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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *MORINGA OLEIFERA* STEM BARK.

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ABSTRACT

Petroleum ether, chloroform, ethyl acetate and carbon tetrachloride extracts of *Moringa oleifera* stem bark were studied for their antimicrobial activities against eleven human pathogenic bacteria (*Shigella dysenteriae*, *S. sonnei*, *Salmonella typhi*, *S. paratyphi*, *Bacillus subtilis*, *B. megaterium*, *B. cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Vibrio cholerae*) and four human pathogenic fungi (*Aspergillus niger*, *A. ochraceus*, *A. ustus* and *Candida albicans*) using disc diffusion and poisoned food method, respectively. Chloroform and ethyl acetate extracts exhibited moderate to good antibacterial and antifungal activity against all the pathogens tested. The ethyl acetate extract exhibited the largest zone of inhibition (21 mm in diameter with 2000 µg/disc extract) against *S. sonnei*. The highest inhibition of fungal radial mycelial growth (52.00% with 100 µg extract/ml medium) was recorded against *C. albicans* with ethyl acetate extract. The ethyl acetate extract exhibited the lowest MIC (750 µg/ml) against *B. megaterium*, *S. dysenteriae*, *V. cholerae* and *E. coli*. For fungi, the lowest MIC was 500 µg/ml against *C. albicans* with crude extract of ethyl acetate.

Key words: Antimicrobial activity, *Moringa oleifera*, crude extract, stem bark.

INTRODUCTION

Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. Still most of the people of rural and urban areas of the world are dependent on plants for the treatment of infectious diseases. The Ayurvedic and Unani system of medicine in the subcontinent of India continue to use them. The natural products have been interesting and important sources of biologically active (antimicrobial) substances and the major sources of which are still left undiscovered. Much of the works

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have been done in India and other countries. But a very little work is done in this field from Bangladesh. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis 1994).

This situation forced scientists to search for new antimicrobial substances from various sources. Secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity (Ahmed *et al.* 2002, Aureli *et al.* 1992, Rahman *et al.* 1999). So, there is a continuing need for new antibacterial and antifungal agents since none of the available drugs is free from adverse effects and limitation. Now-a-days, the natural products have been accepted as important sources of biologically active (antimicrobial) substances and the major sources of which are yet to be another discovered.

Moringa oleifera Lamk. family Moringaceae is a small to medium sized tree with long stringing branches and long cylindrical fruits, planted commonly all over the country. The plant principally contains alkaloids, protein, vitamin, mineral, fixed oil, fatty acid and many carbohydrates, etc. (Ghani 1998). Root is used as stimulant in paralytic affection, intermittent fever epilepsy and as carminative, stomachic, diuretic, cardiac and circulatory tonic, rubefacient in palsy and chronic rheumatism (Ghani 1998).

MATERIALS AND METHODS

Collection and extraction of plant material

Stem Bark of *Moringa oleifera* was collected in fresh condition from Chittagong University campus, Chittagong, Bangladesh. The collected and cleaned samples were cut into small pieces (1-2 cm), dried in air to make it suitable for grinding. The samples were ground to fine powder mechanically and 20g of the dried powder was kept steeped 72 hours in petroleum ether, chloroform, ethyl acetate and carbon tetrachloride. The extracts thus obtained separately were filtered, centrifuged at 5000 rpm for 20 minutes and concentrated to a gummy material under reduced pressure at 50⁰C by rotary vacuum evaporator. The gummy materials were then collected in a small vial, dried as usual. Thus crude extracts were obtained.

Test organisms

The crude extracts obtained from *M. oleifera* were tested for their antibacterial activity against eleven human pathogenic bacteria, viz., *Shigella*

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dysenteriae AE 14396, *S. sonnei* CRL.(ICDDR,B), *Salmonella typhi* AE 14612, *S. paratyphi* AE 14613, *Bacillus subtilis* BTCC 17, *B. cereus* BTCC 19, *B. megaterium* BTCC 18, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* CRL(ICDDR'B), *Escherichia coli* ATCC 25922 and *Vibrio cholerae* AE 14748, and four pathogenic fungi viz., *Aspergillus niger* BTCC 504, *A. ustus* BTCC 503, *A. ochraceus* BTCC 515 and *Candida albicans* BTCC 493.

