

Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against Some Human Pathogenic Bacteria

M. Mashiar Rahman¹, M. Mominul Islam Sheikh¹,
Shamima Akhtar Sharmin¹, M. Soriful Islam¹,
M. Atikur Rahman¹, M. Mizanur Rahman^{2,3} and M. F. Alam^{1*}

¹Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

²Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh

³Dipartimento di Biotechnologie Agrarie, Viale dell'Università 16, 35020-Legnaro (Padova), Italy

*Corresponding author. E-mail: falambt@yahoo.com

ABSTRACT

The antibacterial activity of leaf juice and extracts of Moringa oleifera Lam., belonging to the family Moringaceae, was determined in vitro, using disc diffusion and minimum inhibitory concentration (MIC) determination method against human pathogenic bacteria. The fresh leaf juice (10 µl disc⁻¹), powder from fresh leaf juice, cold water extract of fresh leaf, each of 1175 µg disc⁻¹, displayed a potential antibacterial activity against all the tested four Gram-negative bacteria: Shigella shinga, Pseudomonas aeruginosa, Shigella sonnei and Pseudomonas spp. and six Gram-positive bacteria: Staphylococcus aureus, Bacillus cereus, Streptococcus-B- haemolytica, Bacillus subtilis, Sarcina lutea and Bacillus megaterium. However, ethanol extract (1175 µg disc⁻¹) of fresh leaves exhibited inhibitory effect against all the tested Gram-negative bacteria and Gram-positive bacteria except in S. aureus and Streptococcus-B- haemolytica. The zones of inhibition for fresh leaf juice was 15.23 to 25.2 mm, powder from fresh leaf juice was 29.25 to 42.3 mm, ethanol extract of fresh leaves was 16.25 to 21.5 mm and cold water extract of fresh leaves was 7.75 to 27.5 mm and MIC values were recorded as 1.25 to 2.5 µl disc⁻¹, 229 to 458 µg ml⁻¹, 458 to 916 µg ml⁻¹ and 29.87 to 58.75 mg ml⁻¹, respectively. The consequences of this investigation suggest that the extracts and juice of M. oleifera Lam. can be used to discover antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria responsible for severe illness.

Key words: *Moringa oleifera*, Pathogenic bacteria, Juice, Extract, Antibacterial activity, MIC

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries (Al-Bari et al., 2006). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy et al., 2005; Rojas et al., 2006). For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana et al., 2007). Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections. (Vijaya and Ananthan, 1997; Dilhuydy and Patients, 2003). Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver, 1993). It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Plants are the cheapest and safer alternative sources of antimicrobials (Pretorius and Watt, 2001; Sharif and Banik, 2006; Doughari et al., 2007). *Moringa oleifera* Lam. is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005) which is widely used for treating bacterial infection, fungal infection, anti-inflammation, sexually-transmitted diseases, malnutrition and diarrhoea. *Moringa* species have long been recognized by folk medicine practitioners as having value in the treatment of tumors (Ramachandran et al., 1980). Hence, the present study was undertaken specifically to investigate the role of aqueous and ethanol extracts of *M. oleifera* Lam. leaves as a potential antimicrobial agent against some human pathogenic bacteria.

MATERIALS AND METHODS

Plant material

The fresh leaves of *M. oleifera* Lam. were collected from Rajshahi University Campus, Rajshahi, Bangladesh, in February, 2008 and identified by Mr. Md. Habibur Rahman, taxonomist, National Herbarium, Mirpur, Dhaka-1216, Bangladesh where a voucher specimen DACB32494 has been deposited.

Fresh leaf juice and powder from fresh leaf juice

One hundred grams of fresh leaves of *M. oleifera* Lam. were crushed directly by grinder without adding any solvent, and leaf juice was collected in a clean airtight bottle, and stored for antibacterial activity test. 8 ml of leaf juice were air-dried and 0.94g fine powder was obtained, followed by dissolving in dimethyl sulfoxide (DMSO), and stored in airtight bottle for antibacterial activity test.

