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In vitro Antibacterial and Antifungal Potentials of Petroleum Ether Extract of *Moringa oleifera*

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ABSTRACT

The petroleum ether extract of stem barks of *Moringa oleifera* Lam. was evaluated for antibacterial and antifungal activities. Ten bacterial strains were used in the study for antibacterial activities while six species of fungi were also bioassayed for their response to the extract. Agar disc diffusion method was used for antibacterial and antifungal assay of the petroleum ether extract at 500 μg disc^{-1}. The results were also compared with the standard drugs, amoxicillin (10 μg disc^{-1}) and griseofulvin (10 μg disc^{-1}). The obtained results showed inactivity of the petroleum ether extract to all of the tested bacterial and fungal strains.

Key words: *Moringa oleifera,* petroleum ether, antibacterial activity, antifungal activity

INTRODUCTION

The plant kingdom is considered as a storehouse of traditional medicines that may contribute to development of new medicines for various health problems of human beings. *Moringa oleifera* Lam. belonging to the family of Moringaceae is commonly known as drumstick tree, sajiwan, saijhan, kaai and sajna. This medicinal plant is considered as a rich source of nutrition and natural energy. In addition to this, different pharmacological activities such as antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antidiabetic and hepatoprotective have been revealed from different parts of the plant (Paliwal *et al*., 2011). N-benzyl S-ethyl thioformate, an aglycon of deoxy-niazimicine), isolated from the chloroform fraction of ethanolic extract of *Moringa oleifera* showed more *in vitro* antibacterial activity against *Shigella boydii, Shigella dysenteriae* and *Staphylococcus aureus* with zone of inhibition ranging from 9 to 13 mm in comparison with the crude chloroform extract. Moreover, the isolated compound possessed moderate antifungal activity against some fungal strains such as *Candida albicans* and *Aspergillus flavus* (Nikkon *et al*., 2003). The extract of the plant also exerted its protective effects by reducing liver lipid peroxides and increasing antioxidants (Kumar and Pari, 2003). The leaves and pods of *Moringa oleifera* also possessed different amount of minerals such as Ca, Mg, K, Mn, P, Zn, Na, Cu and Fe. They might be used as important dietary mineral supplements for their high mineral contents (Aslam *et al*., 2005).

In addition to this, *Moringa oleifera* contained higher contents of proteins (40.19%), lipids (41.58%), carbohydrate (9.11%), phytate (10.18 mg/100 g), hydrogen cyanide (0.58 mg/100 g) and saponin (2.052 mg/100 g) (Anhwange *et al*., 2004). Crude α-amylase enzyme obtained from the

seeds of the plant was used to hydrolyze starch (Dahot *et al*., 2001) and molecular weight of amylase from healthy *Moringa oleifera* was 59 kDa. A single polypeptide chain was found in the purified enzymes (Rahman *et al*., 2000). Siddiq *et al*. (2005) reported the antioxidant properties of the plant leaves due to the presence of flavonoids, polyphenolics and tocopherol contents. Moreover, they served as natural sources of antioxidant and nutraceuticals. The plant revealed a dose dependent DPPH free radical scavenging and reducing power capabilities (Ogbunugafor *et al*., 2011). The objectives of the present study were to investigate the antibacterial and antifungal activities of petroleum ether extract of the stem barks of *Moringa oleifera*.

MATERIALS AND METHODS

Preparation of plant extract: The stem bark of *Moringa oleifera* was collected from Patiya, Chittagong on August in 2009 and the plant material was identified by Bangladesh Forest Research Institute (BFRI), Chittagong using standard taxonomical method. After collection of the plant materials, the stem barks were washed with tap water to remove the impurities and were cut into small pieces. They were subjected to air dry for two weeks. The air dried samples were crushed to make powder which was then soaked into two aspiratory bottles with petroleum ether. The mixture was extracted six times with petroleum ether and the extracts were filtered and concentrated under reduced pressure at 50°C through rotary evaporator to yield a dense residue. Finally, the extracts were stored at an air-tight container and kept at 4°C before use.

Bacterial strains: Five gram +ve bacterial strains such as *Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Staphylococcus pyogenes, Staphylococcus aureus* and 5 gram -ve bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella typhi* and *Vibrio cholerae* were included in the study. All of the bacterial samples isolated from clinical cases were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

Fungal species: Six fungal strains such as *Alternaria* spp., *Botryodiplodia* spp., *Trichomonas* spp., *Curvularia* spp., *Fusarium* spp. and *Macrophomina* spp. were used for antifungal activity of the extract. These strains were also collected from ICDDR, B.

