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ANTIMICROBIAL PROPERTIES OF *MORINGA OLIEFERA* AGAINST PATHOGENIC BACTERIA

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ABSTRACT

The research work was investigated to compare the antimicrobial effect of *Moringa oliefera* leaf, bark and seed on pathogenic bacteria. Extracts were prepared in aqueous and organic solvents i.e. ethanol, ethyl acetate, methanol and chloroform. Ethanol and ethyl acetate extract of the leaf of *M. oleifera* were more effective while chloroform showed no zone of inhibition with all bacterial strains except *B. subtilis*. Leaf extract was selected for further study. With antibiotics, maximum growth inhibition was observed in *V. cholerae* (98.8%) in first dilution. MIC values demonstrate that in bacterial strains the most sensitive strain with antibiotic was *B. cereus* (5 mg/ml) and with leaf extract most sensitive strain was *B. subtilis* (16 mg/ml) however with antibiotics less sensitive strains were *S. typhi* (18 mg/ml) and with leaf extract less sensitive strain was *S. typhi* (28 mg/ml).

KEYWORDS: *Moringa oliefera*, antimicrobial, MIC.

INTRODUCTION

Moringa oleifera 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. Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human aliments. Ethanolic leaves extract of *Moringa oleifera* used as hypotensive (Nikkon *et al.*, 2003; Siddiqui and Khan, 1968; Kirtikar and Basu, 1984). The leaves of *M. oleifera* are reported to be used as a hypocholesterolemic agent, and hypoglycemic agent (Dangi *et al*., 2002; Ghasi *et al*., 2000;

Siddiqui and khan, 1968). *M. oleifera* is one of the best known medicinal plant. The *Moringa* plant has been consumed by humans (Iqbal *et al*., 2006). It is one of the richest plant sources of Vitamins A, B, C, D, E and K (Anwar and Bhanger, 2003; Babu 2000; Caceres *et al.*, 1992; Dayrit *et al*., 1990; Delisle *et al*., 1997). The vital minerals present in *Moringa* include Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc. The antimicrobial activities of *M. oleifera* leaves, roots, barks and seeds were investigated in vitro against bacteria, yeast, dermatophytes and helminthes pathogenic to man. Antibacterial effect of aqueous and ethanolic extracts of seeds of *M. oleiferain* the concentration of 1.5 unit and 1.10 unit in volumes 50, 100, 150 and 200 µl were examined against *Staphylococcus aureus, vibrio cholerea, Escherichia coli* (isolated from the organism and the aquatic environment) and *Salmonella enteritidis*. Antibacterial activity (inhibition halo> 13mm) against *S. aureus, V. cholera* and *E. coli* isolated from the white leg shrimp, *Litopenaeous vannmaei*, was detected in aqueous and ethanolic extract of moringa. *E. coli* isolated from tilapia fish and *Oreochrom isniloticus*, were sensitive to the ethanolic extract of *M. oleifera*. During recent years considerable work has been done to investigate the pharmacological actions of the leaves and seeds of *M. oleifera* on scientific lines but only limited work has been reported so far on antibacterial activity of *M. oleifera* root bark though it is reported to possess varied medicinal properties. Therefore, it was considered worthy to investigate the antibacterial activity of *M. oleifera* root bark. Bark used to cure Dental Caries/Toothache, Common cold, External Sores/Ulcer, Anti-Tumor, Snakebite, Scorpion bite, Digestive, Headache, Antinutrietional factors and Scurvy (Fahey, 2005).

The aims and objectives of the present work are to establish a well documented information about the antimicrobial activity of *M. oleifera* extracts against 10 standard microorganisms using reference antibiotics as experimental models.

MATERIAL AND METHODS

Material

1. Test organism

Pure culture of pathogenic 10 bacterial strains, *Shigella shinga, Shigella sonnei, Pseudomonas aeruginosa, Staphyloccocus aureous, Salmonella typhi, Salmonella typhimurium, Bacillus subtilis, Bacillus cereus, Sarcina lutea* and *Vibrio cholerae* were procured from SGMC, Kanpur (U.P.). Pure cultures of the bacterial isolates were maintained in appropriate media for future use.

2.Chemicals: All chemicals and media used in this study were of analytical grade.

3. Antibiotic activity on bacterial strains

Octatadisk of different antibiotics were purchased for antibiotic susceptibility test of all ten pathogenic bacterial strains.

4. Collection of plant material

The fresh leaves, seeds and bark of *Moringa oleifera* were collected from Agra and Jaipur. The plant materials were air-dried in the laboratory for four weeks and then ground into powdered form, using a mortar and pestel, and stored for future use.

