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Antibacterial activity on some gram positive and gram negative bacteria and antihelmintic activity on *Tubifex tubifex* worm of methanol extract of *Macaranga denticulata* (MUELL. ARG.) bark.

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ABSTRACT

The aim of the present study was to evaluate antibacterial activity on three Gram positive and four Gram negative bacteria and antihelmintic activity on *Tubifex tubifex* worm of methanol extract of *M. denticulata* (Muell. Arg.) bark. The extract showed zone of inhibition in highest concentration of 900 µg/ml against Gram-positive bacteria *Staphylococcus aureus* (8mm), *Bacillus subtilis* (12mm), *Bacillus cereus*(8mm)and Gram-negative bacteria *Salmonella typhi* (12mm), *Salmonella paratyphi* (Nil), *Escherichia coli* (13mm), *Pseudomonas aeruginosa* (11mm).*M. denticulata* bark extract exhibited strong anthelmintic activity *in vitro*. Where it paralyzed (6.52±0.53 min) and produced death (12.36±0.81 min) of the *Tubifex tubifex* at 20 mg/ml dose near the value of the standard, Levamisole (3.3±0.38 min and 6.5±0.76 min) at 1 mg/ml. So methanol extract of *M. denticulata* barks showed moderate antibacterial activity and well antihelmintic activity

Key words: *Macaranga denticulata*; bark; disk diffusion method; antihelmintic activity; *Tubifex tubifex* worm.

INTRODUCTION

Nature has been a wellspring of medicinal agents for a great many years and a striking number of current medications have been confined from natural source, numerous in view of their utilization in traditional medicines or phytomedicines. As indicated by the World Health Organisation (WHO) [1,], 65% of the world's populations have incorporated ethnomedicine in their essential health care practice. In some African and Asian countries, 80% of the population depends on traditional medicine for primary health care and about 70% of population in the developed world has used alternative or complementary medicines [2]. In India, traditional healers use about 2500 plant species as a regular source of medicine to treat different diseases [3]. On the other hand, just a little extent of therapeutic plants (10%) has been studied scientifically [4]. For example, of the plants used to treat microbial infections, an estimated 6% have been screened for specific anti-microbial activities and only a small proportion of these have been studied phytochemicals to identify the active constituents and/or blends [5, 6].

Helminth diseases are among the commonest diseases in man, influencing a vast extent of the world's population. In developing countries they represent a noteworthy risk to general wellbeing and add to the predominance of lack of healthy sustenance, anemia, eosinophilia, and pneumonia. Anthelmintics are medications that either kill or expel infesting helminths and the gastrointestinal tract is the abode of many helminthes, although some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is infrequently lethal, but is a major cause of morbidity [7, 8, 9].

The control of this disease has been in light of the utilization of anthelmintics, however because of the advancement of resistance it seems that the efficacy

of some chemical drugs has decreased [10, 11]. The utilization of plants with anthelmintic activity may be an alternative to fluke control, given the colossal assorted qualities of barks. The chance of discovering bioactive mixes with hostile to fluke properties essentially increments in light of the fact that, secondary metabolites (SM) are the most important compounds as new alternatives for parasite control. Some SM such us alkaloids, saponins, skimmiarins A and C, tannins, flavonoids, terpenes (mono, di and sesquiterpenes) have been shown to be active against an extensive variety of parasites [12].

Tubifex tubifex is a cosmopolitan naidid annelid sensu representing one of the major components of the benthic fauna in freshwater communities [13]. Also present in polluted waters, *Tubifex tubifex* is widely used in laboratories for ecotoxicology research [14] and as a model organism for the study of annelid development [15]. *Tubifex tubifex* is characterized by considerable variability in its morphological features [16] and by a mixed reproductive strategy, with parthenogenesis [17], self-fertilization [18], and biparental reproduction through cross-mating [19].