Cold aqueous extracts of fresh and dried leaves

One hundred grams of fresh leaves of *Moringa oleifera* Lam. were weighed out and crushed directly by grinder and dipped into 400 ml cold distilled water into a conical flask stoppered with rubber corks and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whatman no. 1) into a clean conical flask and subjected to water bath evaporation where the aqueous solvent was evaporated at its boiling temperature of 100°C. The standard extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test (Akueshi et al., 2002). In another case, the well air-dried fresh leaves were ground, and 100g sample was dipped in 400ml cold distilled water in a conical flask stoppered with rubber corks and left for 7 days with occasional shaking. The other steps were the same as followed in case of cold aqueous extract of fresh leaves.

Hot aqueous extracts of fresh and dried leaves

Here, same protocol was used as in cold water treatment with 30-min boiling while plant material was dipped in distilled water.

Ethanol (95%) extracts of fresh and dried leaves

Here, also the same procedure was followed as in cold water treatment.

Test microorganisms

10 bacterial strains were used in the study, among these were four Gram-negative, namely, *Shigella shinga* (BMLRU1013), *Pseudomonas aeruginosa* (BMLRU1007), *Shigella sonnei* (BMLRU1015) and *Pseudomonas* spp. (BMLRU1017) and six Gram-positive, namely, *Staphylococcus aureus* (BMLRU1002), *Bacillus cereus* (BMLRU1004), *Streptococcus-B- haemolytica* (BMLRU1006), *Bacillus subtilis* (BMLRU1008), *Sarcina lutea* (BMLRU1012) and *Bacillus megaterium* (BMLRU1010). All the tested strains are reference strains, and were collected from International Centre for Diarrhoeal Disease Research of Bangladesh (ICDDR). Bacteria were grown in Luria-Broth (LB) medium and maintained on nutrient agar slants at 4°C.

Antibacterial assay

Antibacterial activity of the eight different samples; 1: Fresh leaf juice, 2: Powder from fresh leaf juice, 3: Cold water extract of fresh leaves, 4: Hot water extract of fresh leaves, 5: Cold water extract of dried leaves, 6: Hot water extract of dried leaves, 7: Ethanol extracts of fresh leaves, and 8: Ethanol extracts of dried leaves were individually tested against studied bacteria. *In vitro* antibacterial test

was then carried out by disc diffusion method (Bauer et al., 1966; Barry, 1980) using 25 μl of standardized suspension of tested bacteria (10^8 cfu ml^{-1}) spread on LB plates. The discs (6 mm in diameter) were impregnated with 10 μl of fresh leaf juice, followed by air-drying and were placed on seeded agar plates. For samples 2 to 8, the discs (6 mm in diameter) were (sample 1) soaked individually with 10 μl of 117.5 mg ml^{-1} (1175 $\mu\text{g disc}^{-1}$), followed by air-drying and were placed on seeded agar plates. Negative controls were prepared using the same solvents to dissolve the plant extracts. Tetracycline (30 $\mu\text{g disc}^{-1}$) was used as positive controls to determine the sensitivity of bacterial strain. The plates were incubated at 37°C for 24h. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

Minimum inhibitory concentration (MIC)

MIC of eight different samples as mentioned earlier of *M. oleifera* Lam. was determined by two-fold serial dilution method (Chandrasekaran and Venkatesalu, 2004). The dose levels of 5, 2.5 and 1.25 μl for fresh leaf juice, and 117.5 mg ml^{-1} for rest of the samples were serially diluted separately to achieve 58.75, 29.37, 14.68, 7.34, 3.67, 1.83, 0.91 mg ml^{-1} and 0.458, 0.229, 0.114 $\mu\text{g ml}^{-1}$ concentration and were used for MIC determination. Briefly, 0.1 ml of varying concentrations of fresh leaf juice and other samples were added into the test tubes separately, containing 9 ml of standardized suspension of tested bacteria (10^8 cfu ml^{-1}). The test tubes were incubated at 37°C for 24 h. Controls were used with the test organisms, using distilled water instead of the plant extract. The least concentration of the samples with no visible growth was taken as the MIC (Adesokan, 2007).

RESULTS

In Vitro Antibacterial Activity

Table 1 shows diameter of zones of inhibition of bacterial growth at varying concentrations of the fresh leaf juice, powder from fresh leaf juice, cold and hot water extract of fresh leaves, cold and hot water extracts of dried leaves, ethanol extract of fresh leaves, and ethanol extract of dried leaves of *Moringa oleifera* Lam.

