JSR Publications

Available Online

J. Sci. Res. **4** (3), 721-727 (2012)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

In Vitro Cytotoxic and Anthelmintic Activities of Leaf and Root Extracts of *Leonurus sibiricus L.*

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Received 29 February 2012, accepted in final revised form 27 May 2012

Abstract

This study investigated the cytotoxic and anthelmintic activities of *Leonurus sibiricus* L. (commonly known as Raktodrone in Bangladesh) belonging to the family Labiatae. The dried leaves and roots of *L. sibiricus* were extracted with methanol and fractionated by modified Kupchan method. The crude methanolic extracts as well as its soluble fractions of petroleum ether, ethyl acetate and chloroform were screened for cytotoxic activity using brine shrimp lethality bioassay. They were found to possess significant cytotoxic activities. The LC_{50} values of crude extract of leaves and its pet-ether, ethyl acetate and chloroform soluble fractions were 1.0, 2.0, 2.11 and1.33 µg/ml, respectively. On the other hand, the LC_{50} of crude methanolic extract of roots and fractions of pet-ether, ethyl acetate and chloroform were 2.0µg/ml, 2.81 µg/ml, 3.55 µg/ml and 7.58µg/ml, respectively. Vincristine sulphate was used as positive control. The crude methanol extract of leaves and roots also showed very good anthelmintic activities as determined against the earthworms, *Pheretima posthuma*. The study confirms the moderate anthelmintic and potent cytotoxic activities of leaf and root extracts of *L. sibiricus*.

Keywords: *Leonurus sibiricus*; Anthelmintic activities; Brine shrimp cytotoxicity.

doi: http://dx.doi.org/10.3329/jsr.v4i3.9998 J. Sci. Res. **4** (3), 721-727 (2012)

1. Introduction

The history of use of medicinal plants by human beings to treat diverse ailments goes back to thousands of years ago [1, 2]. Though the advent of modern or allopathic medicine has somehow diminished the role of medicinal plants in favor of synthetic drugs, even now a number of modern drug discoveries have been based on medicinal plants used by indigenous people [3]. In recent years, because of the costs as well as serious side-effects of a number of modern drugs, attention has turned back to medicinal plants as a source for discovery of newer drugs with less cost and side effects. The demand for more

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and more drugs from plant sources is continuously increasing. It is therefore essential for systemic evaluation of plants used in traditional medicine for various ailments. [4]

Parasites have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance [5-7]. Consequently the discovery and development of new chemical substances for helminths control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important sources of bioactive substances [8].

L. sibiricus is an herbaceous plant native to Asia but distributed most of the parts of the world. It is an annual or biennial herb with upright stems and about 20 to 80 cm height [9]. *L. sibiricus* L. is also known as Siberian mother-wort; the Hindi name is Guma. Traditionally the roots and leaves of *L. sibiricus* are taken as a tea and febrifuge [10, 11]. The seeds are used in Chinese medicine as a constructive and aphrodisiac; the dried plant is used as a tonic and as a general remedy for puerperal and menstrual disorders. It is a traditional emmenagogue and an antipyretic in China [12]. The leaves are used in the treatment of chronic rheumatism while the juice of the leaves is antibacterial and is extensively used in the treatment of psoriasis, scabies and chronic skin eruptions. *L. sibiricus* is a respiratory stimulant with curare like effect on motor endings [11, 13]. It is used as folk medicine for the treatment of cough and bronchitis [13, 14]. It is popularly used in Brazil for cold, diarrhoea and digestive complaints [15]. Terpenoids**,** phenols and alkaloids are major constituents in *L. sibiricus* [16-21].

In this study we examined the cytotoxic activities of roots and leaves extract of *L. sibiricus* using brine shrimp lethality bioassay. The anthelmintic potential of this plant has also been reported here for the first time.

2. Materials and Methods

2.1. *Collection and identification of the plant*

The plant sample of *L. sibiricus* was collected from the Noakhali and Madaripur district, Bangladesh in February 2011. The plant was identified and a voucher specimen (Accession number DACB: 35900) representing this collection has been deposited in the Bangladesh National Herbarium, Dhaka, for further reference.

