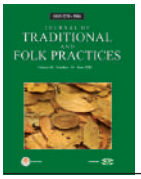

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Evaluation of anthelmintic activity of *Meyna spinosa* Roxb. – A folk medicinal plant of Northeast India

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Abstract

Natural antiparasitics have exhibited cabbalistic effects in healthcare units since ancient time. *Meyna spinosa* Roxb. is a traditional medicinal plant of North east India and is used in the treatment of different worm infections. The present study was designed to investigate *in vitro* anthelmintic activity of the methanolic extract of *Meyna spinosa* against Indian adult earthworm (*Pheretima posthuma*). Worm motility and mortality were assessed in order to evaluate the anthelmintic activity of the extract. Paralysis time and time of death of worms was noted in the presence of different concentrations of extract (50 mg/mL, 100 mg/mL, and 150 mg/mL) and standard drug, albendazole. The results showed the extract exhibited significant *in vitro* anthelmintic activity against *P. posthuma* at different concentrations. Extract (150 mg/mL) exhibited very strong anthelmintic effect which was evidenced by the paralysis and death of worms in lesser time. In conclusion, the findings revealed that the methanolic extract of *Meyna spinosa* leaves has effective anthelmintic activity against *P. posthuma* and indicated that the plant may be a potent natural antiparasitic agent.

Keywords: Anthelmintic activity, Earthworm **(***Pheretima posthuma),* Methanolic extract, *Meyna spinosa*

1. Introduction

Helminthic infection is thriving globally, minutely affecting the tropical countries demography since the last few decades (Antony *et al.,* 2007; Sen *et al.,* 2012). More than 2-3 billion people are affected by parasitic helminths as reported by World Health Organization (Kumar *et al.,* 2010). The helminth parasites largely subsist in the intestinal tract of human, but they are also found in other humans tissue, as their larvae migrate from one part to other parts of the body (Bundy, 1994). In developing countries, helminthic infections are growing rapidly and harming the host with acute and chronic complications like anaemia, eosinophilia, malnutrition, pneumonia, blood loss and damage to the organs. Many researchers reported the major routes of helminthic infections are food deprivation, poor personal care, lack of supply of pure water, climatic conditions etc. (Mali and Mehta, 2008; Mehta *et al.,* 2012; Shukla *et al.,* 2012; Sen *et al.,* 2012). The demand for natural antihelmintics is increasing due to major problems caused by synthetic anthelmintic drugs (synthetic benzimidazole) during management of helminthic diseases (Yadav and Singh, 2001).

Meyna spinosa Roxb. (Family: Rubiaceae) is a deciduous tree, widely occuring in north east India. The plant is famous by virtue of the variety of traditional applications in health care system of ethnic people. The fruits and bark

are used to cure headache and hair washing (Doley *et al.,* 2010) and fruit extract is used to cure diabetes (Khan and Yadava, 2010). Shoots of the plant are also used by folk people to treat jaundice (Anonymous, 2010). Leaves and fruits parts also used for skin infection, intestinal worm infection, painful urination, skin diseases, peptic ulcer, hepatic and healing (Sen *et al.,* 2011, Mali and Borges, 2003; Sen and Chakraborty, 2017).

However, there is no scientific testimony with regard to the anthelmintic activities of the plant. Therefore, the present study was undertaken to investigate *in vitro* anthelmintic activity of methanolic extract of *M. spinosa* leaves.

2. Materials and Methods

2.1. Plant materials

Fresh leaves of *M. spinosa* (Fig. I) were collected from Khowai subdivision of Tripura and identified by a taxonomist, Dr. B K Datta, Department of Botany, Tripura University, Tripura, India (Voucher No. TU/BOT/HB/SS/23072011A).

2.2. Preparation of extract

The fresh leaves of *M. spinosa* were collected and cleaned to remove unwanted materials. Shade-dried plant material was pulverized to fine powder and extracted with methanol in Soxhlet apparatus. The solvent was evaporated to dryness to obtain solvent free methanol extract. Extracts was stored in airtight containers in a cool place for further studies.

Fig. 1. *Meyna spinosa* Roxb.

2.3. Experimental animals

Indian adult earth worms were selected for the study due to their similar anatomical and physiological features with the intestinal roundworm of human beings. For the evaluation of anthelmintic activity, Indian adult earthworm (*Pheretima posthuma*) were collected from North East Green Tech Private Limited, Guwahati, Assam and washed with clean water to eliminate all fecal matter. The selected species size was 3-5 cm length and 0.1-0.2 cm width.

2.4. Evaluation of anthelmintic activity

Total 30 Indian adult earthworms of similar size and length were taken and grouped in the following manner,

Group I: Control (Normal Saline)
Group II: Reference standard albendazole (40 mg/ml)
Group III: Methanol extract of *M. spinosa* leaves (50 mg/ml)
Group IV: Methanol extract of *M. spinosa* leaves (100 mg/ml)
Group V: Methanol extract of *M. spinosa* leaves (150 mg/ml)

Methanol extract of *M. spinosa* leaves was prepared at concentrations of 50 mg/mL, 100 mg/mL and 150 mg/mL and six earthworms of about equal size and length were kept in each petri dish

containing 20 mL of the above test solutions of extract. Albendazole (40 mg/mL) was used as reference standard. Normal saline was used as control. The time required for paralysis and death were recorded for all groups of earthworms. Paralysis time implies loss of motility and death time was noted as the time when earthworms did not react even when shaken vigorously or dipped in warm water (50 ^{0}C), followed by fading of their body colour (Sen *et al.,* 2012).

