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In-vitro anthelmintic activity of leaves of *Mitragyna parvifolia*

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ABSTRACT

The effects of the ethanolic and aqueous extracts of leaves from *Mitragyna parvifolia* were examined for their anthelmintic activity against *Pheritima posthuma*. The different concentrations (10mg/ml, 25mg/ml and 50 mg/ml) of both extracts were studied in the bioassay, which involved determination of time of paralysis and time of death of the worms. The results suggest that the ethanolic and aqueous extracts significantly demonstrated paralysis and also caused death of worms especially at higher concentration of 50 mg/ml, as compared to standard reference, Albendazole (10 mg/ml). These provide scientific evidence to support the isolation and development of biologically active components as anthelmintic.

Key words: *Mitragyna parvifolia*, anthelmintic, *Pheritima posthuma*.

INTRODUCTION

Mitragyna parvifolia (Roxb.) Korth (Family: Rubiaceae) is widespread throught India, in deciduous tree and evergreen forests upto 1200m altitude[1]. The bark and root reportedly used to treat fevers and colic among the santhalis, the bark, ground and made into a paste is applied to relieve muscular pain. In Ayurveda, the bark and fruit are used as substitute for those of Anthocephalus chinensis (Rubiaceae) to treat burning sensation, posining, wounds, gynoaecological disorders, cough oedema, to alleviate kapha and pitta, and as an aphrodisiac. In siddha, the stem, bark, leaf, fruit and seed are used as a substitute for those of Anthocephalus chinensis to treat eye diseases, dropsy, diseases of vatam and urticaria[2]. *M. parvifolia* has been reported, isolation and identification of some alkaloids N-oxides from the leaves[3]. Seven alkaloids from the leaves of *M. parvifolia,* from the sagar district of India, were isolated and the pattern of alkaloid transformation over a period of 12 month is described (4).

A review of the literature revealed that the anthelmintic activity of leaves of *M. parvifolia* has not been subjected to scientific evaluation. The present study was carried out in a experimental animal model to reports the anthelmintic property on leaves of this plant.

MATERIAL AND METHODS

Plant material

The leaves of *M. parvifolia* were collected from the Putkaphar forest Korba, Chhattisgarh in the month of April. The collected material was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. The plant material was also compared with a herbarium specimen maintained at Minor Forest Produce (Trading and Development) Co-Op. Fed. Ltd., Shankar Nagar, Raipur, Chhattisgarh, by Expert Medicinal Plant, Mr. S.N.Khotele.

Preparation of extract

The dried and powdered leaves (250 gm) were successively extracted on a Soxhlet apparatus, employing petroleum ether, ethanol and distilled water respectively. The extracts were further concentrated under reduced pressure with a rotary evaporator. Leaves of *M. parvifolia* yielded 2.3%, 13.5% and 12.6% w/w powdered extract with petroleum ether, chloroform, ethanol and distilled water respectively.

Worms

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil of the Lake view, Bhopal and washed with normal saline to remove all the faecal matter, were used for the anthelmintic study. 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 its anatomical and physiological resemblance with the intestinal roundworm parasites human beings[5,6].

Drugs

Standard drug Albendazole was taken as a gift sample from Zydus Cadila, Ahmedabad.

Anthelmintic assay

All the extracts of *M. parvifolia* were dissolved in minimum amount of DMF and the volume was adjusted to 50 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment. 50 ml formulations containing three different concentrations (10, 25 and 50 mg/ml in saline water), each of crude alcoholic and aqueous extract were prepared and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except 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. Albendazole (10 mg/ml) was used as reference standard while normal saline as the control[7-9].

RESULTS AND DISCUSSION

From the table 1, it is evident that ethanol and aqueous extracts of *M. parvifolia* exhibited anthelmintic activity in dose-dependant manner giving shortest time of paralysis and death with 50 mg/ml concentration. The ethanol extract caused paralysis of 4.97 min. and time of death of 6.86 min., while aqueous extract revealed paralysis of 8.38 min and time of death of 13.02 min. respectively against the earthworm *Pheretima posthuma*. The standard drug Albendazole at 10 mg/ml concentration showed the same at 3.67 and 5.01 minutes, respectively. Table reveals that ethanol extract of leaves of *M. parvifolia* showed the best anthelmintic activity. These parts required the least time for causing paralysis and death of the earthworms.

Table 1: *In-vitro* anthelmintic activity of ethanolic and aqueous extract of *Mitragyna parvifolia*

Treatment	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Ethanolic Extract	10	7.40±0.32	11.23±0.34
	25	6.01±0.29	9.42±0.43
	50	4.97±0.18	6.86±0.26
Aqueous Extract	10	14.49±0.47	21.57±0.37
	25	11.19±0.41	16.42±0.42
	50	8.38±0.41	13.02±0.38
Albendazole	10	3.67±0.18	5.01±0.31
Normal Saline	-	-	-

Results are expressed as Mean ± SEM from six observations; Control worms were alive up to 24 hrs of observation.

The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feaces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at 50 mg/ml as compared with the Albendazole. In conclusion, these plants have been confirmed to display anthelmintic activities. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

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