Methods

Extraction

a) In aqueous solution

The air-dried leaves, bark and seed of *M. oleifera* (50 g) were dipped in 200 ml cold distilled water in a conical flask stoppered with rubber cork and left for 7 days with occasional shaking. Solution was filtered using sterile filter paper (Whattman 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 and as well as by evaporation under vacuum. The standard extracts obtained were stored in a refrigerator at 4°C for antibacterial activity test (Akueshi *et al.*, 2002).

b) In organic solvent

i. Ethanol and Ethyl acetate

Plant material of *M. oleifera* were collected and dried in shade. The dried materials were ground to powder and suspended in petroleum ether and kept in refrigerator overnight. After one week incubation, Solution was filtered using sterile filter paper (Whattman No. 1) into a clean conical flask and subjected to water bath evaporation where the organic solvent was evaporated at room temperature and the residue was dried at room temperature. The residue was further divided into two parts and each part was suspended in ethanol and ethyl acetate respectively in sterile 25 ml conical flasks and kept at 4ºC overnight. After overnight incubation, the supernatant was filtered through Whatman No.1 filter paper and the filtrate was dried to evaporate the organic solvent at room temperature. The standard extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test (Valarmathy *et al.*, 2010).

ii. Methanol and Chloroform

30 g of the plant material powder was thoroughly mixed in 450 ml methanol and chloroform respectively and allowed to stand for one hour before filtering with the help of Whatman filter paper No. 1. The filtrate was overnight dried in hot air oven (45°C). The extraction yielded 6 g (methanol) and 3 g (chloroform). The standard extracts obtained were store in a refrigerator at 4°C for antibacterial activity test (Thilza *et al.*, 2010).

Growth inhibition: The percentage inhibition of bacterial growth was calculated by comparing growth density with antibiotics and plant material extracts and without antibiotics and plant material extracts. Inhibition (%) in growth density was calculated as follows

$$\% \text{ inhibition} = \frac{\text{O.D. Control - O.D. treated tubes}}{\text{O.D. Control}} \text{ x } 100$$

Minimum Inhibitory Concentration (MIC) (mg/l): Well labeled 9 tubes were taken. Add 2 ml of antibiotic solution (100 µg/ml) to the first tube then add 1 ml of sterile broth to all other tubes. Transfer 1 ml from the first tube to the second tube. Using a separate pipette, mix the contents of this tube and transfer 1 ml to the third tube. Continue dilutions in this manner to tube number 8, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette. Remove 1 ml from tube 8 and discard it. The ninth tube, which serves as a control, receives no antibiotic. Suspend to an appropriate turbidity several colonies of the culture to be tested in 5 ml of Mueller-Hinton broth to give a slightly turbid suspension. Dilute this suspension by aseptically pipetting 0.2 ml of the suspension into 40 ml of Mueller-Hinton broth. Add 1 ml of the diluted culture suspension to each of the tubes. The final concentration of antibiotic is now one-half of the original concentration in each tube. Incubate all tubes at 35^{o}C overnight. Examine tubes for visible signs of bacterial growth. The highest dilution without growth is the minimal inhibitory concentration (MIC) (Murray, 2005).

RESULTS

Antibiotic activity on bacterial strains: Octatadisk of different antibiotics were used to check antibiotic susceptibility of all ten pathogenic bacterial strains (*Shigella shinga, Shigella Sonnei, Pseudomonas aeruginosa, Staphyloccocus aureous, Salmonella typhi, Salmonella typhimurium, Bacillus subtilis, Bacillus cereus, Sarcina lutea and Vibrio cholerae*). All

bacterial strains showed different antibiotic susceptibility with various antibiotics (Table-1; Fig. 1).

1. *S. shinga*

Cefoperazone showed maximum zone of inhibition of 37 mm while Ceftazidime shows minimum zone of 14 mm however Cefpodoxime shows no zone against this bacteria. Other antibiotics viz, Moxifloxacin, Amikacin, Gentamycin, Meropenem and Cefepime shows 28 mm, 24 mm, 26 mm, 26 mm, 25 mm zones of inhibition respectively.

2. *S. sonnei*

Cefoperazone showed maximum zone of inhibition of 39 mm while Ceftazidime shows minimum zone of 12 mm however Cefpodoxime shows no zone against this bacteria. Other antibiotics viz, Cefepime, Moxifloxacin, Amikacin, Meropenem and Gentamycin shows 30 mm, 22 mm, 27 mm, 25 mm, 23 mm zones of inhibition respectively.

3. *P. aeruginosa*

Ciprofloxacin shows maximum zone of 28 mm while Imipenum showed minimum zone of 11mm. Other antibiotics viz, Azetreonem, Amikacin, Piperacillin, Gentamicin, Meropenum and Ceftazidime shows 20 mm, 23 mm, 21 mm, 24 mm, 21 mm, and 21 mm zone of inhibition respectively.

4. *S. aureus*

Clindamycin show maximum inhibition zone of 31 mm while Vancomycin shows minimum inhibition zone of 18 mm. Other antibiotics Teicoplanin, Ofloxacin, Azithromycin, Tetracycline, Penicillin and Erythromycin shows 19 mm, 28 mm, 25 mm, 26 mm, 29 mm, 23 mm zone of inhibition respectively.

5. *S. typhi*

Ciprofloxacin showed maximum zone of inhibition of 29 mm followed by Co-Trimoxazole shows maximum zone of 26 mm while Amikacin shows minimum zone of 16 mm. Other antibiotics viz, Ampicillin, Cefuroxime, Cephalothin, Gentamicin, and Ceftriaxome shows 24 mm, 20 mm, 23 mm, 19 mm and 21 mm zone of inhibition.

6. *S. typhimeurium*

Ciprofloxacin and Co-Trimoxazole shows maximum zone of 25 mm while Amikacin shows minimum zone of 18 mm. Other antibiotics viz, Cephalothin, Cefuroxime, Gentamicin,

Ampicillin and Ceftriaxome shows 22 mm, 19 mm, 20 mm, 21 mm and 22 mm zone of inhibition.

