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Research Article

PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ANTHELMINTIC ACTIVITY OF *MIMUSOPS ELENGI* LINN AND *DRYPETES SEPIARIA*

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ABSTRACT

The present study was undertaken to evaluate anthelmintic activity of ethyl acetate, methanol and aqueous crude extract of *M. elengi* roots and *D. sepiaria* leaves on Indian adult earthworms (*Pheretima posthuma*). All the extracts exhibited concentration dependent activity at tested concentrations of 10-80 mg/ml. Higher activities were observed at the higher concentrations. Aqueous extract of *M. elengi* and *D. sepiaria* was more active than methanol and ethyl acetate extracts. At concentrations 80mg/ml the aqueous extracts of both plants showed better activity with paralysis time (8.3, 12.2 min) and death times (14.0, 27.3 min) respectively when compared to the standard piperazine citrate at 10mg/ml. Our study suggests these plants as potent anthelmintics.

Keywords: Anthelmintic activity, *Mimusops elengi*, *Drypetes sepiaria*, *Pheretima posthuma*

INTRODUCTION

Approximately 70-80% of world's population depends on traditional medicinal plants. Plant derived drugs serve as most effective and less toxic medicines for many of the diseases [1]. Helminthic infections are one of the prevalent diseases affecting a large ratio of world's population recognized as much cause of chronic ill health and sluggishness [2]. Anthelmintics from natural sources play a key role in the treatment of these parasitic infections. In view of this, attempts have been made to study the anthelmintic activity of two important medicinal plants *Mimusops elengi* and *Drpetes sepiaria*.

Mimuosops elengi L. (sapotaceae) is a small to large evergreen tree found and distributed throughout the greater parts of India. It is commonly known as Spanish cherry, Medlar and bullet wood in English and Bakul in Sanskrit. Different parts of this plant are used in the indigenous system of medicine for treatment of different ailments. In Ayurveda, the bark, flowers, fruit and seeds are of great value for treating various diseases such as cardiotonic, alexipharmic, stomachiac, astringent cooling, anthelmintic, tonic, and febrifuge properties [3]. The bark and fruits of this plant are used in the treatment of diarrhoea and dysentery and decoction of the bark is used as a gargle [4]. Rinsing mouth with bark decoction is believed to strengthen the gums, reduce inflammation, prevent bleeding of gums, and to stop bad breath caused by pyorrhea and dental caries [5]. Different parts of the plant have also been reported for anti-microbial [6], anti-ulcer [7], anti-anxiety [8], anti-oxidant, hyperglycemic [9], anti hyperlipidemic [10] and anti-helminthic [11]. A recent report of this plant proves that ethanolic extracts of bark contains anti-inflammatory as well as cytotoxic activities [12]. Several triterpenoids, steroids, steroidal glycosides, flavonoids and alkaloids have been identified and reported from this plant [13].

Drypetes sepiaria belongs to the family Putranjivaceae (Euphorbiaceae). It is an evergreen tree commonly grown in foothills and shrub jungles which is widely distributed in Sri Lanka and some places of Tamil Nadu. It is locally known as Kalvirai (Tamil). This plant is used in folk medicine by tribal people of Western Ghats to treat pain and inflammation. The seeds of this plant are used as a wild edible food by Palliyars (A tribal Group) of Western Ghats, India [14]. Recent report on *D. sepiaria* has been stated that the paste of the roots can be used as an antidote for scorpion bite. The decoction of leaves and seeds of this plant is also given for reducing rheumatic inflammation [15]. As per literature reports, till date there is no scientific investigations found on species *D. sepiara* on both of its pharmacological properties as well as phyto constituents present.

MATERIALS AND METHODS

Plant collection and preparation of plant extracts

The Roots of *M. elengi* were collected from VIT University (Coordinates 12°58'09"N 79°09'21"E) and leaves of *D. sepiaria* were collected from Puducherry (Coordinates: 11°58'5"N 79°48'42"E) India, during the month of January, 2011. The plant parts were authenticated by Professor Jayaraman, Director, National Institute of Herbal Science, Chennai. Plants were washed thoroughly with distilled water to remove all contaminated material. The washed material was shade dried to avoid decomposition and pulverized in mixer blender. Sixty grams of the powder was preliminarily defatted with hexane at 60-80°C for 24h in soxhlet apparatus. The defatted material was again subjected to soxhlet extraction with 400 ml of ethyl acetate, methanol and water to obtain ethyl acetate (EA), methanol (ME) and water (AQ) extracts respectively. The extracts were concentrated in a rotary evaporator and lyophilized, weighed and stored in vacuum desiccators.

