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Efficacy of *Moringa pterigosperma Gaertn* leaf extracts on bacterial and fungal pathogens

KEYWORDS: Moringa pterigosperma, leaf extracts, MIC, antimicrobial assay.

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ABSTRACT *In vitro antimicrobial activity of Moringa pterigosperma leaf extracts were studied against selected pathogenic bacteria and fungi by using antimicrobial assay method. Leaves were extracted with various solvents such as acetone, chloroform, methanol, hexane and petroleum ether. Among the five solvents used, acetone extracts of the leaves of M. pterigosperma were found to be effective against the test organisms, where the minimum inhibitory concentration (MIC) ranged between 250 to 1000µg/ml. The chloroform extracts showed MIC of 250 to 1000µg/ml for all test organisms except B. megaterium and E. coli. The extracts of petroleum ether and hexane were ineffective in inhibiting the test organisms. The methanol extracts showed MIC of 750 to 1000µg/ml for both tested bacteria and fungi. The antimicrobial activity of this plant was discussed.*

INTRODUCTION

Natural products, including plants, animals and minerals have been the basis for treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The future of natural products drug discovery involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and community. Several books have been published on Indian medicinal plants (Kirtikar and Basu, 1935; Chopra *et al.* 1996 and Parrotta, 2001). Bhakuni *et al.* (1969) tested 880 plants for their biological activity. Investigations of plants used in traditional and modern medicine in China serve of inspiration and as models for the synthesis of new drugs (Baerheim and Scheffer, 1982). Panthong *et al.*(1986) reviewed the plants possessing the properties of anti-inflammatory, anti-asthmatic and hypertension activities. Panthong *et al.*(1991) described 326 plants exhibiting anti-diarrhoeal, laxative and corminative properties. In recent years, tremendous work has been done in compiling the details of medicinal plants and the studies confirmed many claims of ancient herbalists about the efficacy of several herbs in curing diseases (Bakhru, 2002). Many serious disorders due to microorganisms can be controlled by synthetic drugs. Problems with drug-resistant microorganisms, side effects of modern drugs and emerging diseases, where no medicines are available, have stimulated renewed interest in plants as a significant source of new medicines (Patwardhan *et al.* 2004).

The different biological activities of Moringaceae were well documented in the literature. The family contains only one genus *Moringa* and 12 species. Dayrit *et al.* (1990) studied the activity of aqueous seed extracts of *Moringa pterigosperma* against *Bacillus subtilis*. Armando *et al.* (1991) studied pharmacological and antimicrobial properties of *M. pterigosperma* . Caceres and Lopez (1991) screened antiplasmodic, anti-inflammatory and diuretic activity of *M. pterigosperma* . Antifungal activity of *M. pterigosperma* against *Basidiobolous* and other pathogenic fungi was experimentally proved by Nwosu and Okafor (1995). Nath *et al.* (1997) conducted a survey on indigenous medicinal plants used for abortion and reported that *M. pterigosperma* was used by 75% of population. *Moringa pterigosperma* was found to be used by 75% of population. Rajendran *et al.* (1998) screened the extracts of *Acorus calamus, Cinnamomum zeylanicum, Moringa pterigosperma , Ocimum sanctum, Piperbetle* and *Zingiber officinalis* for antibacterial activity and reported that the aqueous extracts of the seeds of *M. pterigosperma* showed activity against *E. coli, P. aeruginosa, S. aureus* and *Klebsiella spp.* Anti-inflammatory and hepatoprotective activities of fruits of *M. pterigosperma* were studied by Kurma and Mishra (1998). Rao and Mishra (1998) studied the anti-inflammatory and anti hepatoprotective activities of fruits of *M. pterigosperma.* Mughal and Saba (1999) reported food and medicinal values of *M. pterigosperma*. Kumar and Gopal (1999) tested the activity of *M. pterigosperma* against microorganisms of raw water. Anti convalescent, sedative and anti analgesic activities of *M. pterigosperma* were reported by Gupta *et al.* (1999).