7. *B. subtilis*

Methicillin showed maximum zone of 29 mm while Fusidic acid showed minimum zone of 13 mm. Other antibiotics viz, Penicillin, Streptomycin, Tetracycline, Chloramphenicol, Erythromycin and Novobiocin showed 16 mm, 19 mm, 27 mm, 26 mm, 24 mm and 21 mm respectively.

8. *B. cereus*

Methicillin showed maximum zone of 31 mm while Fusidic acid showed minimum zone of 11 mm. Other antibiotics viz, Penicillin, Erythromycin, Streptomycin, Novobiocin, Chloramphenicol and Tetracycline showed 18 mm, 21 mm, 29 mm, 24 mm, 22 mm and 25 mm respectively.

9. *S. lutea*

Ciprofloxacin showed maximum zone of 27 mm while Piperacillin showed minimum no zone. Other antibiotics viz, Azetreonem, Imipenum, Ceftazidime, Gentamicin, Meropenum and Amikacin showed 22 mm, 26 mm, 19 mm, 25 mm, 22 mm, and 23 mm zone of inhibition respectively.

10. *V. cholera*

Penicillin showed maximum inhibition zone of 33 mm while Vancomycin showed minimum inhibition zone of 18 mm. Other antibiotics Erythromycin, Teicoplanin, Azithromycin, Tetracycline, Clindamycin and Ofloxacin showed 21 mm, 27 mm, 27 mm, 22 mm, 30 mm and 23 mm zone of inhibition respectively.

Table-1: Antibacterial activity of different antibiotic against pathogenic bacteria, [ZOI- Zone of inhibition (mm)].

S. shinga		***S. sonnei***		***P. aeruginosa***		***S. aureus***		***S. typhi***	
Antibiotics	**ZOI**	**Antibiotics**	**ZOI**	**Antibiotics**	**ZOI**	**Antibiotics**	**ZOI**	**Antibiotics**	**ZOI**
Cefoperazone	37	Cefoperazon	39	Ciprofloxacin	28	Clindamycin	31	Ciprofloxacin	29
Ceftazidime	14	Ceftazidime	12	Imipenum	11	Vancomycin	18	Co-Trimoxazole	26
Cefpodoxime	-	Cefpodoxime	-	Azetreonem	20	Teicoplanin	19	Amikacin	16
Moxifloxacin	28	Cefepime	30	Amikacin	23	Ofloxacin	28	Ampicillin	24
Amikacin	24	Moxifloxacin	22	Piperacillin	21	Azithromycin	25	Cefuroxime	20
Gentamycin	26	Amikacin	27	Gentamicin	24	Tetracycline	26	Cephalothin	23
Meropenem	26	Meropenem	25	Meropenum	21	Penicillin	29	Gentamicin	19
Cefepime	25	Gentamycin	23	Ceftazidime	21	Erythromycin	23	Ceftriaxome	21
S. typhimeurium		***B. subtilis***		***B. cereus***		***S. lutea***		***V. cholerae***	
Antibiotics	**ZOI**	**Antibiotics**	**ZOI**	**Antibiotics**	**ZOI**	**Antibiotics**	**ZOI**	**Antibiotics**	**ZOI**
Ciprofloxacin	25	Methicillin	29	Methicillin	31	Ciprofloxacin	27	Penicillin	33
Co-Trimoxazole	25	Fusidic acid	13	Fusidic acid	11	Piperacillin	-	Vancomycin	18
Amikacin	18	Penicillin	16	Penicillin	18	Azetreonem	22	Erythromycin	21
Cephalothin	22	Streptomycin	19	Erythromycin	21	Imipenum	26	Teicoplanin	27
Cefuroxime	19	Tetracycline	27	Streptomycin	29	Ceftazidime	19	Azithromycin	27
Gentamicin	20	Chloramphenicol	26	Novobiocin	24	Gentamicin	25	Tetracycline	22
Ampicillin	21	Erythromycin	24	Chloramphenicol	22	Meropenum	22	Clindamycin	30
Ceftriaxome	22	Novobiocin	21	Tetracycline	25	Amikacin	23	Ofloxacin	23