Determination of antibacterial activity

The *in vitro* sensitivity of the bacteria to the test materials was done by disc diffusion method (Bauer *et al.*1966). Sterilized paper discs of 4 mm in diameter and Petri dishes of 150 mm in diameter were used throughout the experiment. The autoclaved Mueller-Hinton agar medium, cooled to 45⁰C, was poured into sterilized Petri dishes to a depth of 3 to 4 mm and after solidification of the agar medium, the plates were transferred to an incubator at 37⁰C for 15 to 20 minutes to dry off the moisture that developed on the agar surface. The plates were inoculated with the standard bacterial suspensions (as of McFarland 0.5 standard) followed by spread plate method and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were treated separately with 20µl (2000 µg /disc) from 10% ethanolic solution of each extract using a micropipette, dried in air under aseptic condition and were placed at equidistance in a circle on the seeded plate. A control plate was also maintained in each case without any test material. These plates were kept for 4-6 hours at low temperature (4-6⁰C) and the test materials diffused from disc to the surrounding medium by this time. The plates were then incubated at 35±2⁰C for 24 hours to allow maximum growth of the organisms. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions in millimeter. Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic ampicillin [20µg/disc, BEXIMCO Pharma Bangladesh Ltd.].

Determination of antifungal activity

The *in vitro* antifungal activity of the crude extracts of the plant was determined by poisoned food technique (Miah *et al.* 1990). Ten percent ethanolic solution (w/v) of the extract was mixed with sterilized melted Sabouraud agar medium to obtain the desired concentration (100 µg/ml) and this was poured in sterilized Petri dishes. At the center of each plate, 5 days old fungal mycelial block (4 mm in diameter) was inoculated and incubated at 27⁰C. A control set was also maintained in each experiment. Linear mycelial growth of fungus was

measured after 3-5 days of incubation. The percentage inhibition of radial mycelial growth of the test fungus was calculated as follows:

$$I = \frac{(C - T)}{C} \times 100$$

Where, I = Percentage of inhibition; C = Diameter of the fungal colony in the control.

T = Diameter of the fungal colony in treatment.

All the results were compared with the standard antifungal antibiotic nystatin [100µg/ml medium, BEXIMCO Pharma Bangladesh Ltd.].

Determination of MIC:

MICs of the crude extracts were determined by broth macrodilution method (Jones *et al.* 1985). The crude extract was dissolved in 30% dimethyl sulfoxide (DMSO) to obtain 10% (w/v) solution. For MIC test, the extract was first diluted in sterilized Mueller-Hinton broth to the highest concentration (0.8% or 8000 µg/ml) and then other dilutions were prepared at concentrations of 2500 µg/ml, 2000 µg/ml, 1500 µg/ml, 1000 µg/ml, 750 µg/ml, 500 µg/ml and 250 µg/ml in screw capped tubes containing Mueller-Hinton broth. Bacterial and fungal suspensions of test organisms were prepared in sterilized Mueller-Hinton broth and potato dextrose broth, respectively. Then 1 ml of this suspension was added to each sterilized screw capped tube containing 1 ml of the compound suitably diluted in the sterilized broth medium to give a final volume of 2 ml. Culture medium without the samples and others without microorganisms were used in the tests as control. Tubes were incubated at 35⁰C for 20-24 hours and growth was indicated by turbidity. MIC was the lowest concentration of antimicrobial agent that completely inhibited growth of the organism in the tubes.

RESULTS AND DISCUSSION

The crude extracts (petroleum ether extract, chloroform extract, ethyl acetate extract and carbon tetrachloride extract) obtained from *Moringa oleifera* were screened for their antibacterial activity against eleven human pathogenic bacteria and compared to that of standard antibacterial antibiotic ampicillin. The results of the sensitivity test are presented in Table 1. Among the four extracts, only chloroform and ethyl acetate extracts exhibited good antibacterial activity against all the bacterial strains tested. But the petroleum ether and carbon tetrachloride extracts did not show any activity against the bacterial strains. The chloroform extract exhibited zones of inhibition from 10 to 17 mm in diameter. In case of ethyl acetate extract, the zones of inhibition varied from 08 to 21 mm in diameter. The ethyl acetate extract exhibited the largest zone of inhibition (21 mm

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in diameter with 2000 µg/disc extract) against *Shigella sonnei*. The ampicillin (20µg/disc) was also found to be active against all the bacteria tested. Similar antibacterial activity of other plant extracts has been reported previously (Ahmed *et al.* 1999, Brantner and Grein 1994, Rahman *et al.* 1998, Rojas *et al.* 1992, Sarker *et al.* 1991).