2.2. *Preparation, extraction and fractionation of plant material*

The dried and powdered leaves and roots (500 gm) were extracted with methanol with occasional shaking and stirring for 7 days. The whole extract was filtered and the solvent was evaporated. A semisolid mass of 35 gm for leaves and 30gm for roots were obtained

and fractionated with petroleum ether, chloroform and ethyl acetate by the modified Kupchan method [22]. The subsequent evaporation of solvents afforded leaf and root extracts of petroleum ether, chloroform and ethyl acetate fraction.

2.3. *Cytotoxicity screening*

Brine shrimp lethality bioassay is widely used for cytotoxicity assay to isolate the bioactive compounds [23]. Here, simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature the nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method [23]. The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) and sea water (3.8% NaCl) to attain concentrations of 5, 10, 20, 25 and 50 µg/ml. A vial containing 50 µl DMSO diluted to 5 ml was used as a control. Standard Vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

2.4. *Screening for anthelmintic activity*

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al* [24] 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. Because of easy availability earthworm has been widely used for the initial evaluation of anthelmintic compounds *in vitro* [25-27]. Adult earthworm (*P. 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 fecal matters. The crude methanolic extracts of leaf and root 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 petridish. The extracts were applied and the paralysis time and death time of the worm were determined. The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The times of death of the worms were recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water (50°C).

4. Results and Discussion

As shown in Table 1, Fig. 1 and Fig. 2, the extracts of both leaves and roots of *L. sibiricus* exhibited very strong cytotoxic activities against brine shrimp nauplii. The leaf extracts

were more cytotoxic than the root extracts. The LC_{50} value of crude methanolic extract of leaves was 1.0 µg/ml whereas it was 2.0 µg/ml in case of root. Among the three fractions obtained by solvent-solvent partitioning, chloroform fraction of leaves was more cytotoxic (LC_{50} value of 1.33 µg/ml) than other two fractions (pet-ether, 2.0 µg/ml; ethyl acetate 2.11 µg/ml). On the other hand, chloroform fraction of roots was less cytotoxic (LC_{50} value of 7.58 µg/ml) than other two fractions (pet-ether, 2.81 µg/ml; ethyl acetate, 3.55 µg/ml). Control group showed 0% mortality. These findings indicate that the extracts may contain antitumor or anticancer entities.

Table 1. LC_{50} values of crude methanolic extracts and their solvent fractions of *L. sibiricus* on brine shrimp lethality bioassay.

Extract/Fraction	LC_{50} values (µg/ml)	
	Leaf	Root
Crude methanolic extract	1.0	2.0
Pet-ether fraction	2.0	2.81
Ethyl acetate fraction	2.11	3.55
Chloroform fraction	1.33	7.58
Vincristine sulfate	0.67	0.67

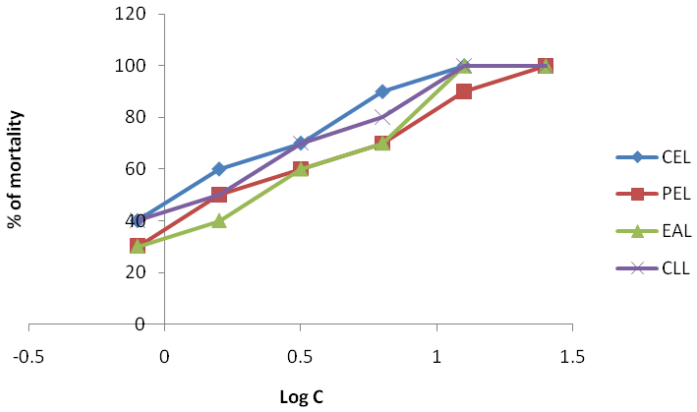

Fig.1. Plot of Log C versus % of mortality of crude methanolic extract of leaves and the petroleum ether, chloroform and ethyl acetate fractions of *L. sibiricus***.** CEL: Crude methanolic extract of leaves, PEL: Pet-ether extract of leaves, EAL: Ethyl acetate extract of leaves, CLL: Chloroform extract of leaves.

Satoh and his co-workers [14] has shown that some ingredients of *L. sibiricus* like furanoditerpenelactones and the diterpene-lactones, leonotinin, leonotin, dubiin and

nepetaefuran exhibited moderate cytotoxic activity with IC_{50} value of 50-60 µg/mL against leukemia cells (L 1210). Cytotoxic activities of methanolic extract of aerial parts of this plant have been reported previously by Maria *et al.* [30]. Cruz and his co-workers [15] found that the dichloromethane extracts from the aerial parts of *L. sibiricus* L killed *Artemia salina* with LC_{50} of 12 ppm. On the other hand, Soberón [31] observed that the aqueous extracts showed no cytotoxicity in the assayed concentrations but the tincture extracts exhibited cytotoxic activities with LD_{50} values of 32 µg/m.