M. 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 [20]. In the mountains of Northern Thailand, *M. denticulata* is used as a fallow enriching species by Karen hill tribe farmers [21]. In folk medicine, traditional healers use fresh or dried leaves of some Macaranga species to treat swellings, cuts, sores, boils and bruises [22,23]. Methanol extract of *M. denticulata* leaves examined for anthelmintic activity on *Pheretima posthuma* [24]. This plant has also thrombolytic and cytotoxic activities [25].

The aim of the present study was to identify the antibacterial and anthelmintic activity of methanol extract of barks of *M. denticulata* bark.

MATERIALS AND METHODS

Collection and identification of plant material: The barks of *M. denticulata* were collected from Comilla cantonment hilly area in November, 2014 then identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Preparation of Extract: The collected barks were washed thoroughly with distill water, chopped, air dried for a week and pulverized in electric grinder (Miyako 3 in One blender, Miyako, China). The powder (500 g) obtained was successively extracted in methanol (55-60ºC) for 10 days with a 2 days interval. The filtrated supernatant was evaporated to dry using a rotary evaporator (RE200, BB Sterling, UK) under reduced pressure. The crude extract (22.5 g, blackish green semisolid, yield 4.5%) was preserved at 4ºC until further use.

Chemicals: All chemicals used were of analytical reagent grade. Methanol was purchased from Merck, Germany. Kanamycin (30ìg/disc, Oxoid, England) was used as a standard antibiotic disc. Levamisole was purchased from ACI Limited, Bangladesh.

***In vitro* Antibacterial activity**

Bacterial strains: Seven bacterial species, gram-positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* gram-negative *Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa*. These microbes were obtained from the department of Pharmacy International Islamic University Chittagong.

Media preparation and maintenance of bacteria: All of the bacterial strains were grown and maintained on Nutrient agar (Merck, India) media at 37 °C and pH (7.4±0.2). The bacteria were sub cultured overnight.

Preparation of concentration: In the study of the antibacterial activity, the extract was diluted in methanol. The concentrations to the extract given in Table1 are expressed in terms of mg/ disk.

Preparation of discs: The sample discs of about 5 mm in diameter were cut by punching machine (Kangaro 280) from Whatman No. 1 filter paper (Made in China). The discs were taken in a Petri dish and sterilized by autoclave (Daihan Labtech Co., LTD Model: LIB-060M: ISO 9001 certified) dried in oven at 180°C.

Antimicrobial screening by disk diffusion technique: The antibacterial assay was performed by using the disc diffusion method [26, 27]. Seven pathogenic bacteria were used as test organisms for antibacterial activity of *M. denticulata* extract. The test organisms were inoculated on 10 ml previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile Petri dish in an aseptic condition using a sterile loop. Prepared sample and standard solutions were applied to the corresponding Petri dish. The plates were incubated for overnight at 37^0 C. After proper incubation, clear zone of inhibition around the point of application of sample solution were measured which is expressed in millimeter (mm).

***In-vitro* Anthelmintic Assay:** The anthelmintic activity of extract of bark of *M. denticulata* was carried out as per the procedure [28] with some minor modifications. The aquarium worm *Tubifex tubifex* were used in the present study because it has anatomical similarity and belongs to the same group of intestinal worm i.e. annelida.[29, 30, 31] The worms were collected from the local market of Chittagong, average size of worms 2-2.5 cm in length were used for the study. The standard drug levamisole and three different concentrations of different extracts (5, 10 and 20 mg/ml) in double distilled water [32] were prepared just before experiment and used for the study of anthelmintic activity. One group was composed of water and it was considered as controlled group. The anthelmintic activity was determine at two different stage 'time of paralysis' and 'time of death' of the worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors. [33] Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had stopped [34].

Statistical analysis: The results were expressed as mean (integer) (n=3) from triplicate experiment for zone of inhibition from triplicate experiments for Antibacterial activity. The data on *in vitro* study of Antihelmintic activity was reported as mean ± S.E.M. (n = 3). Data were analyzed using one way factorial ANOVA tests using SPSS followed by Dennett's tests on each group except control for anthelmintic. $P<0.05$, $P<0.01$ and $P<0.001$ were considered as statistically significant. Statistical program used was SPSS and GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) and Microsoft Excel, 2007, used for graphical presentation.