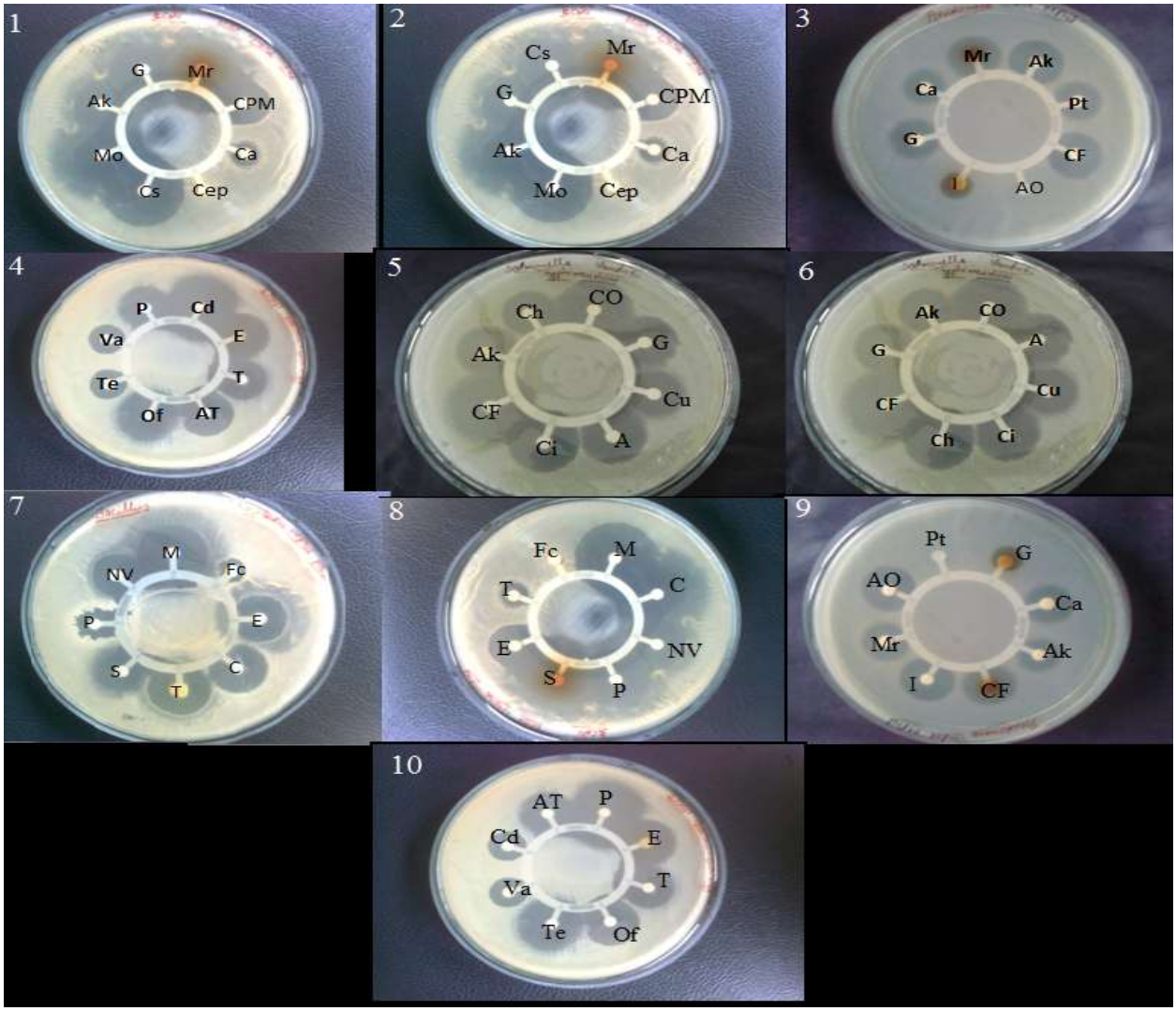

Fig.1: Antibacterial activity of different antibiotic against pathogenic bacteria; 1. *Shigella shinga,* 2. *Shigella sonnei,* 3. *Pseudomonas aeruginosa,* 4. *Staphyloccocus aureous,* 5. *Salmonella typhi,* 6. *Salmonella typhimurium,* 7. *Bacillus subtilis,* 8. *Bacillus cereus,* 9. *Sarcina lutea,* 10. *Vibrio cholera*

Extracts activity of bacterial strains: To screen the antibacterial activity of leaves, bark and seeds of *M. oleifera*, all selected pathogenic bacterial strains were taken as test microbes. Antibacterial activity was assayed by agar well diffusion method. Tetracycline was used as a positive control and all organic solvents were used as negative control. The appearance of zone of inhibition is indicate that extracts of *M. oleifera* were inhibiting the growth of bacteria, thereby revealing the presence of antibacterial activity in the plant material extracts. Ethyl acetate extracts showed stronger antibacterial activity against all pathogenic bacterial strains, while chloroform showed no zone of inhibition with all bacterial strains except *B. subtilis*. Leaf extracts showed good activity in comparison to bark and seed (Table-2; Fig. 2).

1. *S. shinga*: Best activity was found in ethyl acetate extract of leaf (21.7 mm) followed by ethanol and water extracts of leaf (15.3, 11.2 mm) respectively. Minimum zone of inhibition was found with water extract of seed (6.7 mm) followed by water extract of bark (9.6 mm). Activity was not found in methanol and chloroform extracts.

2. *S. sonnei*: Showed activity only in ethanol and ethyl acetate extracts. Best activity was found in ethyl acetate extract of leaf (18.3 mm) followed by ethanol extract of leaf (16.7 mm). Activity was not found in water, methanol and chloroform extracts.

3. *P. aeruginosa*: Activity was found in all selected extracts except chloroform. Best activity was found in ethyl acetate extract of leaf (23.7 mm) followed by bark and seed (18.5, 15.2 mm). In methanol extract activity was not found in bark while in water extract activity was found only in leaf extract.

4. *S. aureous*: Activity was found in ethyl acetate and methanol extracts. Best activity was found in ethyl acetate extract of leaf (19.2 mm) followed by seed and bark (17.7, 16.5 mm). Activity was not found in water, methanol and chloroform extracts.

5. *S. typhi*: Activity was found in all selected extract except chloroform. While in methanol extract activity was found only in leaf extract. Best activity was found in water extract of leaf (19.4 mm) followed by ethanol extract of leaf and water extract of seed respectively (17.4, 16.9 mm).

6. *S. typhimurium*: Activity was not found in water, methanol and chloroform extracts. Best activity was found in ethanol extract of leaf (15.5 mm) followed by ethyl acetate extracts of leaf and ethanol extract of seed respectively (13.9, 12.1 mm).

