been reported which includes cholesterol, β-sitosterol, β-amyrinand quercetin [13]. Phyto-constituents analysis of the root extract also showed the presence of tannin, polyphenol and other constituents (Table 2). It is reported that different polyphenolic and tannin compounds possess antiparasitic activity through either interfering with energy generation of parasites or binding of GIT free glycoprotein [14]. In the current study, the ethanol extract of *P. sylvestris*

root also contains polyphenolic and tannin compounds which might contribute to similar anthelmintic activity. The cytotoxic effect presented in Table 3 indicates that *P. sylvestris* root extract posses low toxicity (IC_{50} 58.745 µg/ml) in comparison to positive control vincristine sulphate (IC50 0.6449 µg/ml). The results supported that the death of parasites is not due to the toxicity of the crude extracts.

In conclusion, the traditional claim of root of *P. sylvestris* as an anthelmintic have been supported as the crude extract showed activity against the *H. contortus* worms used in the study. However, further studies are needed to be carried out to isolate the active constituents responsible for this activity of the extract as well as its mechanism of action.

Statistical Analysis: All measurements were repeated three times. The results are expressed as mean ± standard error of mean. The results were statistically analyzed student's t-test.

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Figure 1: Physical observation of parasite for their movement with and without treatment with and without treatment with *P. sylvestris* and positive control albendazole.

Table 1: Anthelmintic activity of root extract of *Phoenix sylvestris* on the basis of paralysis time and death time of *H. contortus* parasite.

Group	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Control	--	--	--
Standard (Albendazole)	10	5.34 ±0.06*	6.23± 0.09*
	15	4.27 ± 0.07*	5.45± 0.04*
P. sylvestris extract	25	6.39 ± 0.14*	8.23± 0.06*
	50	3.33± 0.08*	6.28 ±0.08*
	100	2.16 ± 0.05*	4.41 ±0.08*
	200	1.30± 0.06*	2.45± 0.06*

All Values are represented as mean ± SEM, SEM= Standard error for mean, n= number of parasites(6), * P<0.001 vs. standard, Student's t-test.

Table-2: Phytochemical constituents found in *Phoenix sylvestris* Roxb root extract.

Constituents	Reducing sugar	Tannins	Flavonoid	Saponin	Gums	Steroids	Alkaloids	Glycoside	Proteins	Terpenoids
Result	+	+	+	-	-	+	+	+	+	+

+ = Presence, - = Absence

Table 3 : IC_{50} for *P. Sylvestris* (test sample) and vincristine sulphate (standard) with lower and upper limit calculated by Ldp line software.

Treatment	**IC_{50} (µg/mL)**
P. sylvestris	58.745
Vincristine sulphate	0.6449

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