RESULTS

***In vitro* Antibacterial activity:** Antibacterial activity of the extract was tested against seven pathogenic bacteria and were compared with the standard antibiotic Kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm) showed in table 1. All the found zone of inhibition are significant (P<0.001) as compared with standard Kanamycin (30µg/disc).

Table 1: Antibacterial activity of Methanol extract of *M. denticulata* bark

Name of the bacteria	Diameter of zone of inhibition (mm)			
	Methanol extract of *M. denticulata* bark			Standard (Kanamycin) (30µg/disc)
	500µg/disc	700µg/disc	900µg/disc	
Gram Positive				
Staphylococcus aureus	0	0	8*	30
Bacillus subtilis	8*	9*	12*	27
Bacillus cereus	0	7*	8*	28
Gram Negative				
Salmonella typhi	8*	10*	12*	33
Salmonella paratyphi	0	0	0	30
Escherichia coli	7*	12*	13*	28
Pseudomonas aeruginosa	9*	10*	11*	27

Values are mean (integer) inhibition zone (mm) of three replicates. The superscripted (*) value has significantly (*$P < 0.001$) as compared with standard (Kanamycin) in same row.

Antihelmintic activity: Results of study were recorded as shown in Table 2 and Figure 1 as in the form of time required getting consecutive attacks of paralysis and at the end time required for complete death of worm. From the observations made, higher concentration of the extract produced paralytic effect much earlier and the time to death was shorter for all worms. From the above study it was seen that this

methanol extract showed dose dependent anthelmintic activity as compared to a standard drug Levamisole. Different treatment showed different anthelmintic activity. Methanol extract of *M. denticulata* bark showed very good anthelmintic activity. Where it paralyzed (6.52±0.53min; P<0.001) and produced death (12.36±0.81 min; P<0.001) of the *Tubifex tubifex* at highest 20 mg/ml dose, which near the value of the standard (paralysis time, 3.3±0.38 min and produced death, 6.5±0.76 min) at 1 mg/ml dose.

Table 2: Anthelmintic activity of methanol extract of bark of *M. denticulata*.

Treatment	Time taken for paralysis (min)	Time taken for Death (min)
Control(Water)	0	0.00
Levamisole (1 mg/ml)	3.3±0.38	6.5±0.76
M. denticulata (20 mg/ml)	6.52±0.53[c]	12.36±0.81[c]
M. denticulata (10 mg/ml)	10.16±0.88[b]	21.47±1.24[b]
M. denticulata (5 mg/ml)	17.42±1.04[b]	48.19±1.45[b]

Values are mean ± SEM, (n = 3); [a]*P* < 0.05, [b]*P* < 0.01 and [c]*P* < 0.001, Dennett's test as compared to positive control (Levamisole, 1 mg/ml). Statistical representation of the effective paralysis and dead time by methanol extract of *M. denticulata* bark, positive anthelmintic control (Levamisole ,1 mg/ml) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis by using SPSS for windows, version 16.0.

