

In vitro investigation of anthelmintic activity of *Mitragyna parvifolia* (Roxb.) Korth. (Rubiaceae)

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Abstract

In the present study anthelmintic activity of *Mitragyna parvifolia* (Roxb.) Korth. (Rubiaceae) stem-bark was performed in vitro against earthworms. Methanol extract of dried stem-bark was used for the study. The methanolic extract of *M. parvifolia* stem bark at the concentration of 100 mg/ml produced significant anthelmintic activity, whereas 20 mg/ml lower concentration did not produce significant results when compared with standard ($P < 0.01$). The result of anthelmintic activity of methanolic extract produced were evaluated by observing paralysis time and death time of earthworms and were found to be dose dependent. This finding proves usefulness of stem-bark as a anthelmintic drug.

Keywords: *Mitragyna parvifolia*, Rubiaceae, Anthelmintic activity

Introduction

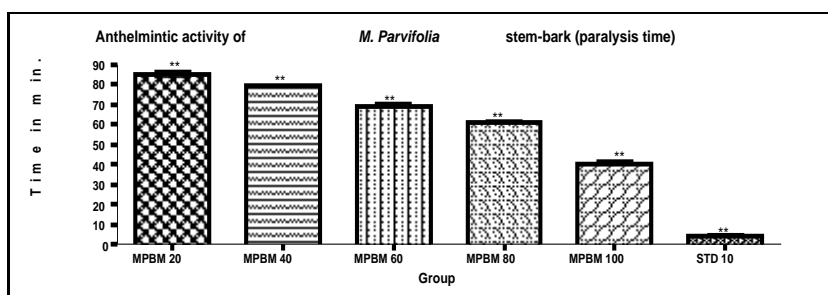
Parasites have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance (Coles, 1999, Geerts & Gryseels, 2000, Sangster, 1999). Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health (Padilha, 1996, Turnipseed et al., 1999). 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 (Hamond et al., 1997).

Mitragyna parvifolia (Roxb.) Korth (Rubiaceae) is a deciduous tree commonly known as Kaim, found in well drained deep soil (The wealth of India, 2003). It is credited with innumerable medicinal properties and is widely used by tribal peoples and other ayurvedic practitioners. The bark and roots are used to treat fever, colic, muscular pain, burning sensation, poisoning,

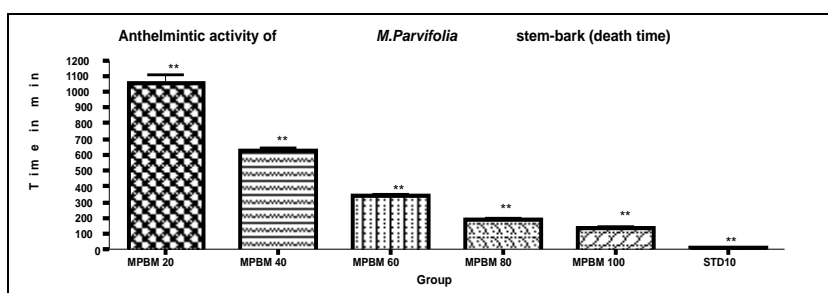
gynecological disorders, cough, edema and as aphrodisiac. Wounds and ulcers are dressed with its leaves to alleviate pain, swelling and for better healing (Shellard and Houghton, 1971, Prajapati et al., 2003, Panwar and Tarafdar, 2006, Pandey, et al., 2006). It is very rich in alkaloids and many have been isolated from the leaves, stem bark, and root-bark. The leaves of the plant *M. parvifolia* have afforded two alkaloids, 16,17-dihydro-17b-hydroxy isomitraphylline and 16, 17-dihydro-17b-hydroxy mitraphylline, together with two known alkaloids, isomitraphylline and mitraphylline (Pandey et al., 2006). The major alkaloids are mitraphylline, rotundifoline, rhynchophylline, isorotundifoline, rhynchociline, mitragynine and speciociliatine.

