In vitro anthelmintic activity of leaves and stems extract of Biophytum Sensitivum linn.

Rajanikant T. Kakade*, Nagesh Sandu and K. L. Senthilkumar

Padmavathi College of Pharmacy, Periyannahalli, Dharmapuri, Tamilnadu, India

ABSTRACT

Biophytum Sensitivum Linn., leaves and stems were extracted with Petroleum ether, Ethyl acetate and Methanol by successive hot extraction. All the extracts were evaluated for Anthelmintic activity with Indian earthworm (Pheretima posthuma). Leaves ethyl acetate extract has shown significant activity for concentration 25 mg/ml, 50 mg/ml, 100mg/ml with paralysis time \(85.0 \pm 1.9\), \(66.0 \pm 1.3\), \(40.8 \pm 1.0\) and Death time \(91.3 \pm 1.1\), \(81.2 \pm 1.7\), \(50.5 \pm 0.8\) respectively. The anthelmintic activity may be shown due to biflavonoids and tannins present in ethyl acetate extract. Other extracts also shows comparatively low activity.

Keywords: Biophytum Sensitivum Linn., Pheretima posthuma, Anthelmintic activity, Biflavonoid.

INTRODUCTION

Biophytum Sensitivum is wildly grown plant from the region of tropical Asia, mainly in Indian hot region. Macroscopic study shows following characteristics of plant. It is a annual herb up to 25 cm tall, leaves sensitive to touch, pinnately compound, crowded into rosette atop the stem. Leaflets 3–12 pairs, opposite, oblong, the terminal pair the largest. In Ayurveda the plant is reported to possess tonic, stimulant, antimicrobial and styptic properties and is used to treat chest complaints, insomnia, convulsions, cramps and inflammatory tumours and its ash is used in stomach ache [1]. The plant is bitter, thermogenic, diuretic, lithinotriptic, suppurrative, expectorant, stimulant and tonic. It is useful in strangury, urinary calculi, hyperdipsia in bilious fever, wounds, abscesses, gonorrhea, asthma, phthisis, stomachalgia and snake bite [2]. Different activities of this plant is due to presence of active principle constituents like flavonoids, phenolic organic acid and tannins [3].

Helminthiasis, the condition resulting from worm infestation, is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for wide spread nature of this disease in the developing countries. Helminthiasis is prevalent globally (1/3 of world's population harbours them), but is more common in developing countries with poorer personal and environmental hygiene [4]. Anthelmintics or antihelmintics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them [5]. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs; therefore, there is a foremost problem in treatment of helminthes diseases [6]. Moreover, these drugs are unaffordable because of their high cost. These factors paved the way for herbal remedies as alternative anthelmintics.

The present study aimed to check in-vitro anthelmintic activity of Biophytum sensitivum. Petroleum ether, ethyl acetate and methanolic extracts of leaves and stems were used.
MATERIALS AND METHODS

Drugs and Plant material
Leaves and stems of *Biophytum sensitivum* Linn. were collected from Chambharleni region of Nashik, in the state of Maharashtra (India). The plant was identified Dr. P. S. N. Rao, Joint Director, Botanical Survey of India, Pune, Maharashtra. (Wide voucher no.BSI/WC/Tech/2012/731). The herbarium of plant has been deposited in our department for future reference. All the chemicals and reagents were procured from S.D.Fine Chemicals (Mumbai, India). All the chemicals were of analytical grade. The drug piperazine citrate was procured from Glaxo Smithkline Ltd, Mumbai.

Preparation of plant extracts
The powdered leaves and stem material of plant was successively extracted with solvent petroleum ether, ethyl acetate and methanol. After complete extraction extracts were collected and dried by using Rotavac evaporator. Extract was preserved in refrigerator for future use.

Worm collection and authentication
Indian earthworm *Pheretima posthuma* (Pheretimidae) have been used widely for the initial evaluation of anthelmintic activity, because of their easy availability. Indian adult earthworms (*Pheretima posthuma*) were collected from the water logged areas of soils and washed with normal saline to remove all filthy matter. They were authenticated by Head, Department of Zoology, K.T.H.M. College, Nashik, Maharashtra. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [7-11].

Phytochemical test
The freshly prepared crude extracts of leaves and stems of *Biophytum sensitivum* Linn. were subjected to standard phytochemical screening tests for various constituents [12-15].

Anthelmintic Assay
The anthelmintic activity was carried out on adult Indian earthworms, *Pheretima posthuma* as per Ajaiyeoba [16] with minor modification. The dose suspensions were prepared using Carboxy methyl cellulose (1% CMC), which is nontoxic and nonirritant used in oral and other formulations. All extracts and standard drugs suspensions were freshly prepared before starting the experiment. Eight groups of approximately equal size earthworms consisting of six earthworms in each group were used for the present study.

Group-1: Control (Normal saline)
Group-2: Standard (Piperazine citrate- 10mg/ml)
Group-3: Pet. Ether leaves extract of different concentration (25mg/ml, 50mg/ml, 100mg/ml)
Group-4: Ethyl acetate leaves extract of different concentration (25mg/ml, 50mg/ml, 100mg/ml)
Group-5: Methanolic extract of different concentration (25mg/ml, 50mg/ml, 100mg/ml)
Group-6: Pet. Ether stems extract of different concentration (25mg/ml, 50mg/ml, 100mg/ml)
Group-7: Ethyl acetate stems extract of different concentration (25mg/ml, 50mg/ml, 100mg/ml)
Group-8: Methanolic stems extract of different concentration (25mg/ml, 50mg/ml, 100mg/ml)

Observations were made for the time taken for paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C followed with fading of their body colour [17] [18]. Three sets of experiments were done statistical significance.

RESULTS AND DISCUSSION

The anthelmintic activity was carried out on adult Indian earthworms, *Pheretima posthuma* in view of its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

The results of preliminary phytochemical screening of different extracts of leaves and stems of *Biophytum sensitivum* for detection of various chemical constituents are given in Table 1.
The leaves and stem extract of *Biophytum sensitivum* Linn. consists of sterol, triterpenoids and saponin in pet ether extract, flavonoids and phenolic compound in ethyl acetate extract, sugar, glycosides and alkaloids and traces of phenolic compounds in methanolic extract. Leaf part of plant showed rich content of polar compound which are soluble in methanol and ethyl acetate.

Extracts exhibited more potent activity at higher concentration (100 mg/ml) against *Pheretima posthuma* (earthworm). Evaluation of anthelmintic activity was compared with reference standard piperazine citrate as shown in Table 2.

Stem and leaf extracts of *Biophytum Sensitivum* showed the significant activity against Indian earthworm (*Pheretima posthuma*). Earthworm in normal saline solution has not showed paralysis or death which gives ideal about selection of solvent is suitable. The isolation and characterization of the active principle phytoconstituent from ethyl acetate extract is under progress.

**Statistical Analysis**

The results are presented as mean SEM. “One-way ANOVA” using graph pad prism was performed.
Figure 1: Graphical representation for the anthelminthic activity of *Biophytum sensitivum* Linn. compared to standard drug (Paralysis)

- Concentration (mg/ml)
- Paralysis time (Minute)

Figure 2: Graphical representation for the anthelminthic activity of *Biophytum sensitivum* Linn. compared to standard drug (Death)

- Concentration (mg/ml)
- Death time (Minute)

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