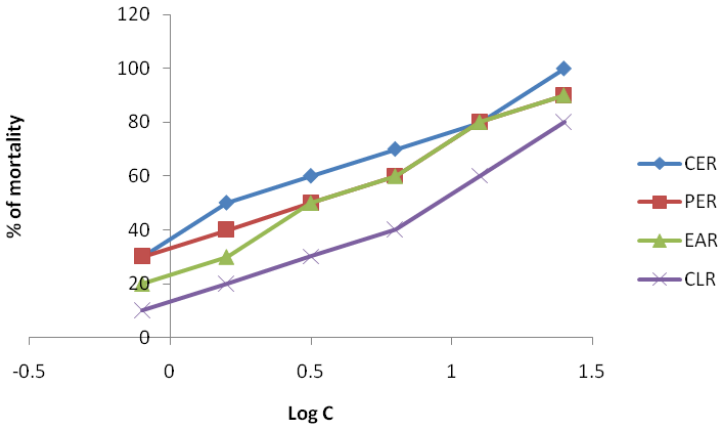

Fig 2. Plot of Log C versus % of mortality of crude methanolic extract of roots and the petroleum ether, chloroform and ethyl acetate fractions of *L. sibiricus*. CER: Crude methanolic extract of roots, PER: Pet-ether extract of roots, EAR: Ethyl acetate extract of roots, CLR: Chloroform extract of roots.

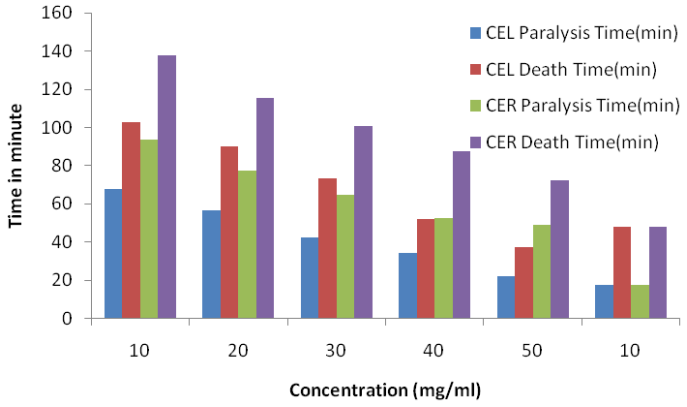

Fig 3. Anthelmintic activity of crude methanolic extracts of leaves and roots of *P. posthuma*. Values are expressed as mean±SEM (n = 3). CEL: Crude metahnolic extract of leaves, CER: Crude methanolic extract of roots.

To determine the anthelmintic potential only the crude methanolic extract of leaves and roots were employed. The result has been shown in Fig. 3. We see a linear correlation of paralysis and death time and concentration of extracts. Increasing concentration of extracts results in decrease in death time of the earth worm, *P. posthuma*. The crude methanol extract of leaves caused paralysis and death within 70 minutes and around 100 minutes, respectively after treatment at the lowest concentration of 10 mg/ml. Whereas, standard albendazole paralyzed the earthworms at concentration of 10 mg/ml within 20 minutes. At 50 mg/ml concentration, paralysis occurs at around 22 minutes and death occurs at 37 minutes i.e., time difference between paralysis and death is 15 min. Upon exposure with same concentration of methanol extract of roots death and paralysis occurs at 49 and 72 minutes, respectively. The methanolic extract of roots also showed inhibitory effect but the effect was less than the leaves extract. Paralysis occurs at 49 minutes after exposure of methanolic extract of roots at 50mg/ml concentration. The difference between paralysis time and death time is 23 min. These results indicate that the leaves are more active against earthworms than the roots.

Though, there are some previous reports of cytotoxic and antitumor activities of *L. sibiricus* [15, 28, 29, 31], anthelmintic activities have not been studied yet. The very good wormicidal effect of both leaf and root extracts indicated that the extract can be used as to kill parasitic worms of human.

Acknowledgment

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

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