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In vitro Antimicrobial Activity of Methanolic Extract of *Moringa oleifera* Lam. Fruits

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The present study was designed to investigate antibacterial and antifungal activity of Methanol extract from the fruits of *Moringa oleifera*. The extract were screened for antibacterial and antifungal activity by Disc diffusion method. The fruit extract of *Moringa oleifera* showed a broad-spectrum antibacterial activity and antifungal activity. The highest zone of inhibition was found at the concentration of 200 µg/disc for *Pseudomonas aeruginosa* (22 mm) and *Colletotrichum* Sp (14 mm). Methanol extract possessed moderate antibacterial activity against bacterial strains- *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera*, *Bacillus cereus*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Klebsiella species* and *Proteus species* and antifungal activity against pathogenic fungi- *Alternaria* SP, *Colletotrichum* SP, *Curvularia* sp and *Fusarium* SP.

Keyword: *Moringa oleifera*, Antibacterial and Antifungal.

1. Introduction

Moringa oleifera commonly referred as Moringa. It is an exceptionally nutritious vegetable tree with a variety of potential uses. These leaves have high medicinal value.^[1]

Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti tumor,^[2] antipyretic, antiepileptic, anti inflammatory, antiulcer,^[3] antispasmodic, diuretic,^[4,5] antihypertensive, cholesterol lowering, antioxidant, antidiabetic,

hepatoprotective, antibacterial and antifungal activities,^[6] and are being employed for the treatment of different ailments in the indigenous system of medicine.^[7]

In traditional medicine *Moringa oleifera* tree is known as a Miracle tree as almost every part of this tree possesses product useful for humans. The leaves and pods are eaten. The plant is also reported to be medicinally important and almost all parts of the *Moringa oleifera* tree are considered to possess medicinal properties and

are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant. Leaves are also known to have anti-oxidant properties and are known to cure hallucinations, dry tumors, hiccup and asthma.^[8] The root and bark are useful in treatment of heart complaints, eye disease, inflammation, dyspepsia and enlargement of spleen. The flowers are known to cure inflammation and muscle diseases. Seed oil is to be useful in the treatment of leprosy ulcers.

Richa Gupta *et al.*, (2005) reported chelating property against arsenic toxicity.^[9] A total of forty four compounds were isolated from the leaves of *Moringa oleifera* (Lam.).^[10] and four pure compounds were also isolated which showed a blood pressure lowering effect.^[11] Moringine and Moringinine were the two alkaloids isolated from the stem bark of *Moringa oleifera* (Lam.).^[12] Methanol extract of *Moringa oleifera* (Lam.) root showed central nervous system depressant action was reported by Gupta *et al.*, (1999)^[13]. The aqueous extract of *Moringa oleifera* (Lam.) root possess antifertility property.^[14] *Moringa oleifera* (Lam.) seed also exhibited cyanobacteriacid activity.^[15] *Moringa oleifera* (Lam.) leaves possess hypolipidaemic and antiatherosclerotic activities.^[16] *Moringa oleifera* (Lam.) seed has excellent coagulation properties for treating waste water proved by Anselmendabigengesere *et al.*, (1995).^[17] *Moringa oleifera* (Lam.) pods was studied for free radicals scavenging abilities.^[18] Due to availability in the local area of Chittagong, the plant *Moringa oleifera* was chosen for study. Hence, the present study was undertaken specifically to investigate the antimicrobial and antifungal activity of sequential extracts of *Moringa oleifera* (Lam.) fruit.

2. Materials and Methods

2.1 Plant materials

Fruits of *Moringa oleifera* were collected during March 2010 from the local market in Bahadderhat Bazar of Chittagong city, Chandanish pourashava, and Bagichar hat, Chandanish, Chittagong and identified by

National Herbarium, Mirpur, Dhaka-1216, Bangladesh where a voucher specimen DACB32494 has been deposited.

2.2 Extraction

The fruits *Moringa oleifera* were cut in ½ inches and dried in air and finally in mechanical drier at 60-70 °C. The dried samples were grinded to course powder with a mechanical grinder and extracted with methanol for 7 days with occasional shaking in a beaker. The extract was filtered. The filtrate was dried at 50 to 60 °C and the yielded percentage was calculated.

2.3 Bacterial Media (Agar Media)

36 gm Nutrient Agar Media was mixed with distilled water and then sterilized in autoclave at 15 lb pressure for 15 minutes. The sterilized media were poured into petri dishes. The solidified plates were bored with 5mm diameter cork bearer. The plates with wells were used for the antibacterial studies.

