BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2012; 7: 50-53
ISSN: 1991-0088

Evaluation of *in vitro* anthelmintic activities of leaf and stem extracts of *Justicia gendarussa*

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Article Info

Received: 3 April 2012
Accepted: 9 May 2012
Available Online: 13 May 2012

DOI: 10.3329/bjp.v7i1.10558

Cite this article:
Saha MR, Debnath PC, Rahman MA, Islam MAU. Evaluation of *in vitro* anthelmintic activities of leaf and stem extracts of *Justicia gendarussa*. Bangladesh J Pharmacol. 2012; 7: 50-53.

Abstract

This present study explored the anthelmintic activities of crude methanolic extract of dried leaves and stems of *Justicia gendarussa*, an evergreen plant belonging to the family Acanthaceae. This plant is commonly known as Bishjarul in Bangladesh. Five concentrations (10, 20, 30, 40 and 50 mg/mL) of each extract were studied in the bioassay to determine the paralysis time and death time of the worm, *Pheretima posthuma*. Methanolic extract of leaves (50 mg/mL) caused paralysis of the worms at 35.3 min and death at 70.7 min while methanolic extract of stem of *J. gendarussa* caused paralysis at 41.3 min and death at 89.3 min. Albendazole (positive control) paralyzed and killed the worms at 17 and 48 min, respectively. The study confirms the significant anthelmintic activities of leaves and stems extract of *J. gendarussa* and therefore demands the isolation of active principles thorough bioassay. This is the first report of anthelmintic activities of *J.gendarussa*.

Introduction

Nature always acts as a great source of salvation for human being by providing different remedies from its plants, animals, and other sources to cure all ailments of mankind (Dubey et al, 2004). Medicinal plants provide affordable means of health care for poor and marginalized people. But they are also potential sources of many therapeutic active compounds. The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many plants which has been unexplored in the field of medicine or science (Reddy et al, 2010). Because of such great opportunities in medicinal plants, continuous approaches are going on to isolate various therapeutically active compounds. Many active drugs have been derived from different medicinal plants, and the process is going on. According to an analysis, 23 new drugs derived from natural sources have been launched on the market during 2000-05 (Chin et al, 2006). Besides new drugs launched on the market from 2000 to the present, there are a variety of new chemical entities from natural sources undergoing clinical trials.

Justica gendarussa Burm (Family- Acanthaceae, English name, Black adusa) is a shade loving, quick growing, evergreen shrub found throughout India and also in all Asian countries like Malaysia, Indonesia, Sri Lanka and Bangladesh (Rantnasooriya et al, 2007). The plant is used traditionally for the treatment of chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever (Rantnasooriya et al., 2007; Mrunthunjaya, 2007). *Justicia* has been found to contain lignans, naturally occurring phenolic dimmers (Mrunthunjaya, 2007) and triterpenoids (Chakravarty et al., 1982). In Bangladesh, it is not cultivated but found in almost everywhere. Uddin and his co-workers (2011) reported the isolation of stigmasterol, lupeol and 16 hydroxylupeol from a methanol extract of *J. gendarussa.* They also reported the anti-oxidant, anti-microbial and