7. *B. subtilis*: Except methanol extract, activity was found in all selected extracts. Best activity was found in ethanol extract of leaf (21.6 mm) followed by ethyl acetate and water extract of leaf and ethanol extract of seed respectively (19.2, 18.5, 18.5 mm).

8. *B. cereus*: Activity was not found in water and chloroform extract. Best activity was found in ethyl acetate extract of leaf (24.3 mm) followed by ethanol extract of leaf and seed (19.8, 18.5 mm). Minimum zone of inhibition was found in methanol extract of bark (9.7 mm).

9. *S. lutea*: Activity was not found in water, methanol and chloroform extracts. Best activity was found in ethyl acetate extract of leaf (16.3 mm) followed by ethanol extract of leaf and ethyl acetate extract of bark respectively (13.1, 11.2 mm).

10. *V. cholerae*: Best activity was found in ethyl acetate extract of leaf (17.4 mm) followed by ethyl acetate extract of bark and ethanol extract of leaf respectively (15.9, 15.3 mm). Activity was not found in water, methanol and chloroform extracts.

Table-2: Antibacterial activity of *M. oleifera* leaf, bark and seed extracts against pathogenic bacteria

Strains	Plant Material	Zone of inhibition (mm) with different extracts					Positive control Tetracycline
		Water	Ethanol	Ethyl acetate	Methanol	Chloroform	
S. shinga	Leaf	11.2±0.06	15.3±0.14	21.7±0.11	-	-	13.6±0.12
	Bark	09.6±0.12	12.5±0.04	14.2±0.17	-	-	
	Seed	06.7±0.08	12.4±0.12	16.4±0.09	-	-	
S. sonnei	Leaf	-	16.7±0.22	18.3±0.32	-	-	17.7±0.23
	Bark	-	13.6±0.14	11.5±0.16	-	-	
	Seed	-	12.5±0.17	13.6±0.24	-	-	
P. aeruginosa	Leaf	08.3±0.09	13.4±0.21	23.7±0.04	10.6±0.09	-	17.5±0.13
	Bark	-	07.8±0.12	18.5±0.33	-	-	
	Seed	-	09.5±0.17	15.2±0.15	7.08±0.08	-	
S. aureous	Leaf	-	-	19.2±0.13	08.5±0.11	-	13.3±0.17
	Bark	-	-	16.5±0.33	06.5±0.07	-	
	Seed	-	-	17.7±0.11	06.7±0.05	-	
S. typhi	Leaf	19.4±0.21	17.4±0.13	15.6±0.07	12.4±0.14	-	19.4±0.34
	Bark	13.5±0.14	13.4±0.11	11.0±0.23	-	-	
	Seed	16.9±0.17	15.7±0.08	14.3±0.15	-	-	
S. typhimurium	Leaf	-	15.5±0.22	13.9±0.27	-	-	15.7±0.22
	Bark	-	11.3±0.09	09.4±0.13	-	-	
	Seed	-	12.1±0.14	08.7±0.17	-	-	
B. subtilis	Leaf	18.5±0.13	21.6±0.27	19.2±0.26	-	09.6±0.08	10.9±0.27
	Bark	12.3±0.16	15.7±0.17	14.5±0.16	-	08.2±0.02	
	Seed	13.7±0.11	18.5±0.34	13.6±0.36	-	07.8±0.09	
B. cereus	Leaf	-	19.8±0.19	24.3±0.15	14.6±0.13	-	10.3±0.11
	Bark	-	16.7±0.21	16.7±0.21	09.7±0.12	-	
	Seed	-	18.5±0.14	17.9±0.19	10.3±0.11	-	
S. lutea	Leaf	-	13.1±0.18	16.3±0.15	-	-	8.7±0.15
	Bark	-	09.6±0.04	11.2±0.14	-	-	
	Seed	-	08.2±0.06	10.8±0.09	-	-	
V. cholerae	Leaf	-	15.3±0.12	17.4±0.17	-	-	11.7±0.06
	Bark	-	11.8±0.13	12.3±0.22	-	-	
	Seed	-	11.5±0.16	15.9±0.14	-	-	

Note- 1. Values are presented as mean ± SD of triplicate experiments.

2. Diameter of inhibition zone including of 5 mm agar well (tested at a volume of 10 µg/well at a concentration of 1026 µg/well).

