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In-vitro anthelmintic activity of stem bark of *Mimusops elengi* Linn.

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ABSTRACT - The aim of present study was to evaluate anthelmintic potential of crude alcoholic extract of bark of *Mimusops elengi* and its different fractions namely ethyl acetate, n-butanol and methanol using *Pheretima posthuma* and *Ascardia galli* as test worms. Various concentrations (10 – 100 mg/ml) of alcoholic extract and its various fractions were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control. The results of present study indicated that the crude alcoholic extract and its ethyl acetate and n-butanol fractions significantly demonstrated paralysis, and also caused death of worms especially at higher concentration of 100 mg/ml, as compared to standard reference Piperazine citrate. In conclusion, the traditional use of bark of the plant *M. elengi* as an anthelmintic have been confirmed and further studies are suggested to isolate the active principle/s responsible for the activity.

KEY WORDS: *Mimusops elengi*, anthelmintic, *Pheretima posthuma*, *Ascardia galli*, Piperazine citrate.

INTRODUCTION

Mimusops elengi Linn (Sapotaceae) commonly known as Bakul, is a small to large evergreen tree found all over the different parts of India. It is cultivated in gardens as an ornamental tree. It has been used in the indigenous system of medicine for the treatment of various ailments. Several therapeutic uses as cardiotonic, alexipharmic, stomachic, anthelmintic and astringent have been ascribed to the bark of *Mimusops elengi* (1). Phytochemical review shows the presence of taraxerol, taraxerone, ursolic acid, betulinic acid, α-spinosterol, ß-sitosterol, lupeol, alkaloid isoretronecyl tiglate and mixture of triterpenoid saponins in the bark of *Mimusops elengi* (2-5). Literature search revealed that there is no report available regarding anthelmintic activity of *M. elengi* bark. The present study was, therefore undertaken to evaluate the *in vitro* anthelmintic activity of crude extract of *M. elengi* bark (70 % alcoholic extract) and its different fractions against *Pheretima posthuma* and *Ascardia galli*.

MATERIALS AND METHODS

Plant Collection and Authentication

The stem bark of *M. elengi* was collected from mature trees and its botanical identification was confirmed from Botanical Survey of India (BSI), Koregaon Road, Pune. A voucher specimen RGM-A2 was deposited in the herbarium of BSI, Pune.

Plant Extraction

The plant material (stem bark) was dried for several days and powdered with the help of an electric grinder. After defatting the bark powder (250 g) using petroleum ether (40 – 60 °C), it was air dried and extracted exhaustively with 70 % alcohol. The liquid extract was evaporated in vacuum to yield 34.5 % dark brown residue. The dried residue was taken in minimum quantity of water and was successively extracted with ethyl acetate, n-butanol and methanol to yield 4.92 % w/w, 7.85 % w/w and 8.20 % w/w residue, respectively. Alcoholic extract and all the fractions were preserved in refrigerator.

Worms Collection and Authentication

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil and *Ascardia galli* (Nematode) worms were obtained from freshly slaughtered fowls (*Gallus gallus*). Both worm types were identified at the P.G. Department of Zoology, Pratap College, Amalner.

Preparation of Test Sample

Samples for in-*vitro* study were prepared by dissolving 2.5 gm of each crude alcoholic extract and its ethyl acetate, n-butanol and methanol fractions in 25 ml of

distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 50 and 100 mg/ml.