2.4 Fungal Media (Potato dextrose sugar)

200 gm of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20 gm of dextrose was mixed with potato infusion. 20 gm of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm diameter cork borer. The plates with wells were used for antifungal studies.

2.5 Antimicrobial and Antifungal screening (*in vitro*)

The antimicrobial activity of the compounds *Moringa oleifera*, were measured by disk diffusion method (Beur *et al.* 1966 and Barry *et al.* 1980).^[19, 20] The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar surface with 6 mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2 °C for 24 hours for bacterial and 25±2 °C for 48 hours for fungal activity. The plates were observed for the zone clearance around the wells.

The methanol extract was dissolved in sterile distilled water to form dilution such as 30 µg, 50 µg and 100 µg. Each concentration of the plant extract was tested against different bacterial pathogens. The antifungal activity was observed at the concentration of 100 µg/disc, 150 µg/disc and 200 µg/disc. It was demonstrated by well diffusion assay (Bauer *et al.*, 1996). The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

3. Results

The results of the antimicrobial assay of the *Moringa oleifera* (Methanol extract) indicated that these plant exhibited antimicrobial activity against the tested microorganisms at concentrations of 30, 50 and 100 µg/disc. The potential sensitivity of the extract was obtained against all the microorganisms tested and the zone of inhibition was recorded and presented below in the tabulation drawn (Table 1). Fruit extract of *Moringa oleifera* (Methanol extract) showed mild to moderate antifungal activity against a number of tested fungi at concentration of 100, 150 and 200 µg/disc (table 2)

Table 1: *In vitro* Antimicrobial activity of the fruit extract of *Moringa oleifera*

Name of the Bacteria	Zone of the inhibition in mm			
	Kanamycin disc (Standard)	<i>Moringa oleifera</i> Lam. Fruit. (Methanol extract)		
	30 µg/disc	30 µg/disc	50 µg/disc	100 µg/disc
<i>Staphylococcus aureus</i>	30 mm	12	15	16
<i>Bacillus subtilis</i>	29 mm	13	14	16
<i>Vibrio cholera</i>	25 mm	11	13	14
<i>Bacillus cereus</i>	28 mm	12	14	19
<i>Salmonella typhi</i>	30 mm	11	13	15
<i>Shigella dysenteriae</i>	30 mm	0	0	18
<i>Pseudomonas aeruginosa</i>	27 mm	0	18	22
<i>Klebsiella species</i>	30 mm	12	13	15
<i>Proteus species</i>	24 mm	13	14	16

Table 2: *In vitro* Antifungal activity of the fruit extract of *Moringa oleifera* Lam.

Name of the Fungi	Zone of the inhibition in mm			
	Nystatin (Standard)	<i>Moringa oleifera</i> Lam. Fruit (Methanol extract)		
	30µg/disc	100 µg/disc	150 µg/disc	200 µg/disc
<i>Alternaria</i> SP.	30 mm	10	11	13
<i>Colletotrichum</i> SP.	27 mm	11	12	14
<i>Curvularia</i> SP.	26 mm	10	11	12
<i>Fusarium</i> SP	30 mm	8	9	11

4. Discussion

In the present investigation, the antimicrobial and antifungal activity of the fruit extract of *Moringa oleifera* was assayed against nine potentially pathogenic microorganisms *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera*, *Bacillus cereus*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Klebsiella species* and *Proteus species* and antifungal activity against four pathogenic fungi- *Alternaria Sp*, *Colletotrichum Sp*, *Curvularia Sp* and *Fusarium Sp* at different concentrations of the extract to understand the most effective activity. The fruit extract of *Moringa oleifera* showed a broad-spectrum antibacterial activity with a zone of inhibition of 0 to 22 mm and antifungal activity with a zone of inhibition of 8 to 14 mm. For methanol extract, the maximum zone of inhibition was obtained for *Pseudomonas aeruginosa* and *Bacillus cereus* at a concentration of 200 µg/disc. While the maximum zone of inhibition was found for antifungal activity at a concentration of 200 µg/disc for *Colletotrichum Sp*. and *Alternaria Sp*. All the tested microorganisms and fungi exhibited good sensitivity against above three concentrations except *Shigella dysenteriae*, *Pseudomonas aeruginosa*.

5. Conclusion

These findings suggest a new pathway in elucidating a potent antimicrobial agent from *Moringa oleifera*. Present study indicates that the plant contains antimicrobial compound that can be further developed as phytomedicine for the therapy of infection. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule at the onset of drug discovery will pay off later in drug development. Lastly, to conclude the extracts were found to inhibit the growth of Gram-positive bacteria as well as the Gram-negative bacteria and the fungal species.

6. References

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