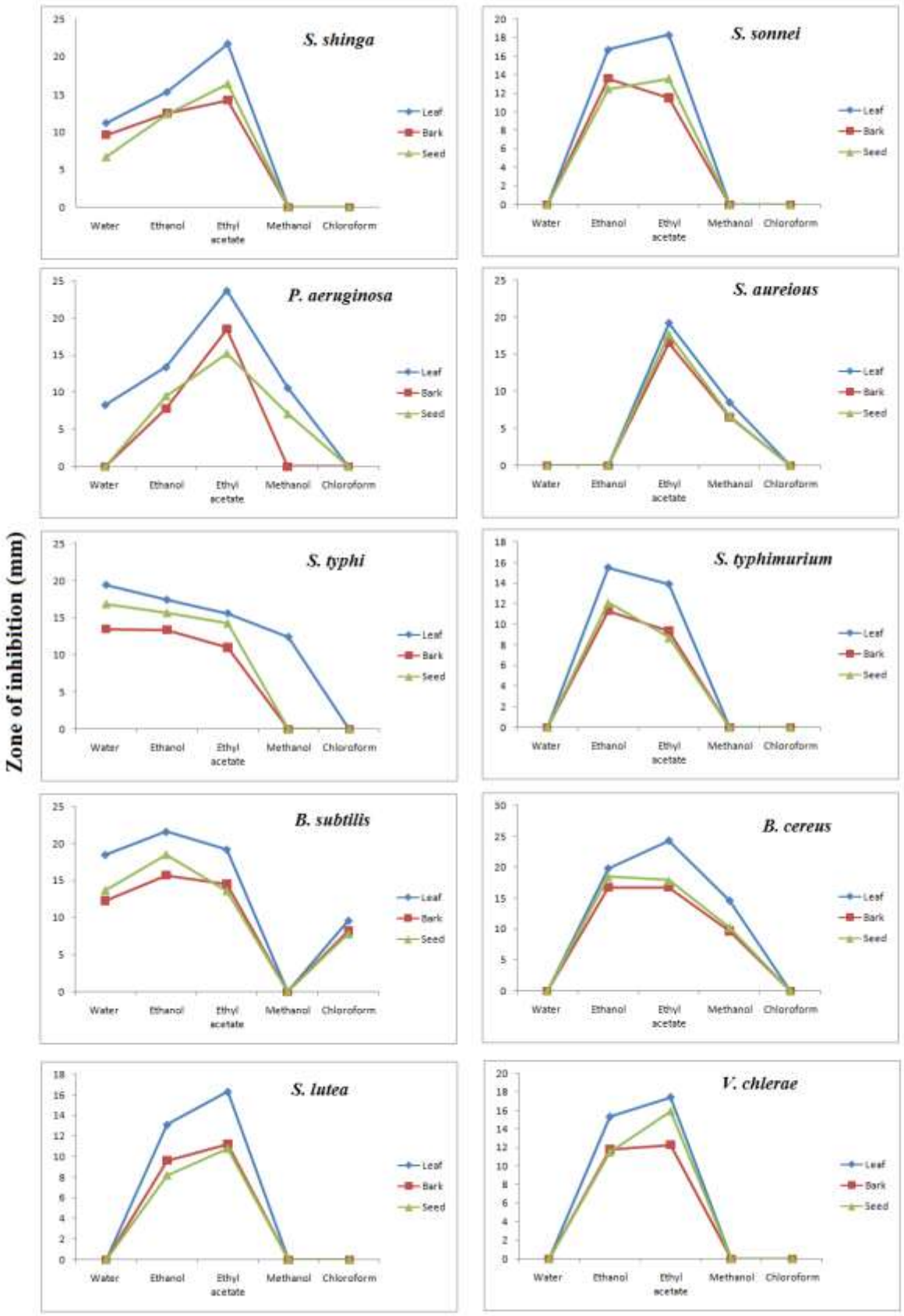

Fig. 2: Graphical view of antibacterial activity of *M. oleifera* leaf, bark and seed extracts against pathogenic bacteria

Growth inhibition (%) in the presence of antibiotics and extracts

All selected pathogenic bacterial strains showed growth inhibition (%) in all dilutions of leaf extract and antibiotic. However growth was reduced in higher to lower form in all dilutions (Table- 3, 4; Fig. 3 a, b).

In leaf extract maximum growth inhibition was recorded in *S. typhi* (96.3%) followed by *B. cereus, P. aeruginosa* and *S. lutea* respectively in first dilution (96.3, 93.6, 93.5%). Minimum growth inhibition was recorded in *S. typhimeurium* and *S. sonnei* (5.3, 5.5%) followed by *B. subtilis* and *S. shinga* respectively in last dilution (7.6, 8.9%).

With antibiotics, maximum growth inhibition was observed in *V. cholerae* (98.8%) followed by *S. typhimeurium* and *S. typhi* in first dilution respectively (97.4, 96.7%). Minimum growth inhibition was recorded in *S. sonnei* (5.2%) followed by *S. lutea* and *S. aureus* respectively in last dilution (6.3, 6.4%).