Anthelmintic Assay

The anthelmintic assay was carried as per the method of Ajaiyeoba E.O. *et al* (6) with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (7-10). Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in *vitro* (11-14). *Ascardia galli* worms are easily and plentily available from freshly slaughtered fowls and its use, as a suitable model for screening of *anthelmintic* drug was advocated earlier (15-17). 50 ml formulations containing three different concentrations, each of crude alcoholic extract and its various fractions (10, 50 and 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. This was done for both types of worm. 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 (50 °C) (18-19). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the -control.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of crude alcoholic extract and its different fractions revealed the presence of alkaloids, saponins, flavonoids and tannins. As shown in Table 1, alcoholic extract of *M. elengi* and its different fractions exhibited anthelmintic activity in dose-dependant manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration, for both types of worms. The alcoholic extract of *M. elengi* caused paralysis of 12 min and time of death of 29 min while ethyl acetate and n-butanol fractions revealed paralysis of 13 and 14 min. and time of death of 31 and 30 min. respectively against the earthworm *P. posthuma*. The reference drug Piperazine citrate showed the same at 23 and 60 minutes, respectively.

Ascardia galli worms were also shown sensitivity to the alcoholic extract and different fractions significantly higher concentration of 100 mg/ml. The alcoholic extract caused paralysis at 8 min. and time of death of 18 min. Ethyl acetate and n-butanol fractions showed paralysis at 9 and 11 min and the time of death were 19 and 21 min. respectively. Piperazine citrate exhibited similar effects at 16 and 33 min. respectively. The predominant effect of Piperazine citrate on worm is to cause a flaccid paralysis those results in expulsion of the worm by peristalsis.

Table 1: Anthelmintic activity of alcoholic extract of Mimusops elengi and its fractions

Test subs	Concentration mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minute			
		P. posthuma		*A. galli*	
		P	D	P	D
Alcoholic extract	10	25 ± 0.2	65 ± 0.8	20 ± 0.1	39 ± 0.9
	50	18 ± 0.1**	40 ± 0.7***	15 ± 0.8	29 ± 0.1**
	100	12 ± 0.3***	29 ± 0.5***	08 ± 0.1***	18 ± 0.2***
Ethyl acetate fraction	10	26 ± 0.3	64 ± 0.2	21 ± 0.8	42 ± 0.3
	50	20 ± 0.4	50 ± 0.1***	14 ± 0.6	30 ± 0.4
	100	13 ± 0.5***	31 ± 0.3***	09 ± 0.7***	19 ± 0.1***
n - Butanol fraction	10	27 ± 0.1	63 ± 0.3	19 ± 0.1	40 ± 0.2
	50	19 ± 0.2**	49 ± 0.5***	15 ± 0.1	29 ± 0.3**
	100	14 ± 0.1***	30 ± 0.2***	11 ± 0.5***	21 ± 0.2***
Methanol fraction	10	28 ± 0.1	68 ± 0.7	22 ± 0.6	44 ± 0.2
	50	25 ± 0.7	59 ± 0.2	19 ± 0.8*	38 ± 0.1
	100	20 ± 0.4	41 ± 0.3***	15 ± 0.2	31 ± 0.4*
Piperazine citrate	10	23 ± 0.8	60 ± 0.5	16 ± 0.4	33 ± 0.4

*All values represent Mean ± SEM; n=6 in each group. Values are significantly different from reference standard (Piperazine citrate) *p<0.05; **p<0.01; ***p<0.001*

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis (20). The stem bark extract of *M. elengi* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extracts revealed the presence of tannis among the other chemical constituent contained within them. Tannins were shown to produce anthelmintic activities (21). Chemically tannins are polyphenolic compounds (22). Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (23). It is possible that tannins contained in the extracts of *M. elengi* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (24) or glycoprotein on the cuticle of the parasite (25) and may cause death.

The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. It is in this context that the people consume several plants or plant-derived preparations to cure helminthic infections (26). The origin of many effective drugs has been found in the traditional medicines practices and in view of this it is important to undertake studies pertaining to screening of the folklore medicinal plants for their proclaimed anthelmintic efficacy.

In conclusion, the traditional use of stem bark of *Mimusops elengi* as an anthelmintic have been confirmed as the stem bark extracts displayed profound arithemetic activity in the study. Further, it would be interesting to isolate the possible phyto constituents which may be possible responsible for the anthelmintic activity and to possible the mechanism (s) of action.

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