Some of these alkaloids, particularly mitraphylline and mitragynine, possess a local anaesthetic activity, induce a fall of blood pressure, and increase cardiac frequency. It has been observed that environmental factor has a vital role to play in modifying the alkaloid content and the structure (Chatterjee et al., 1982, Avadhoot and Varma, 1991). Various indolic and oxindolic alkaloids have been reported from this species are of significant biological importance (Shellard et al., 1969b, Stuppner et al., 1993). *M. parvifolia* (Roxb.) Korth. is a much-investigated species, stem-bark of the plant yet has not explored for anthelmintic activity. So in this study we have attempted to investigate the anthelmintic activities of *Mitragyna parvifolia* stem-bark for its methanolic extract.

Graph 1: Effect of *M. parvifolia* stem-bark extract on paralysis time of earthworms



Graph 2: Effect of *M. parvifolia* stem-bark extract on Death time of earthworms



Values represent mean \pm SEM, One way ANOVA, $P < 0.0001$, followed by Dunnett's t-test, $**P < 0.01$

Materials and methods

Plant material:

Mitragyna parvifolia (Roxb.) Korth. (Rubiaceae) stem-bark was collected from Toranmal Hills of Satpuda region from Maharashtra, India, during the month of June. The plant got identified and authenticated by Botanical Survey of India, Pune, and a voucher specimen of the sample (MPVB11) has deposited in the Herbarium collection at Department. The stem-bark was cleaned and dried in the shade, and then it was cutted in small pieces and powdered to 40 mesh and stored in an airtight container.

Preparation of extract:

Methanolic extract: Dried stem-bark powder (900 gm) was extracted with 3 L of methanol by continuous hot extraction using soxhlet extractor. The methanol containing extract was filtered and distilled on a water bath. The resulting Methanol extract solution was concentrated in vacuum using a Rota vapor to obtain a brown powder 16% (144 g).

Preparation of test sample and standard drug:

Extract obtained was used as a test sample and prepared by using distilled water to give a different concentrations ranging from 20, 40, 60, 80 and 100 mg/ml. further Levamisole hydrochloride tablets were crushed and powdered to make a standard solution of 10 mg/ml.

Animal:

Adult earthworms (*Pheretima posthuma*) having average size 6-9 cm and weighing 0.9 to 4.0 g were used to evaluate anthelmintic activity in vitro.

Anthelmintic activity:

The anthelmintic assay was carried out as per the method of according to Hukkeri et. al. (1993). The assay was performed in vitro using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity. Test samples of the extract 25 ml and six worms i.e. *Pheretima posthuma*, of approximately equal size (same type) were placed in each 9 cm Petri dish. Levamisole hydrochloride (10 mg/ml) was used as reference standard and distilled water as control. Observations were made for the time taken 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 worms neither moved when shaken vigorously nor when dipped in warm water (50°C). All the results are shown in Graph 1,2 and expressed as a mean \pm SEM of six worms in each group.

Results and Discussion

The data revealed that total methanolic extracts

of stem-bark of the plant *M. parvifolia* showed significant anthelmintic activity at 100 mg/ml concentrations. Results are comparable with standard drug Levamisole hydrochloride 10 mg/ml concentration. Graph 1 and 2 reveals that total methanolic extract of *M. parvifolia* stem-bark showed good effect on paralysis time and death time respectively. The results shows that methanol extract of stem-bark of plant *M. parvifolia* took the least time to cause paralysis and death of the earthworms at higher concentration only. Results of preliminary phytochemical tests suggest that methanol extract of stem-bark shows presence of flavonoids, glycosides, tannins and alkaloids, which might be in part responsible for this anthelmintic effect. Since *M. parvifolia* belongs to family Rubiaceae which has proven anthelmintic activities in various plants. Also the different parts of the plant have shown presence of active principles such as tannis and alkaloids. It is suggested that further experiments incorporating in vivo purification of extracts and toxicological investigations should be carried out.

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