2.5. Statistical analysis

The results were expressed as mean ± SEM (n = 6).

3. Results and Discussion

The present study was investigated to determine the anthelmintic activity of *M. spinosa* leaves. Table 1and Fig. 2 revealed that the methanolic extract of *M. spinosa* leaves exhibited significant anthelmintic activity. The methanol extract of *M. spinosa* leaves at 150 mg/ml showed more potent anthelmintic effect than the other two concentrations (50 mg/ml, 100 mg/ml). The paralysis and death time of earthworms (150 mg/ml) against earthworms were observed 12.40 and 30.36 min respectively which is almost similar to the effect of albendazole exhibited in this study. Albendazole (40 mg/kg) produced paralysis after 12.23 min and death of earthworm occurred after 25.12 min.

Table 1. *In vitro* anthelmintic effect of methanolic extract of *M. spinosa* leaves extract against Indian adult earthworm *(Pheretima posthuma)*

Group	Treatment	Concentration	Paralysis time (min)	Death time (min)
Group –1	Control (Normal saline)	-	-	-
Group –2	Standard drug (Albendazole)	40 mg/ml	12.23 ± 1.45	25.12 ± 1.12
Group –3	Methanol extract of *M. spinosa*	50 mg/ml	30.35 ± 2.12	50.24 ± 3.12
Group –4		100 mg/ml	22.15 ± 3.13	35.13 ± 2.34
Group –5		150 mg/ml	12.40 ± 2.45	30.36 ± 1.45

Results were expressed as mean ± SEM (n= 6)

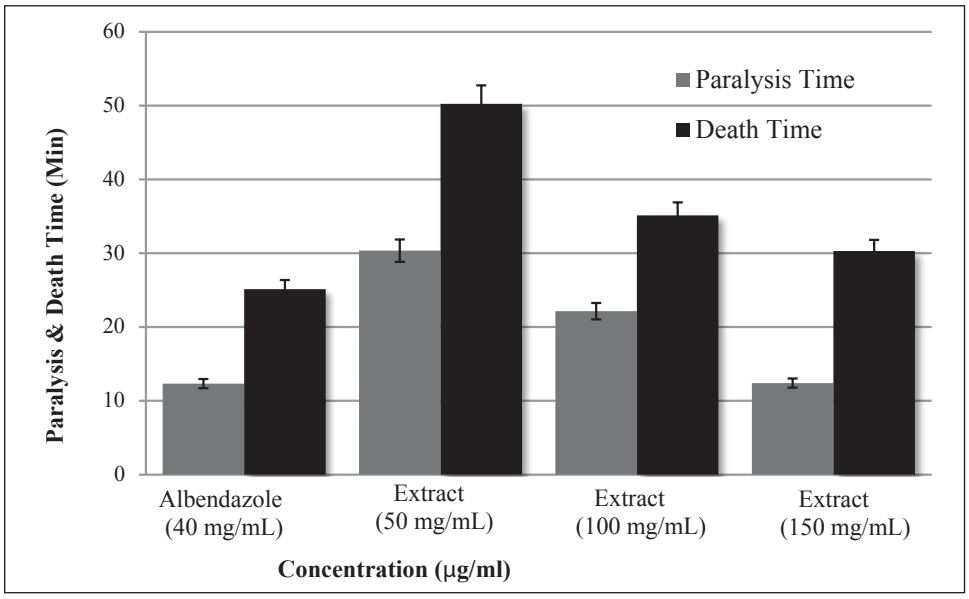

Fig. 2. Graphical representation of the anthelmintic activity of *M. spinosa* leaves extract

Albendazole and its related compounds from benzimidazole family act by entering into the helminths by passive diffusion through the exterior surface of helminths where they directly induce disruption of the tegumental and muscle layers, prevent polymerization of helmintic β-tubulin and interfere with microtubule-dependent functions like glucose uptake and glycogen depletion (Chander *et al.,* 2014). Now a days, resistance to the available synthetic drugs is a major problem. Therefore, in recent years there is a hunt for plant-derived natural compounds, which are the primary alternative of investigators as natural compounds and are believed to have more compatibility with physiological flora and lesser side effects (Mehta *et al.,* 2012; Sen *et al.,* 2012). In the search for a new compound with anthelmintic activity, diverse species of worms like earthworm, *Nippostrongylus, Ascaris,* and *Heterakis* are used by the investigators. However, due to the easy accessibility and similarity with intestinal worms in their reaction to anthelmintics, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (Eguale *et al.,* 2011; Goswami *et al.,* 2011; Sen *et al.,* 2012). Plant extracts are potential source of anthelmintic drugs and have been extensively studied for anthelmintic activities (Ayaz *et al.,* 2014).

Our findings revealed that time taken for paralysis and death were declining with the increasing concentration of *M. spinosa* leaves extract. Helminthe infection affected large proportion of world's population. The present study revealed the anthelmintic potential of methanolic extract of *M. spinosa* leaves. All the concentrations of the extract asserted significant anthelmintic activity and increasing concentration of *M. spinosa* leaves extract showed better paralysis and death time against earthworms.

4. Conclusion

In conclusion, the findings revealed that the methanolic extract of *Meyna spinosa* leaves has effective anthelmintic activity against *Pheretima posthom* and indicated that the plant is a potent natural antiparasitic. However, further study is essential to detect and isolate phytochemicals from *Meyna spinosa* leaves responsible for its anthelmintic activity.

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