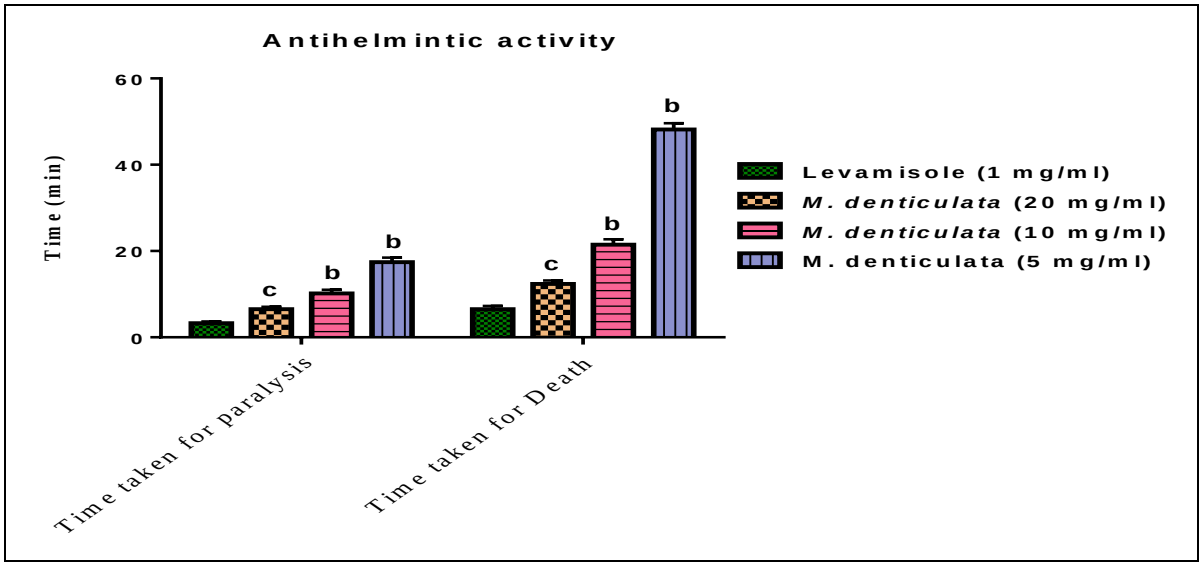

Figure 1: Anthelmintic activity of methanol extract of bark of *M. denticulata*.

Values are mean ± SEM, (n = 3); [a]*P* < 0.05, [b]*P* < 0.01 and [c]*P* < 0.001, Dennett's test as compared to positive control (Levamisole, 1 mg/ml). Statistical representation of the effective paralysis and dead time by methanol extract of *M. denticulata* bark, positive anthelmintic control (Levamisole ,1 mg/ml) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis by using SPSS for windows, version 16.0.

DISCUSSIONS

The knowledge of medicinal property of plants has been accumulated in the course of many centuries. The local inhabitants have inherited rich traditional knowledge on the use of many plants or plant parts for treatment of common disease. Medicinal plants provide accessible and culturally relevant sources of primary health care. The remedies based on these plants often have minimal side effect [35]. The bioactive substances in plants are produced as secondary metabolites, which may not only be developmental stage specific but also organ and tissue specific. While plant leaf, bark and root extracts have been widely evaluated for bioactive compounds, screening of plant flower has not been extensive. Secondary metabolites belonging to polypeptide and nonribosomal peptide families constitute a major class of natural products with diverse biological functions and they have a variety of pharmaceutically important properties. Experimental studies have shown that the

biosynthetic mechanism for polypeptide and nonribosomal peptides involves multi-functional megasynthases [36, 37, 38].

The antibacterial activity of *M. denticulata* barks were carried out. Methanol extract shows an antibacterial activity against the human pathogens such as gram-positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and gram-negative *Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa*. This extract of barks has shown the activity, which also showed by its methanol extract of leaves.

In this study, antihelmintic activity of *M. denticulata* barks examined on *Tubifex tubifex*. Different dose of this extract showed good antihelmintic activity, which proved that previous study on its leaves for antihelmintic activity on *Pheretima posthuma* is real. So this plant must possessed antihelmintic activity. So present studies suggested that methanol extract of *M. denticulata* barks possessed moderate antibacterial activity and strong antihelmintic activity.

CONCLUSIONS

This study delineates that methanol extract of *M. denticulata* barks possesses moderate antibacterial effect. Since, this extract of *M. denticulata* showed low zone of inhibition against several bacteria. On the other hand, it showed strong antihelmintic activity against *Tubifex tubifex*. So methanol extract of *M. denticulata* barks showed well antihelmintic activity. 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.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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