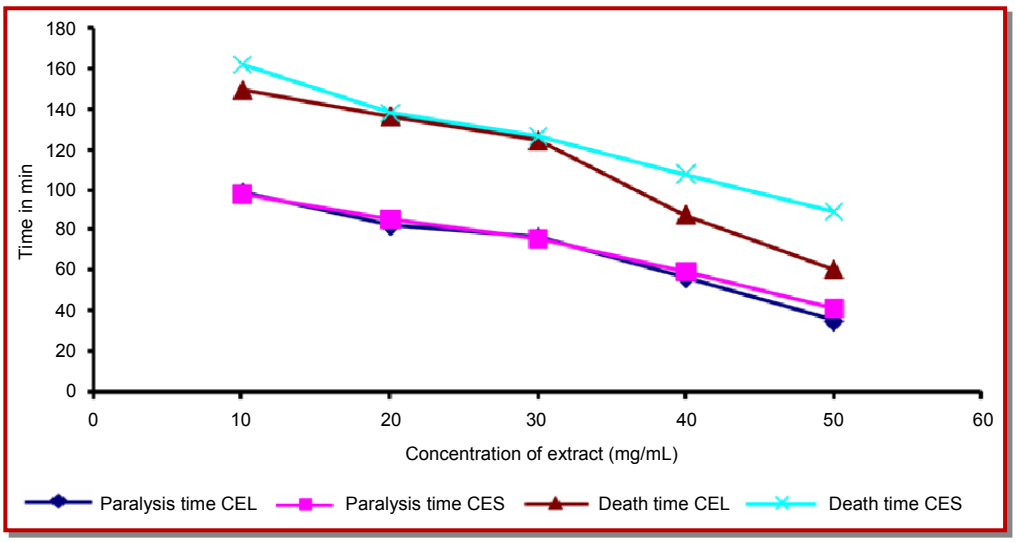

Figure 1: Plot of concentration of plant extractives versus paralysis time and death time of *Pheretima posthuma*. Where, CEL: Crude methanolic extract of leaves; CES: Crude methanolic extract of stem

Table I

Anthelmintic activity of crude methanolic extracts of leaves and stems of *Justicia gendarussa* against *Pheretima posthuma*

Conc. (mg/mL)	Paralysis time (min)				Albendazole		
	CEL	CES	CEL	CES	Conc. (mg/mL)	Paralysis time (min)	Death time (min)
10	98.7 ± 0.7	97.7 ± 0.5	149.3 ± 0.7	161.7 ± 0.5	10	17.7 ± 0.5	48 ± 0.5
20	82.3 ± 0.7	85.3 ± 0.7	136.3 ± 0.7	137.7 ± 0.5			
30	76.7 ± 0.7	75.7 ± 0.7	124.3 ± 0.5	126.7 ± 0.7			
40	56.7 ± 0.7	59.7 ± 0.7	87.7 ± 0.7	107.7 ± 0.7			
50	35.3 ± 0.7	41.3 ± 0.7	70.7 ± 0.3	89.3 ± 1.0			

Values are expressed as mean ± SEM (n=3). CEL: Crude methanolic extract of leaves; CES: Crude methanolic extract of stem

cytotoxic properties of the various extractives of the whole plant for the first time. However, so far no study has been conducted to determine the anthelmintic potential of *J. gendarussa*. In the present study, leaves and stems of *J. gendarussa* were extracted with methanol and the extractives were investigated for their anthelmintic activities.

Materials and Methods

Collection and identification of the plant

The plant sample of *J. gendarussa* was collected from the Noakhali district, Bangladesh in June 2011. The plant was identified and a voucher specimen (Accession number DACB: 36060) representing this collection has been deposited in the Bangladesh National Herbarium, Dhaka, for further reference.

Preparation, extraction and fractionation of plant material

The dried and powdered leaves and stems (500 g) were extracted with methanol with occasional shaking and stirring for 7 days. The whole extract was filtered and the solvent was evaporated and a semisolid mass of 30 g and 25 g for leaves and stems were obtained, respectively.

In vitro anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al. (2001) with minor modifications. Adult earthworms were used to study the anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being (Vidyarthi, 1967; Thorn et al., 1977; Vigar, 1984; Chatterjee, 1967; Kumar BSA et al., 2010; Kumar et al., 2010). Because of availability of earthworms, they are widely used as effective tools for anthelmintic study (Sollmann, 1918; Jain et al., 1972; Szewezuk et al., 2003; Dash et al., 2002). Adult earthworm (*Pheretima posthuma*) were collected (3-5 cm in length and 0.1-0.2 cm in width weighing about 0.8-3.04 g) from moist soil of a road side canal of Noakhali Science and Technology University, Sonapur, Noakhali. All the worms were washed with normal saline to remove all fecal matters. Extracts were weighed and

dissolved in 10 mL of distilled water to obtain the concentrations of 10, 20, 30, 40 and 50 mg/mL. Albendazole was used as reference standard (10 mg/mL). Earthworms were divided into twelve groups (each containing three) in petri dish. Extract of leaves and stems were applied to the petri dishes and observations were made for determining paralysis time and death time of the worm. Paralysis was said to occur when the worms do not move even in normal saline. Death was confirmed when the worms lose their motility followed with fading away of their body color.