Chemicals

Piperazine citrate was obtained from local medical shop. Hexane, ethyl acetate, methanol is of AR grade purchased from SD-Fine Chem limited, India.

Phytochemical Analysis

Preliminary screening of the crude extracts was carried out qualitatively for the presence of alkaloids, saponins, flavonoids, tannins, amino acids, carbohydrates, steroids, phenols, anthraquinones by following the standard methods [16].

Earth Worms

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity of the plant extracts. The adult earthworms were collected from moist soil of Christian medical college, Vellore, Tamil Nadu, India and washed thrice with normal saline solution to remove all the faecal matter. The worms of 5-6cm in length and 0.2-0.3cm in width were used for complete experiment.

Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al [17] on Indian adult earthworms (*P. posthuma)* with minor modifications. The assay was carried on Indian earthworms (*P. posthuma)* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. The ethyl acetate, methanol and aqueous extracts of *M. elangi* root and *D. Sepiaria* leaf were suspended in normal saline to prepare 20, 40, 60, 80 mg/ml concentrations. Piperazine citrate (10 mg/ml) was used as a standard drug. Normal saline was used as a control.

The worms were divided in to twenty six groups with each containing three worms (N=3) were placed in 10mL of desired formulation. One group serve as control and one group serves as standard and twelve sets of two different groups was treated with extracts of desired concentrations. The observations were made for paralysis time and death time for each earthworm and mean time was taken for all the extracts. The paralysis time was said to occur when there is no sort of movement except when shaken vigorously. The time of death was recorded after ascertaining that worms neither moved when given external stimuli nor dipped in warm water (50°C) [18].

Statistical analysis

The results were analyzed for statistical significance using one way ANOVA followed by student t-test. The P value (<0.001) was considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical analyses of *M. elengi* revealed the presence of saponins, flavonoids, alkaloids and carbohydrates in all the extracts. Whereas, *D. sepiaria* revealed the presence of saponins, anthraquinones in all the extracts, alkaloids in petroleum ether and aqueous extracts, while flavonoids and carbohydrates in methanol and aqueous extracts, steroids in petroleum ether and ethyl acetate extracts.

The aqueous extracts of *M. elengi* and *D. sepiaria* exhibited a dose dependent manner of anthelminthic activity as shown in Graphs 1 and 2. It is observed that the Indian adult earthworms were more sensitive to the extracts and it is evident that aqueous extracts of *D. sepiaria* exhibited paralysis as well as death in less time and showed potent anthelmintic activity when compared to all the extracts of *M. elengi*, which took long time for death of worms.