Moringa pterigosperma Gaertn. is a small medium sized tree up to 10 m tall with thick, soft, corky, deeply fissured bark and tomentose twigs; roots pungent. Leaves usually tripinnate, 45 cm long; pinnae and pinnules opposite, deciduous; leaflets 1.2 – 2 cm long 0.6-1 cm wide, the later elliptic and the terminal obovate. Flowers white, fragrant in large pannicles, fruits pendulous, green 22-50 cm or more in length triangular, 9-ribbed, seeds trigonous and the wings angled. Different parts of *M. pterigosperma* are used to treat different diseases. Root is used to treat asthma, gout, rheumatism, epilepsy, intermittent fever and also used as cardiac tonic. Leaf is used to cure scurvy and wounds. Fruit is used to treat liver and spleen disorders

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and tetanus. Seeds are antipyretic (Maheswari, 1965).

The principle aim of the present work was to study the antimicrobial activity of Moringa pterigosperma leaf extracts in different organic solvents such as acetone, chloroform, methanol, hexane and petroleum ether against seven bacteria and three fungal species.

EXPERIMENTAL

Plant material

The leaves of *Moringa pterigosperma* were collected from Gorantla, Guntur district of Andhra Pradesh, India (Figure 1). The voucher specimen was deposited at Department of Botany, St. Ann's College for women, Guntur, Andhra Pradesh, India for future reference.

Figure 1 Leaf of *Moringa pterygosperma*

Leaf Extract Preparation:

Plants were collected, shade dried and milled to coarse powder and subjected to soxhlet extraction. Leaves were extracted with different organic solvents such as acetone, chloroform, methanol, hexane and petroleum ether for 8 h or until plant material was discoloured in the soxhlet apparatus. The extracts were filtered and concentrated to dryness under vacuum. The different solvent extracts thus obtained were tested for antimicrobial activity at different concentrations such as 100 µg, 250 µg, 500 µg, 750 µg and 1000 µg using the antibiotic sensitivity test (Harikrishna Ramaprasad Saripalli *et al.,* 2013). The zones of growth inhibition around the wells were measured and the area of inhibition zone was calculated. Simultaneously the activity of six standard antibiotics named Streptomycin, gentamicin, chloromphenicol, vancomycin, rifampicin, kanamycin and nystatin were also tested against the bacteria and fungi in required conditions, so as to compare the degree of inhibition exhibited by the plant extracts. Well fed with corresponding solvents served as controls.

Test organisms:

Tests were performed on seven species of selected bacteria such as *Bacillus megaterium* (ATCC 23564), *B.subtilis*(ATCC 6633), *Escherichia coli*(ATCC 25922), *Enterobacter faecalis* (ATCC 35550), *Proteus vulgaris*(ATCC 638), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus*(ATCC 25923) *and three fungal species Asperigillus niger* (NCIM 596), *A.fumigatus*(NCIM 291) and *Candida albicans*(NCIM 670).

Table 1 List of the selected test organisms (bacteria and fungi)

S. No	Name of the Microorganisms	Characteristic feature	Diseases caused by the organisms
1	Bacillus megaterium (ATCC 23564)	Gram + ve	Intestinal disturbances.
2	Bacillus subtilis (ATCC 6633)	Gram + ve	Food poisoning, Oppurtunistic Pathogen.
3	Escherichia coli (ATCC 25922)	Gram – ve	Gasteroenteritis, urinary tract disease.
4	Enterobacter faecalis (ATCC 35550)	Gram – ve	Oppurtunistic human pathogen.
5	Proteus vulgaris (ATCC 6380)	Gram – ve	Urinary tract infections.
6	Pseudomonas aeruginosa (ATCC 27853)	Gram – ve	Wounds and urinary tract infections.
7	Staphylococcus aureus (ATCC 25923)	Gram + ve	Chronic osteomyletis, Meningitis, endocarditis.
8	Aspergillus niger (NCIM 596)	Dichotomously branching, filamentous	Allergy, Asthma
9	Aspergillus fumigatus (NCIM 291)	Monomorphic filamentous fungi	Pulmonary haemorrhage, pnemonia.
10	Candida albicans (NCIM 670)	Dimorphic fungi	Oral thrush, Gastritis, Cutaneous infection.