Results

The crude methanolic extracts of leaves and stems of *Justicia gendarussa* were used to evaluate anthelmintic activities. As shown in the Figure 1 and Table I , crude methanol extract of leaves of *J. gendarussa* showed significant anthelmintic activity at the concentration of 10, 20, 30, 40 and 50 mg/mL in a dose-dependant manner giving shortest time of paralysis and death time at 50 mg/mL.

The crude methanol extract of stem also exhibited significant anthelmintic property at dose of 50 mg/mL. Methanolic extract of leaves of *J. gendarussa* at concentration of 50 mg/mL caused paralysis of *Pheretima posthuma* at 35.3 min and death at 70.7 min while the methanolic extract of stem at the same concentration caused paralysis at 41.3 min and death at 89.3 min. Albendazole (positive control) caused paralysis and death at 17 and 48 min, respectively at 10 mg/mL.

Discussion

Cytotoxic activities of various fractions of the whole plant extract of *J. gendarussa* have been described by Uddin et al. (2011). According to their observation, the petroleum ether soluble materials of whole plant demonstrated highest toxicity with LC_{50} value of 1.3 µg/mL. This was the first report of isolation of compounds from *J. gendarussa* and its anti-oxidant, antimicrobial and cytotoxic properties. We also found that the chloroform fractions of both stem and leaves were more toxic than petroleum ether fractions. The LC_{50} values of chloroform fractions of leaves and stem were 0.7 and 1.8 µg/mL, respectively, whereas, for petroleum ether fractions the values were 1.1 and 2.7 µg/mL (data not shown).

Antihelminthic activities of *J. gendarussa* have not been studied before. Adult earthworms *(Pheretima posthuma*) were used in this study to determine the anthelmintic activity due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human being (Vidyarthi, 1967; Thorn et al., 1977; Vigar, 1984; Chatterjee, 1967; Kumar et al., 2010; Kumar et al., 2010). Most of *in vitro* experiments regarding anthelmintic activity of plants have been based on their toxic effects on this earthworm (Gaind et al., 1967; Ali et al., 1970; Kokate et al., 1971; Dixit et al., 1975; Banerjee et al., 1978; Girgune et al., 1978; Agarwal et al., 1979; Girgune et al., 1979; Mishra et al., 1979; Mehta et al., 1981; Garg et al., 1982, Dengre, 1982; Nanda et al., 1987; Siddiqui et al., 1990; Garg et al., 1992). It has been demonstrated that all anthelmintic are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic (Sollmann, 1918). We used albendazole was used as positive control. The crude extracts of stem and leaves of *J. gendarussa* exhibited significant anthelmintic activities. At 50 mg/mL concentration, the methanolic crude extracts of leaves and stem caused death of *Pheretima posthuma* at 60 and 89 min, respectively.

As studied previously by Uddin et al. (2011), the plant contains stigmasterol, lupeol and 16-hydroxylupeol in the methanol extract which may be responsible for the anthelmintic activities of this plant. However, further study should be carried out to determine the compounds present in the methanolic extract. *In vivo* study using animal model may also be used to determine the possible antitumor activities of the extracts. Now our next aim is to explore the compounds liable for aforementioned activity from this plant.

Acknowledgement

The authors would like to thank the Chairman, Department of Pharmacy, Noakhali Science and Technology University, Noakhali, Bangladesh for providing laboratory facilities to conduct the research.

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