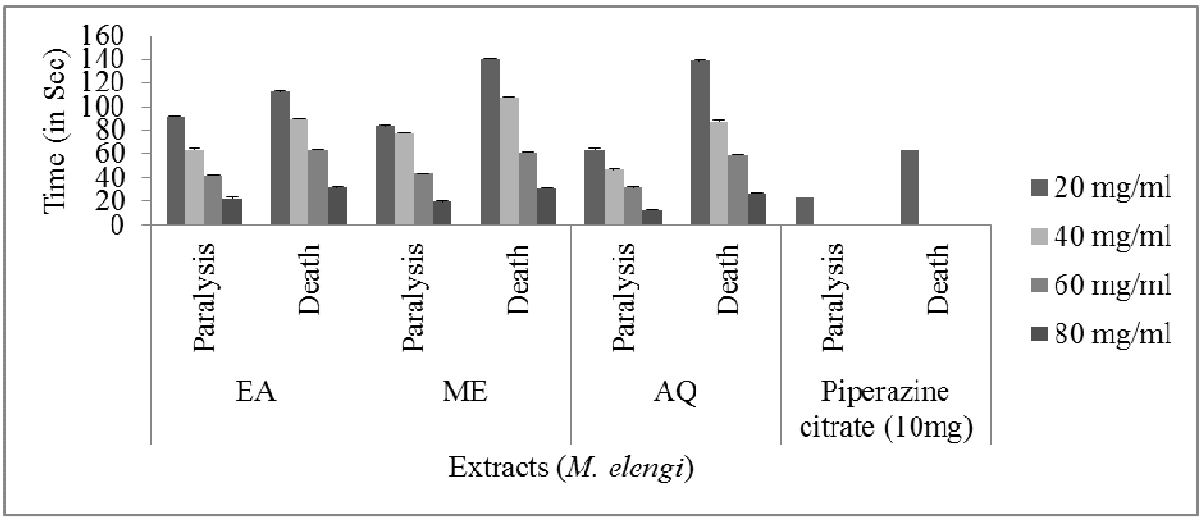

Fig. 1: Paralysis and death time for different extracts of *M.elengi*

Each value represents mean ± SEM (N=3). P<0.001significantly different compared with reference compound, Piperazine citrate

The aqueous extracts of *D. sepiaria* and *M. elengi* caused paralysis at 72.2, 63.33 min (at 20 mg/ml); 42.3, 47.6 min (at 40 mg/ml); 12.6, 31.33 min (at 60 mg/ml); 8.3, 12.2 min (at 80 mg/ml) respectively. Whereas the paralysis time for ethyl acetate and methanol extracts were high when compared to aqueous extracts. These aqueous extracts of *D. sepiaria* and *M. elengi* extracts caused death at 148.5, 139.3 min (at 20 mg/ml); 101.3, 87.5 min (at 40 mg/ml); 30.7, 59.6 min (at 60 mg/ml); 14.0, 27.3 min (at 80 mg/ml) respectively when compared to the standard reference drug piperazine citrate (10 mg/ml) which showed paralysis time of 23.4 min and death time of 63.2 min. This activity may be due to the presence of phenolic and alkaloid group of compounds [19]. Both the plants aqueous extracts were more effective in causing the paralysis and mortality of the worms compared with the piperazine citrate. These findings suggest that the two plants possess potent anthelmintic activity and can be used as an alternate to the use of synthetic drugs.

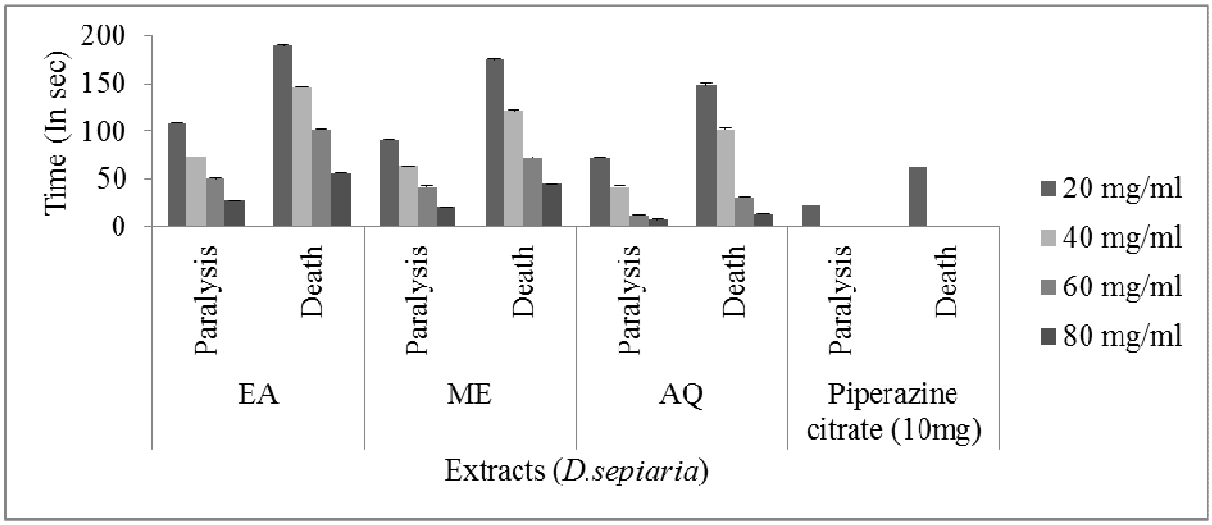

Fig. 2: Paralysis and death time for different extracts of *D.sepiaria*

Each value represents mean ± SEM (N=3). P<0.001significantly different compared with reference compound, Piperazine citrate

CONCLUSION

From this study, two important medicinal plants which investigated were found to be active as anthelmintics with respect to both paralysis and death times. It validates their uses in ethno medical importance. However, the isolation of the active constituents responsible for the activity is under progress in our laboratory.

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