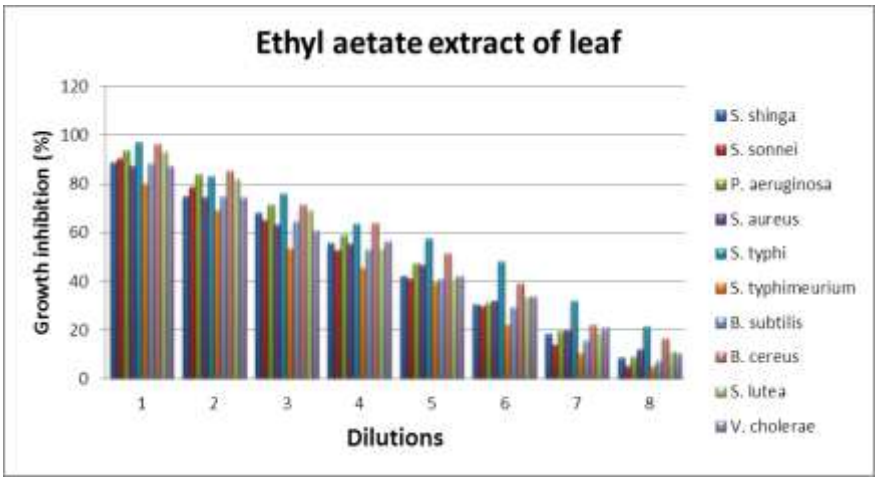

Fig. 3 (a): Graphical view of growth inhibition (%) in leaf extract

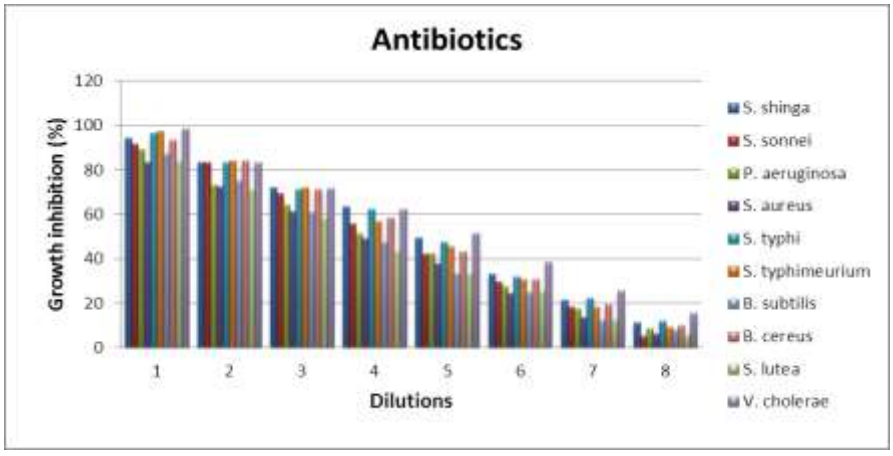

Fig. 3 (b): Graphical view of growth inhibition (%) in antibiotics

Table- 3: Growth inhibition of pathogenic bacterial strains in ethyl acetate extract of *M. oleifera* leaf

Dilution	Ethyl acetate extract of leaf									
	S. shinga	***S. sonnei***	***P. aeruginosa***	***S. aureus***	***S. typhi***	***S. typhimeurium***	***B. subtilis***	***B. cereus***	***S. lutea***	***V. cholerae***
1	88.7	90.3	93.6	87.3	97.2	80.2	88.3	96.3	93.5	87.4
2	74.9	78.7	84.1	74.5	83.1	69.3	75.1	85.4	82.1	74.6
3	68.3	65.1	71.5	63.2	75.9	53.6	64.7	71.5	69.2	61.2
4	55.7	52.6	59.1	55.4	63.6	45.7	53.2	64.2	53.4	56.7
5	42.1	41.1	47.6	46.7	57.7	39.6	41.3	51.6	41.6	42.1
6	30.6	29.7	31.2	32.0	48.1	22.5	29.5	39.3	33.7	33.9
7	18.5	14.2	19.6	20.1	32.2	10.7	15.9	22.4	19.5	21.2
8	08.9	05.5	09.3	12.3	21.7	05.3	07.6	16.7	11.4	10.6

Table- 4: Growth inhibition of pathogenic bacterial strains in standard antibiotics

Dilution	Antibiotics									
	S. shinga	***S. sonnei***	***P. aeruginosa***	***S. aureus***	***S. typhi***	***S. typhimeurium***	***B. subtilis***	***B. cereus***	***S. lutea***	***V. cholerae***
1	94.2	91.6	89.2	83.4	96.7	97.4	87.2	93.5	84.4	98.8
2	83.6	83.3	73.4	72.4	83.4	84.1	75.4	84.2	71.6	83.4
3	72.1	69.4	64.5	61.1	71.3	72.1	61.2	71.3	58.7	71.7
4	63.6	55.7	51.7	49.2	62.6	57.2	47.8	58.6	43.9	62.6
5	49.7	42.1	42.6	37.8	47.5	45.6	33.7	43.2	33.5	51.7
6	33.2	29.9	28.0	24.6	32.1	31.1	25.2	31.1	25.7	38.6
7	21.7	18.6	17.6	13.8	22.4	18.6	12.6	19.5	13.2	25.8
8	11.6	05.2	08.7	06.4	12.1	09.4	08.5	10.3	06.3	15.6

S. shinga – Ceftazidime, ***S. sonnei*** - Ceftazidime, ***P. aeruginosa*** - Imipenum, ***S. aureus*** - Vancomycin, ***S. typhi*** - Amikacin,

S. typhimeurium - Amikacin, ***B. subtilis*** - Fusidic acid, ***B. cereus*** - Fusidic acid, ***S. lutea*** - Ceftazidime, ***V. cholerae*** - Vancomycin

Table-5: MIC value (mg/ml) of leaf extracts and antibiotics

Strains	Leaf extract	Antibiotics						
		Ceftazidime	Imipenum	Vancomycin	Amikacin	Fusidic acid	Ceftazidime	Vancomycin
S. shinga	21	10	-	-	-	-	-	-
S. sonnei	23	8	-	-	-	-	-	-
P. aeruginosa	18	-	7	-	-	-	-	-
S. aureous	24	-	-	11	-	-	-	-
S. typhi	28	-	-	-	18	-	-	-
S. typhimurium	22	-	-	-	16	-	-	-
B. subtilis	16	-	-	-	-	10	-	-
B. cereus	25	-	-	-	-	5	-	-
S. lutea	19	-	-	-	-	-	10	-
V. cholerae	18	-	-	-	-	-	-	7

MIC value

MIC values demonstrate that in bacterial strains the most sensitive strain with antibiotic was *B. cereus* (5 mg/ml) followed by *P. aeruginosa* and *V. cholerae* respectively (7 mg/ml) and with leaf extract most sensitive strain was *B. subtilis* (16 mg/ml) followed by *P. aeruginosa* and *V. cholerae* (18 mg/ml) respectively. With antibiotics less sensitive strains were *S. typhi* and *S. typhimurium* (18, 16 mg/ml) respectively and with leaf extract less sensitive strain was *S. typhi* (28 mg/ml) followed by *B. cereus* and *S. aureous* (25, 24 mg/ml) respectively.