The fresh leaf juice showed stronger antibacterial activity against studied Gram-negative bacteria and Gram-positive bacteria, the respective diameter zones of inhibition were 20.2 ± 0.04 , 17.00 ± 0.66 , 25.1 ± 0.12 , 25.2 ± 0.04 and 15.23 ± 0.05 , 22.4 ± 0.28 , 18.0 ± 0.04 , 21.6 ± 0.04 , 18.1 ± 0.04 , 19.0 ± 0.04 mm, respectively.

Powder from fresh leaf juice (dissolved in DMSO) exhibited a potent inhibitory effect against all the tested Gram-negative and Gram-positive bacteria and their respective diameter zones of inhibition were 36.20 ± 0.08 , 39.60 ± 0.49 , 33.5 ± 0.12 , 42.3 ± 0.16 and 36.4 ± 0.08 , 29.25 ± 0.2 , 35.15 ± 0.12 , 33.75 ± 0.2 , 34.4 ± 0.44 , 39.25 ± 0.2 mm, respectively.

Cold water extract of fresh leaves displayed a relatively better antibacterial effect against *S. shinga*, *P. aeruginosa*, *S. sonnei*, *Pseudomonas* spp. and *S. aureus*, *B. cereus*, *S.-B-hemolytica*, *B. subtilis*, *S. lutea*, *B. megaterium* with

their individual diameter zones of inhibition recorded 7.75 ± 0.56 , 15.00 ± 0.34 , 13.45 ± 0.04 , 27.5 ± 0.21 and 12.0 ± 0.12 , 8.00 ± 0.42 , 10.75 ± 0.24 , 17.25 ± 0.14 , 8.50 ± 0.09 , 14.75 ± 0.04 mm, respectively.

However, hot water extract of fresh leaves and cold and hot water extracts of dried leaves did not show any inhibitory action against the tested bacteria.

Ethanol extract of fresh leaves also showed the extensive antibacterial effect against all the tested Gram-negative bacteria (*S. shinga*, *P. aeruginosa*, *S. sonnei*, *Pseudomonas* spp.) and some Gram-positive bacteria (*B. cereus*, *B. subtilis*, *S. lutea*, *B. megaterium*) and their respective diameter zones of inhibition were 17.5 ± 0.34 , 21.21 ± 0.05 , 21.50 ± 0.08 , 21.25 ± 0.13 and 16.25 ± 0.04 , 20.23 ± 0.56 , 19.50 ± 0.21 , 20.50 ± 0.04 mm, respectively. But no inhibitory effect of ethanol extracts of dried leaves was noticed. In all cases, the activity of the extracts was compared with standard antibiotic tetracycline.

In this study, fresh leaf juice, cold water extract of fresh leaves and ethanol extract of fresh leaves exhibited higher antibacterial activity compared to tetracycline. But only the powder (dissolved in DMSO) from fresh leaf juice exhibited the highest antibacterial activity against all the studied bacteria.

Table 1. Antibacterial activity of *Moringa oleifera* Lam. leaf juice and extracts against some human pathogenic bacteria.

Bacteria		Zone of Inhibition (mm)								
		Fresh leaf juice		Aqueous extracts ^a		Aqueous extracts ^a		Ethanol extracts ^a		Positive control Tetracycline
Liquid ^b	Powder ^a dissolved in DMSO	Fresh leaves		Dried leaves		Fresh leaves	Dried leaves			
		Cold	Hot	Cold	Hot					
Gram-Negative	Shigella shinga	20.2±0.04	36.2± 0.08	7.75±0.56	+	+	+	17.5±0.34	+	12.20±0.13
	Pseudomonas aeruginosa	17.00±0.66	39.60±0.49	15.00±0.34	+	+	+	21.21±0.05	+	18.30±0.12
	Shigella sonnei	25.1±0.12	33.5±0.12	13.45±0.04	+	+	+	21.50±0.08	+	14.16±0.23
	Pseudomonas spp.	25.2±0.04	42.3±0.16	27.5±0.21	+	+	+	21.25±0.13	+	20.16±0.19
Gram-Positive	Staphylococcus aureus	15.23±0.05	36.4±0.08	12.0±0.12	+	+	+	+	+	12.76±0.02
	Bacillus cereus	22.4±0.28	29.25±0.2	8.00±0.42	+	+	+	16.25±0.04	+	9.34±1.3
	Streptococcus-B-haemolytica	18.0±0.04	35.15±0.12	10.75±0.24	+	+	+	+	+	8.33±2.01
	Bacillus subtilis	21.6±0.04	33.75±0.2	17.25±0.14	+	+	+	20.23±0.56	+	7.25±0.08
	Sarcina lutea	18.1±0.04	34.4±0.44	8.50±0.09	+	+	+	19.50±0.21	+	8.66±0.13
	Bacillus megaterium (Enter)	19.0±0.04	39.25±0.2	14.75±0.04	+	+	+	20.50±0.04	+	20.16±0.43