Antimicrobial and antifungal assay: The study of *in vitro* antibacterial and antifungal activity of the petroleum ether extract of the stem bark of the plant was done by agar disc diffusion method (Bauer *et al*., 1966) with minor modifications.

Standard drugs: Amoxicillin and griseofulvin were used as reference standard drugs for comparison of the antibacterial and antifungal assay, respectively. The concentration used for both of the standard drugs was 10 μg $disc^{-1}$ while 500 μg $disc^{-1}$ of the plant extract was used for investigation of antimicrobial and antifungal properties.

Statistical analysis: Three replicates of each sample were used for statistical analysis and the results of the experiment were expressed as Mean±Standard Deviation (SD).

RESULTS AND DISCUSSION

The results for antibacterial activity of the stem bark extract of the plant are shown in Table 1 and the activity was measured in terms of zone of inhibition in mm. Results obtained from

Table 1: Antibacterial activity of the petroleum ether extract of the stem barks of *Moringa oleifera*

		Diameter of zone of inhibition (mm)	
Bacterial strains	Blank	Petroleum ether extract of *Moringa oleifera* (500 μg disc^{-1})	Standard drug, amoxicillin (10 μg disc^{-1})
Gram positive			
B. megaterium	-	-	27.07±0.33
B. subtilis	-	-	15.25±0.54
B. cerus	-	-	12.45±0.29
S. pyogenes	-	-	25.02±0.39
S. aureus	-	-	16.08±0.43
Gram negative			
E. coli	-	-	15.97±0.16
P. aeruginosa	-	-	12.52±0.34
S. dysenteriae	-	-	21.72±0.61
S. typhi	-	-	14.70±0.21
V. cholerae	-	-	16.07±0.37

Data were represented as Mean±SD of triplicate determination, -: No inhibition

Table 2: Antifungal activity of the petroleum ether extract of the stem barks of *Moringa oleifera*

		Diameter of zone of inhibition (mm)	
Fungal strains	Blank	Petroleum ether extract of *Moringa oleifera* (500 μg disc^{-1})	Standard drug, griseofulvin (10 μg disc^{-1})
Alternaria spp.	-	-	26.57±0.42
Botryodiplodia spp.	-	-	19.08±0.31
Colirio trichomonas	-	-	-
Curvularia spp.	-	-	36.00±0.82
Fusarium spp.	-	-	31.92±0.72
Macrophomina spp.	-	-	37.78±0.61

Data were represented as Mean±SD of triplicate determination, -: No inhibition

the *in vitro* antibacterial activity showed that the petroleum ether extract of the plant showed no antibacterial activity against either gram (+) or gram (-) bacteria. Even no antifungal activity was observed at 500 μg disc^{-1} of the plant extract which are shown in Table 2. Saadabi and Abu Zaid (2011) reported that the petroleum ether extract of the seeds of *Moringa oleifera* did not show any antibacterial activity against the tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*. No inhibition was also found against the fungal strains such as *Aspergillus niger* and *Candida albicans*. They also reported that the methanolic extract of the plant seeds showed little inhibition to the tested bacterial strains and superior activity was identified by the aqueous extract in inhibiting the bacterial growth. N-benzyl S-ethyl thioformate, an aglycon of deoxy-niazimicine, isolated from the chloroform fraction of ethanolic extract of *Moringa oleifera* showed more *in vitro* antibacterial activity against *Shigella boydii, Shigella dysenteriae* and *Staphylococcus aureus* with zone of inhibition ranging from 9 to 13 mm in comparison with the crude chloroform extract. Moreover, the isolated compound possessed moderate antifungal activity against some fungal strains such as *Candida albicans* and *Aspergillus flavus* (Nikkon *et al*., 2003).

The present study results showed that the petroleum ether extract of the stem barks of *Moringa oleifera* was found to be inactive against all of the tested microorganisms. Similarly, no

susceptibility to the fungal strains was detected at 500 μg $disc^{-1}$ of the plant extract. Finally, it was concluded that the absence of antibacterial and antifungal activities of the petroleum ether extract of the stem barks of the plant may be due to polar characteristics of the bioactive compounds which were not extracted by the petroleum ether.

CONCLUSION

Further research is necessary to isolate and elucidate the structures of bioactive components responsible for antimicrobial and antifungal properties of the plant extract which may attribute to the development of new medicines.

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