(Ref: Harikrishna Ramaprasad S, 2007)

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All the above test bacterial species were maintained on nutrient agar medium. 36 hours old bacterial culture was inoculated into nutrient broth and incubated on a rotary shaker (Janked And Kunkel Ika, Labortechnik, Germany) at 35 ± 2 °C at 100 rpm. After 36 hours of incubation, the bacterial suspension was centrifuged at 10000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1×10^8 cfu/ml using UV Visible Spectrophotometer (Hitachi U-2000, Japan). By reading the OD of the solution to 0.45Å (610nm) it was used for further studies. Fungal colonies were harvested from 9 -10days old cultures, which were maintained on potato dextrose agar. The spores were suspended in sterile distilled water and the spore suspension was adjusted to 1×10^8 spores/ml.

Antimicrobial assay:

Different concentrations of the above mentioned solvent extracts of the leaf were tested for antimicrobial activity by using antibiotic sensitivity test. Microbial suspension was evenly mixed with sterile agar medium and poured into the sterile Petri plates. After allowing the media to solidify at room temperature, wells of 6mm diameter were bored in the agar with sterile cork borer. Each concentration was checked for antimicrobial activity by introducing equal amounts of the sample (40µl) into wells. The method was repeated in five plates. Plates were allowed to stand at room temperature for 1 hour, for extract to diffuse into agar media and then incubated at 37 °C for 24 to 48 hours. The zone of growth inhibition around the wells was measured and area of inhibition zone was calculated. Simultaneously the activity of seven standard antibiotics such as Streptomycin(10µg/ml),Gentamycin(10µg/ml),Cholorompheni col(30µg/ml),Vancomycin(10µg/ml),Rifimapicin(5µg/ml),Kanamycin(10µg/ml),Nystatin(10µg/ml) were also tested against seven species of bacteria and three species of fungi. Agar wells fed with corresponding solvents served as control .Minimum inhibitory concentration which was determined as the lowest concentration of leaf extracts inhibiting the growth of organisms was determined based on the readings.

RESULTS AND DISCUSSION

Acetone extracts of the leaves of *M. pterigosperma* as inhibitory to all the test organisms except *B. megaterium* (Table 1). The minimum inhibitory concentration was found to be 250 µg for *E. coli, E. faecalis* and *S. aureus*, while it was 500 µg for *B. subtilis*, 750 µg for *P. aeruginosa, A. niger, A. fumigatus* and *C. albicans* and 1000 µg for *P. vulgaris* . Among the microbial species tested, *S. aureus* was found to be highly sensitive to the acetone extracts of *M. pterigosperma* .

Figure 2 Antibacterial activity of acetone extracts of leaves of Moringa pterygosperma against Bacillus subtilis

The chloroform extracts of the leaves of *M. pterigosperma* are inhibitory to all test organisms except *B. megaterium* and *E. coli* (Table 2). The minimum inhibitory concentration was found to be 250 µg for *S. aureus*, while it was 500 µg for *B. subtilis, E. faecalis* and *C. albicans*; 750 µg against *P. vulgaris, A. niger* and *A. fumigatus*; 1000 µg against *P. aeruginosa*. The methanol extracts of the leaves of *M. pterigosperma* are inhibitory to *B. subtilis, E. coli, E. faecalis, P. vulgaris, S. aureus* and the three fungi. The minimum inhibitory concentration was found to be 750 µg for *B. subtilis, E. coli, E. faecalis* and *P. vulgaris*, while it was 1000 µg for *S. aureus* and the three fungi (Table 3). The extracts of petroleum ether and hexane did not show inhibitory activity against the test organisms .The acetone extracts of the leaves of *M. pterigosperma* were found to be effective against the test organisms, followed by chloroform, methanol extracts.