Values are presented as mean $\pm$ S.E. of triplicate experiments. + = Growth

^a Diameter of inhibition zone including diameter of disc 6 mm (tested at a volume of 10 μ l/disc at a concentration of 1175 μ g disc⁻¹).

^b Diameter of inhibition zone including diameter of disc 6 mm (tested at a volume of 10 μ l disc⁻¹).

DMSO = Dimethylsulfoxide

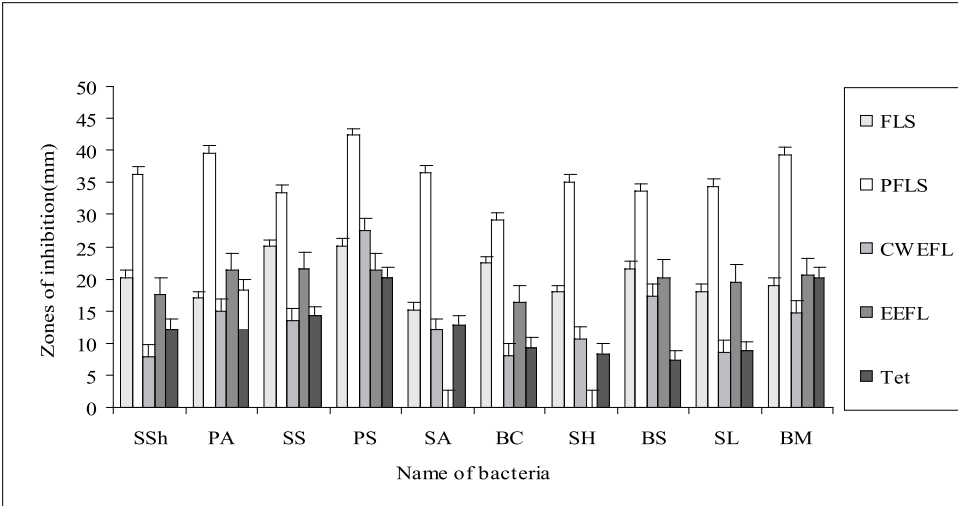


Figure-1:The comparison of antibacterial potential of *Moringa oleifera* Lam. leaf extracts and standard antibiotic tetracycline against some human pathogenic bacteria. FLS = Fresh leaf juice, PFLS = Powder from fresh leaf juice, CWEFL = Cold water extract of fresh leaf, EEFL = Ethanol extract of fresh leaf, Tet = Tetracycline, SSH = *Shigella shinga*, PA = *Pseudomonas aeruginosa*, SS= *Shigella sonnei*, PS=*Pseudomonas spp.*, SA=*Staphylococcus aureus*, BC=*Bacillus cereus*, SH=*Streptococcus-B-haemolytica*, BS=*Bacillus subtilis*, SL=*Sarcina lutea*, BM= *Bacillus megaterium*.

Minimum Inhibitory Concentration

As shown in Table 2, display of strong inhibition of fresh leaf juice against all the tested bacteria were noticed (volume 1.25 to 2.5 μ l disc-1). Powder from fresh leaf juice (dissolved in DMSO) was noticed to be vulnerable to all tested bacteria and their MIC values were ranged from 229- 458 μ g ml⁻¹. For cold aqueous extracts of fresh leaves, have inhibitory activity against all the tested bacteria and their MIC values were ranged from 29.87-58.75 mg ml⁻¹. The ethanol extracts of fresh leaves were also noticed to be more susceptible to *S. shinga*, *P. aeruginosa*, *S. sonnei*, *Pseudomonas spp.*, *B. cereus*, *B. subtilis*, *S. lutea*, and *B. megaterium* and their respective MIC values were ranged from 458-916 μ g ml⁻¹.