TABLE 1: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS FROM *MORINGA OLEIFERA*

Name of bacteria	Diameter of inhibition zone in mm. (Crude extract 2000µg/disc)				Ampicillin* 20µg/disc
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethyl acetate	
<i>Bacillus subtilis</i>	-	-	10	08	19
<i>B. megaterium</i>	-	-	13	10	16
<i>B. cereus</i>	-	-	11	09	18
<i>Staphylococcus aureus</i>	-	-	16	18	22
<i>E. coli</i>	-	-	13	18	10
<i>Vibrio chlorae</i>	-	-	17	17	15
<i>Shigella dysenteriae</i>	-	-	13	08	20
<i>S. sonnei</i>	-	-	16	21	19
<i>Salmonella typhi</i>	-	-	11	14	20
<i>S. paratyphi</i>	-	-	12	13	17
<i>Pseudomonas aeruginosa</i>	-	-	14	12	-

*Standard antibacterial antibiotic

(-) minus mean no inhibition

The antifungal activity of crude extract (100 µg/ml medium) against four human pathogenic fungi were studied and compared with that of standard antifungal antibiotic nystatin. Chloroform extract, and ethyl acetate extracts of *M. oleifera* inhibited the radial mycelial growth of all the test fungi at the concentration of 100 µg/ml medium. The highest inhibition (52.00%) of fungal radial mycelial growth was recorded against *C. albicans* with ethyl acetate extract at a concentration of 100 µg/ml medium (Table 2). Antifungal antibiotic nystatin (100µg/ml medium) exhibited inhibitions of radial mycelial growth of all the four fungi, but it was much better active against the fungi tested compared to that of

the crude extracts. Similar antifungal activities on plant extracts of other plants have also been previously reported (Anwar *et al.* 1994, Miah *et al.* 1990, Naidu and John 1981, Shetty and Shetty 1987, Stange *et al.* 1993).

The MIC values of the crude extracts obtained from *M. oleifera* are summarized in Table 3. Ethyl acetate extract exhibited comparatively the lowest MIC values against bacterial pathogens which were 2000 µg/ml, 750 µg/ml, 1500 µg/ml, 2000 µg/ml, 750 µg/ml, 750 µg/ml, 750 µg/ml, 1500 µg/ml, 1500 µg/ml, 1500 µg/ml and 1500 µg/ml against *Bacillus subtilis*, *B. megaterium*, *B. cereus*, *S. aureus*, *E. coli*, *Vibrio cholerae*, *Shigella dysenteriae*, *S. sonnei*, *Salmonella typhi*, *S. paratyphi* and *Pseudomonas aeruginosa*, respectively (Table 3). The lowest MIC (750 µg/ml) was recorded against *B. megaterium*, *S. dysenteriae*, *E. coli* and *Vibrio cholerae* with ethyl acetate extract. In case of fungi, ethyl acetate extract exhibited the lowest MICs (500 µg/ml) compared to that of other extracts, was recorded against *C. albicans*.

TABLE 2. ANTIFUNGAL ACTIVITY OF THE CRUDE EXTRACTS FROM *M. OLEIFERA*.

Name of fungi	Percentage inhibition of fungal mycelial growth ^a (100 µg/ml medium)				
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethyl acetate	Nystatin*
<i>Aspergillus niger</i>	-	-	23.5	24.5	62.50
<i>A. ochraceus</i>	-	-	49.0	49.0	68.00
<i>A. ustus</i>	-	-	49.5	48.0	72.00
<i>Candida albicans</i>	-	-	50.0	52.0	78.50

* Standard antifungal antibiotic;

^aGrowth measured- radial growth in cm

Natural products from plants are known to control some infectious diseases. The finding of the active compound may be interesting in the search for new efficacious and safe antimicrobial agent against a variety of pathogenic bacteria and fungi. The present investigation confirms that there are antibacterial and antifungal properties in the crude extract of *M. oleifera* stem bark. However, it is important to point out that crude extract such as this needs to be further processed to obtain pure compound(s) which can then be tested for antimicrobial activity.

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TABLE 3. MICs OF CRUDE EXTRACTS FROM *M. OLEIFERA*.

Bacteria / fungi	MIC (Crude extract µg/ml medium)			
	Petroleu m ether	Carbon tetrachloride	Chloroform	Ethyl acetate
A. Bacteria:				
<i>Bacillus subtilis</i>	NF	NF	1500	2000
<i>B. megaterium</i>	NF	NF	1000	750
<i>B. cereus</i>	NF	NF	1500	1500
<i>Staphylococcus aureus</i>	NF	NF	2000	2000
<i>E. coli</i>	NF	NF	1000	750
<i>Vibrio cholerae</i>	NF	NF	1500	750
<i>Shigella dysenteriae</i>	NF	NF	1500	750
<i>S. sonnei</i>	NF	NF	1000	1500
<i>Salmonella typhi</i>	NF	NF	1500	1500
<i>S. paratyphi</i>	NF	NF	1000	1500
<i>Pseudomonas aeruginosa</i>	NF	NF	1500	1500
B. Fungi:				
<i>Aspergillus niger</i>	NF	NF	2000	1500
<i>A. ochraceus</i>	NF	NF	2000	750
<i>A. ustus</i>	NF	NF	1500	750
<i>Candida albicans</i>	NF	NF	750	500

NF – not found up to 2500 µg/ml

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