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In vitro anthelmintic activity of methanolic extract of *Macaranga denticulata* leaves in *Pheretima posthuma*

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Abstract

Parasitic diseases cause extreme horribleness by influencing population in endemic areas with major economic and social outcomes. More than half of the population of the world suffers from various types of infection and majority of cattle suffer from worm infections. So there is a need to find new drugs to treat parasitic diseases. Methanolic extract from the leaves of *Macaranga denticulata* Muell. Arg. (Euphorbiaceae) was investigated for their anthelmintic activity against *Pheretima posthuma*. Three concentrations (25, 50 and 100 mg/ml) of the extract were studied, which involved the determination of time of paralysis and time of death of the worm. It was found that this extract exhibited significant anthelmintic activity. Albendazole in 10 mg/ml concentration was included as standard reference and 0.5% CMC as a control. Results showed that the methanolic extract was potent as it took near time of highest concentration (100 mg/ml) to cause paralysis and death of the earthworms as compared to the standard reference drug.

Keywords: Anthelmintic, *Macaranga denticulate*, *Pheretima posthuma*, Methanolic extract, Albendazole.

Introduction

Since the beginning of human history, plants have been in practice as folk medicine. All natural plants are a rich source of medicinal agents, commonly in traditional medicine.¹ One or more parts of medicinal plants having substances that can be useful for the therapeutic purpose.²

Helminthiasis is the condition resulting from worm infestation, and is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for wide spread nature of this disease in the developing countries. Helminthiasis is prevalent globally (1/3 of the world's population harbours them), but is more common in developing countries with poorer personal and environmental hygiene.^{3,4} Anthelmintics or anthelmintics are drugs that expel parasitic worms (helminthes) from the body, by either stunning or killing them.⁵ The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in the treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics.⁶

Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminthes^{7,8} against conventional anthelmintics is a foremost problem in the treatment of helminthes diseases^{9,10}. Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelmintic activity.

Macaranga denticulata Muell. Arg. (Euphorbiaceae) is a small to medium-sized, evergreen tree and is a common pioneer species in moist open areas and secondary forests.¹¹ In the mountains of Northern Thailand, *M. denticulata* is used as a fallow enriching species by Karen hill tribe farmers.¹² In folk medicine, traditional healers use fresh or dried leaves of some *Macaranga* species to treat swellings, cuts, sores, boils and bruises.¹³ A phytochemical review of literatures indicates the genus *Macaranga* to be a rich source of the isoprenylated, geranylated and farnesylated flavonoids and stilbenes. Furthermore, more classes of secondary metabolites like terpenes, tannins, coumarins and other types of compounds are known to be isolated from different species of the genus *Macaranga*. Flavonoids and stilbenes are regarded as the major constituents and are most likely responsible for most of the activities found in the plants of this genus.

Materials and Methods

Plant material

The leaves of *M. denticulata* were collected from the Chittagong city area in front of Chittagong Medical college hostel gate of Bangladesh in October, 2014 then identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh. Voucher specimens, collection id: CTG 121, for *M. denticulata* kept in the Department of Pharmacy, International Islamic University Chittagong, Chawkbazar, Chittagong-4203, Bangladesh for further reference.

Preparation of Extract

The collected plant was washed thoroughly with water and air dried for a week at 35 to 40 °C and pulverized in an electric grinder. The obtained powder was successively added to methanol with vigorous shaking at 55 to 60 °C temperature. The extracts were made to dry by using rotary evaporator under reduced pressure. The extract was preserved at 4°C for further use.

Anthelmintic Activity

Experimental worms

All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil (Vatiary, Chittagong, Bangladesh) and identified by local zoologist. *Pheretima posthuma* washed with water to remove all fecal matters.

Administration of Albendazole

Albendazole (10 mg/ml) was prepared by using 0.5% w/v of CMC as a suspending agent as administered as per the method of extract.

Administration of extract

The suspension of Methanolic extract of leaves of *M. denticulate* of different concentration (25,50,100 mg/ml) were prepared by using 0.5% w/v of CMC as a suspending agent and final volume was made

Table 1: Anthelmintic potency of methanolic extract of *M. denticulate*.

| Group | Concentration (mg/ml) | Time taken for Paralysis (P) in min. | Time taken for death (D) in min. |
|---|-----------------------|--------------------------------------|----------------------------------|
| Control (0.5% CMC) | - | - | - |
| Albendazole | 10 | 19.51±1.29 | 42.25±2.26 |
| Methanolic extract of <i>M. denticulata</i> | 25 | 32.36±3.19 | 63.59±2.12 |
| | 50 | 26.17±1.30 | 50.08±0.45 |
| | 100 | 21.26±1.06 | 44.23±0.58 |

All Values represent Mean± SD; n=6 in each group. Comparisons made between standard versus treated groups, P<0.05 was considered significant

Discussion

In the current study anthelmintic tests were performed in vitro. One of the main advantages of analyzing the biological properties of plant extracts in vitro is that, the process is cost effective and includes rapid turnover allowing the screening of plants at large scale.¹⁵

Some of the phyto-constituents like alkaloids, tannins, phenols etc. may be accountable to have a significant anthelmintic activity. It was reported that, tannins may to interfere with energy generation of worms by uncoupling oxidative phosphorylation or they binds to the

up to 10 ml for respective concentration. Albendazole was used as standard. Groups of approximately equal size worms consisting of two earthworms individually in each group were released into in each 10 ml of the desired concentration of drug and extracts in the petridish.

Experimental Design

The anthelmintic activity was performed according to the method.¹⁴ On adult Indian earth worm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Pheretima posthuma* was placed in petridish containing three different concentrations (25, 50 &100 mg/ml) of methanolic extract of leaves of *M. denticulata*. Each petridish was placed with 2 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the timely death of the worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with Reference compound Albendazole (10 mg/ml) treated samples.

Statistical analysis

Values were expressed as mean ± Standard error for mean (± SEM). P < 0.05 - 0.01 were considered as statistically significant.

Result

From the observation made extracts exhibited more potent activity at higher concentration (100 mg/ml) against *Pheretima posthuma* (earthworm). Evaluation of anthelmintic activity was compared with reference standard Albendazole. The methanolic leaves extract of *M. denticulata* produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death as shown in Table 1. The activity was concentration dependent of the extract.

In the present study, it was observed that the methanolic extract of *M. denticulata* have exhibited positive and potent response like Albendazole.

free protein of the gastrointestinal tract of the worms and lead to death.¹⁶ In another study, alkaloids were reported to cause paralysis of the worms by acting on its central nervous system.¹⁷ The literature review ensured that, *Macaranga* genus have both tannins and alkaloids.¹⁸ The prime effect of albendazole is to cause a flaccid paralysis of the worm which results in expulsion of the worm by peristalsis. Albendazole acts to increase chloride ion conductance of worm muscle membrane which produces hyperpolarization and excitability reduction that leads to muscle relaxation and flaccid paralysis of worms.¹⁹

Conclusion

The results of the present study clearly indicated that the methanol extract of *M. denticulata* did produce anthelmintic activity against Indian earthworm *Pheretima posthuma*.

The plant possesses significant Anthelmintic activity at 100 mg/ml concentration measured by the time taken for paralyse / death of the earth worms. The current investigation leads to conclusion that the leaves of *M. denticulata* have potent anthelmintic activity when compared with the conventionally used drug. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminthes. Further studies using *in vivo* models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rational for the use of *M. denticulata* as an anthelmintic drug.

Conflict of interest

We declare that we have no conflict of interest.

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