Conclusion:

Phytomedicines are effective in treating most of the infectious diseases mainly skin infections. Most of the secondary metabolites, serve as a plant defence mechanism against microrganisms, insects and herbivores (Harikrishna R. P. Saripalli *et al*., 2013). Antimicrobial activity of tested medicinal plants can be attributed to any of these constituents. The tabular reports indicated that acetone extracts were effective than other extracts. In the present study organic solvent extracts of leaves of *M. pterigosperma* exhibits better antimicrobial activity as compared with standards. Hence the detailed phytochemical investigations and antimicrobial screening of secondary metabolites from this plant may yield promising antimicrobial agents.

Table 1. Antibacterial activity of acetone extracts of the leaves of Moringa pterygosperma

Product (µg)	Area of inhibition zone (mm²)									
	A	B	C	D	E	F	G	H	I	J
Control	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
100	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
250	12.60	12.60	31.43	31.43	12.60	12.60	31.43	12.60	12.60	12.60
500	12.60	31.43	88.00	56.57	12.60	12.60	56.57	12.60	12.60	12.60
750	12.60	56.57	106.07	88.00	12.60	31.43	88.00	21.21	21.21	31.43
1000	12.60	125.7	125.7	125.7	88.00	56.57	169.7	31.43	31.43	47.99
Standard	190	172	148	143	148	180	143	83.00	92.00	138.00

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Table 2. Antibacterial activity of chloroform extracts of the leaves of Moringa pterygosperma

Product (µg)	Area of inhibition zone (mm²)									
	A	B	C	D	E	F	G	H	I	J
Control	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
100	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
250	12.60	12.60	12.60	12.60	12.60	12.60	31.43	12.60	12.60	12.60
500	12.60	31.43	12.60	31.43	12.60	12.60	56.57	12.60	12.60	21.21
750	12.60	56.57	12.60	56.57	31.43	12.60	88.00	21.21	21.21	31.43
1000	12.60	125.7	12.60	125.7	88.00	31.43	169.7	31.43	31.43	43.21
Standard	190	172	148	143	148	180	143	83.00	92.00	138.00

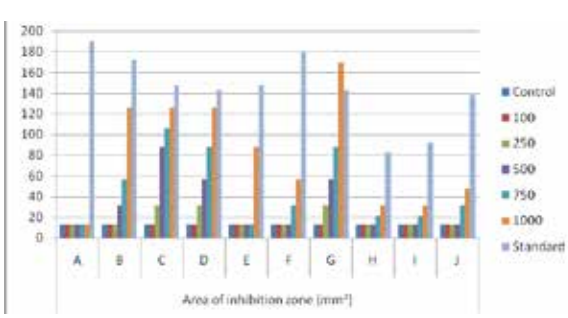
Table 3. Antibacterial activity of Methanol extracts of the leaves of Moringa pterygosperma

Product (µg)	Area of inhibition zone (mm²)									
	A	B	C	D	E	F	G	H	I	J
Control	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
100	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
250	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
500	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
750	12.60	31.43	31.43	56.57	31.43	12.60	12.60	12.60	12.60	12.60
1000	12.60	88.00	88.00	125.7	56.57	12.60	31.43	21.21	21.21	21.21
Standard	190	172	148	143	148	180	143	83.00	92.00	138.00

(A) Bacillus megaterium (B) B. subtilis (C) Escherichia coli (D) Enterobacter faecalis (E) Proteus vulgaris (F) Pseudomonas aeruginosa (G) Staphylococcus aureus (H) Aspergillus niger (I) Aspergillus fumigatus (J) Candida albicans

Standard: Streptomycin (30µg/ml) for *E. coli*; gentamicin (10 µg/ml) for *P. aeruginosa* and *P. vulgaris*; chloromphenicol (40 µg/ml) for *S. aureus*; vancomycin (20 µg/ml) for