In this study leaf extract showed the highest antibacterial activity against bacterial strains. Comparative table of MIC value of leaf extract and antibiotic are given in Table-5.

DISCUSSION

Our results demonstrate that the antimicrobial activity of the plant extracts of *M. oleifera* affected predominantly pathogenic bacterial strains. The antimicrobial activity of extract might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane (Jabeen *et al.,* 2008). The extracts of *M. oleifera* leaves, bark and seed showed antimicrobial activity with all selected bacterial strains. *M. oleifera* leaves extracts worked in doze dependent manner, as the concentration of the extract decreased the activity also decreased, indeed different MIC values were observed against different bacterial strains. This is due to susceptibility of the species towards concentration of the extracts, after which this extract damage, the species which is not tolerable for it (Ordonez *et al.,* 2006).

In the last few years various study has been done for its antimicrobial activity from the extract made using chloroform, ethanol. Where as in the present study antimicrobial activity was observed in plant material extracts prepared with water, ethanol, ethyl acetate, methanol, and chloroform. However there are no reports of antimicrobial activity against ethyl acetate extract. In present study ethyl acetate extract show zone of inhibition against all selected pathogenic bacterial strains *S. shinga, S. sonnei, P. aeruginosa, S. aureous, S. typhi, S. typhimurium, B. subtilis, B. cereus, S. lutea* and *V. cholerae.*

The ethanolic extract of *M. oleifera* leaves has been demonstrated to exhibit anthelmintic activity against Indian earthworm (Rastogi *et al.,* 2009), antifungal activity against dermatophytes (Chuang *et al.,* 2007), antifertility (Prakash, 1998; Shukla *et al.,* 1981) and hypoglycemic potential (Jaiswal *et al.,* 2009). A study on evaluation of *M. oleifera* leaves extract on ovariectomy induced bone loss in rats records that the ethanolic extract of *M. olifera* leaves possess osteoprotective effect comparable with estradiol (Burali *et al.,* 2010) and has been reported to reduce cyclophosphamide induced immunodepression by stimulating cellular and humoral immunity in mice (Gupta *et al.,* 2010; Siddarth and Gupta, 2007).

The aqueous extract of *M oleifera* leaves have been demonstrated to exhibit protective effect on ulcerated gastric tissue induced by aspirin, cerebral nodular lesion and cold stress in rats (Patel *et al.,* 2008), wound healing property in rats (Makkar and Becker, 1996) significant hypoglycemic and antidiabetic potential (Jaiswal *et al.,* 2009), antifertility activity (Prakash, 1998; Shukla *et al.,* 1981) and the regulatory control on thyroid hormone status in adult Swiss rats (Rathi *et al.,* 2006).

Folkard and Sutherland (2005) proposed utilization of *Moringa* seeds as food since it sterilizes the food and destroys *S. typhii* which lives in the intestinal tracts of man. The antibiotic nature of *Moringa* seeds is due to an oil it contains which on consumption forms a thin film over the intestinal wall thus reducing or preventing the pathogen (by inhibition) from penetrating the walls (Caceres and Lopez, 1991; Caceres *et al.*,1991; Nwosu and Okafor, 1995). Other studies have also shown that *M. oleifera* seeds produce a gum that is antityphoid in antibacterial activity (Fuglie, 1999; Harristoy *et al*., 2005). The antibacterial activity of the plant has been demonstrated against both gram-negative and gram-positive bacteria and this is in agreement with our findings (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007; Mashiar *et al.*, 2009). According to Madsen *et al*. (1987) *Moringa* have been characterized as basic polypeptides which can bind suspended particles in water that contain colonies of bacteria such as *V. cholerae*. The charged protein molecules can serve as nontoxic natural polypeptides to settle mineral particles and organics in destruction of specific bacteria (Aney et al., 2009).

CONCLUSION

When the obtained results were compared to antibiotics findings; it could be concluded that the ethanol and ethyl acetate extract of the leaves obtained from *M. oleifera* was more effective than the standard antibiotics used. According to high antimicrobial activity of the *M. oleifera* leaf extracts further research work should be done using this plant. More studies are needed to isolate and characterize the active compounds to be tested *in vivo* to determine the toxicity and the optimum dose to be used as effective as antibiotics. Selected extracts could be promising natural antimicrobial agents with potential applications in controlling bacteria that cause diseases. The extracts can provide a cheap and sustainable method toward disease reduction and can eventually improve the quality of life of the rural and peri-urban poor in developing countries. However, *Moringa* extracts should not be regarded as a panacea for reducing the disease incidences since issues of safety and toxicity need to be evaluated.

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