In this study, powder from fresh leaf juice showed the highest antibacterial activity against the bacteria tested with the lowest MIC value of 229 μ g ml⁻¹.

Table 2. Minimum inhibitory concentration (MIC) of *Moringa oleifera* Lam. leaf

extracts against some human pathogenic bacteria.

Bacteria		Minimum inhibitory concentration (MIC) ^{abc}							
		Fresh leaf juice		Aqueous extracts				Ethanol extracts	
		Liquid ^a	Powder ^b	Fresh leaves ^c		Dried leaves ^c		Fresh Leaves ^b	Dried Leaves
				Cold	Hot	Cold	Hot		
Gram-negative	<i>Shigella shinga</i>	1.25	458	58.75	nd	nd	nd	916	nd
	<i>Pseudomonas aeruginosa</i>	1.25	229	29.87	nd	nd	nd	458	nd
	<i>Shigella sonnei</i>	1.25	229	29.87	nd	nd	nd	458	nd
	<i>Pseudomonas spp.</i>	1.25	458	58.75	nd	nd	nd	458	nd
Gram-positive	<i>Staphylococcus aureus</i>	1.25	458	58.75	nd	nd	nd	nd	nd
	<i>Bacillus cereus</i>	2.5	458	58.75	nd	nd	nd	916	nd
	<i>Streptococcus-B-haemolytica</i>	1.25	458	58.75	nd	nd	nd	nd	nd
	<i>Bacillus subtilis</i>	1.25	458	58.75	nd	nd	nd	458	nd
	<i>Sarcina lutea</i>	1.25	458	58.75	nd	nd	nd	916	nd
	<i>Bacillus megaterium</i>	1.25	458	58.75	nd	nd	nd	458	nd

^aMinimum inhibitory concentration (values in $\mu\text{l disc}^{-1}$). nd: no detection.

^bMinimum inhibitory concentration (values in $\mu\text{g ml}^{-1}$).

^cMinimum inhibitory concentration (values in mg ml^{-1}).

DISCUSSION AND CONCLUSION

The present study was conducted to obtain preliminary information on the antibacterial activity of juice, water and ethanol extracts of *Moringa oleifera* Lam. leaves in Bangladesh. The disc diffusion method was applied to be used in this study. The powder from fresh leaf juice (dissolved in DMSO) has greater antibacterial activity than fresh leaf juice, ethanol and water extracts while fresh leaf juice and ethanol extract of fresh leaves showed higher antibacterial potential than the corresponding water extracts (Figure 1). This result is interesting because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Nair et al., (2005). In our investigation, highest zones of inhibition were found in powder from fresh leaf juice against all the bacteria tested which was more than one and a half to twice as much effective as known antibiotic tetracycline ($30 \mu\text{g /disc}$) while fresh leaf juice showed relatively higher inhibitory potency on all the tested bacteria except *B. megaterium* and this trend was also phenomenal against all the bacteria employed except *S. aureus* and *S.-B- haemolytica* (Figure 1). Gram-negative bacteria have been found to be less susceptible to plant extracts in earlier studies done by other researchers (Kuhnt et al., 1994; Afolayan and Meyer, 1995). In this study, we observed that powder from fresh leaf juice, fresh leaf juice, ethanol and cold water extracts of fresh leaves were more active against all the Gram-negative bacteria tested along with employed Gram-positive bacteria. The lowest

MICs were determined in terms of two Gram-negative bacteria such as *S. sonnei* and *P. aeruginosa*. These consequences suggest that *M. oleifera* Lam. leaves used contain bio-components whose antibacterial potentials are highly comparable with that of the antibiotic tetracycline against all Gram-negative and Gram-positive bacteria tested. The activity of the plant against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007). Today, most pathogenic organisms are becoming resistant to antibiotics (Chandarana et al., 2005). Moringa leaves have been reported to be a good source of natural antioxidants such as ascorbic acid, avo-noids, phenolics and carotenoids (Dillard and German, 2000). To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus, *M. oleifera* Lam. could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

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