B. subtilis; rifampicin (5 µg/ml) for *E. faecalis*; kanamycin (20 µg/ml) for *B. megaterium*;nystatin (20 µg/ml) for *Aspergillus niger, A. fu*



migatus and *Candida albicans*

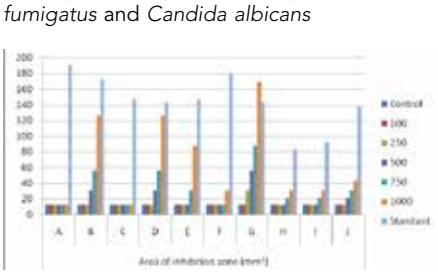
Graph 1 Antibacterial activity of acetone extracts of the leaves of Moringa pterygosperma

(A) *Bacillus megaterium* **(B)** *B. subtilis* **(C)** *Escherichia coli* **(D)** *Enterobacter faecalis* **(E)** *Proteus vulgaris* **(F)** *Pseudomonas aeruginosa* **(G)** *Staphylococcus aureus* **(H)** *Aspergillus niger* **(I)** *Aspergillus fumigatus* **(J)** *Candida albicans*

Standard: Streptomycin (30µg/ml) for *E. coli*; gentamicin (10 µg/ml) for *P. aeruginosa* and *P. vulgaris*; chloromphenicol (40 µg/ml) for *S. aureus*; vancomycin (20 µg/ml) for

B. subtilis; rifampicin (5 µg/ml) for *E. faecalis*; kanamycin (20 µg/ml) for

B. megaterium;nystatin (20 µg/ml) for *Aspergillus niger, A. fumigatus* and *Candida albicans*



Graph 2 Antibacterial activity of chloroform extracts of the leaves of Moringa pterygosperma

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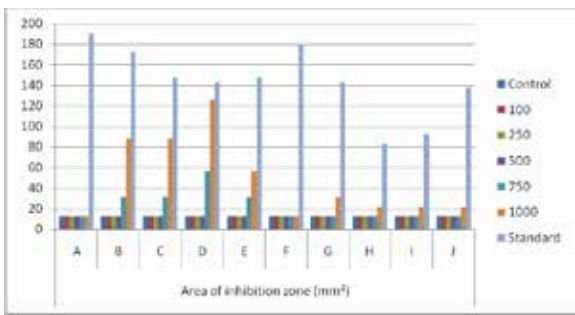
(A) *Bacillus megaterium* **(B)** *B. subtilis* **(C)** *Escherichia coli* **(D)** *Enterobacter faecalis* **(E)** *Proteus vulgaris* **(F)** *Pseudomonas aeruginosa* **(G)** *Staphylococcus aureus* **(H)** *Aspergillus niger* **(I)** *Aspergillus fumigatus* **(J)** *Candida albicans*

Standard: Streptomycin (30µg/ml) for *E. coli*; gentamicin (10 µg/ml) for *P. aeruginosa* and *P. vulgaris*; chloromphenicol (40 µg/ml) for *S. aureus*; vancomycin (20 µg/ml) for

B. subtilis; rifampicin (5 µg/ml) for *E. faecalis*; kanamycin (20 µg/ml) for



Graph 3 Antibacterial activity of Methanol extracts of the leaves of Moringa pterygosperma

(A) *Bacillus megaterium* **(B)** *B. subtilis* **(C)** *Escherichia coli* **(D)** *Enterobacter faecalis* **(E)** *Proteus vulgaris* **(F)** *Pseudomonas aeruginosa* **(G)** *Staphylococcus aureus* **(H)** *Aspergillus niger* **(I)** *Aspergillus fumigatus* **(J)** *Candida albicans*

Standard: Streptomycin (30µg/ml) for *E. coli*; gentamicin (10 µg/ml) for *P. aeruginosa* and *P. vulgaris*; chloromphenicol (40 µg/ml) for *S. aureus*; vancomycin (20 µg/ml) for

B. subtilis; rifampicin (5 µg/ml) for *E. faecalis*; kanamycin (20 µg/ml) for

B. megaterium;nystatin (20 µg/ml) for *Aspergillus niger, A. fumigatus* and *Candida albicans*

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