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Research Article

Antimicrobial Activity and Phytochemical Analysis of *Moringa oleifera* Lam. Crude Extracts Against Selected Bacterial and Fungal Strains

Patel N*, Mohan J S S

Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India

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ABSTRACT

The n- Hexane, ethyl acetate, methanol and distilled water Leaf, Stem, Flower and Fruit extracts of *Moringa oleifera* were evaluated for their antibacterial activity against six Gram-Negative and Gram-Positive bacteria and antifungal activity against seven fungal strains using Ciprofloxacin, Doxycycline, Fluconazole and Ketacozole as positive control. The activity was analyzed by well diffusion and two-fold serial broth dilution method of different extract. The study revealed that all the extracts irrespective of their types, in different concentrations inhibited growth of the test pathogens to varying degrees. Ethyl acetate extract showed maximum activity against all the bacterial strains followed in descending order by methanol, n-.Hexane and distilled water extracts. Ethyl acetate extract showed high antibacterial activity against *Serratia marcescens* (22mm) Methanol and n-Hexane extract were effective against *Enterococcus faecalis and Bacillus subtilis* (10mm) respectively. Aqueous extract showed maximum number of inhibition against *Staphylococcus aureus* (27mm) and *Micrococcus luteus* (18mm). Ethyl acetate extract showed maximum inhibition against *Trichoderma harzianum* (16mm) than other extracts were ineffective against selected fungus. MIC values were recorded as 0.125 to 4mg/ml. The phytochemical screening revealed the presence of phenols and flavonoids. Expression to these results it may be concluded that *M. oleifera* may be a potential source for the curing of various infectious diseases caused by the resistant microbes.

Keywords: *Moringa oleifera*, extract, antibacterial activity, antifungal activity, MIC, Phytochemical.

INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards to genetic resources of medicinal plants[1]. A survey conducted by the All India Coordinated Research Project on Ethnobiology (AICRPE) during the last decade recorded over 8000 species of wild plants used by the tribals and other traditional communities in India for treating various health problems[2]. Medicinal plants and plant-derived medicines are widely used in different traditions all over the world and they are becoming increasingly popular in modern scientific communities as natural alternatives to synthetic chemicals[3]. The trees and shrubs have wide range of benefits to human beings amongst other include medicine and foods[4-8]. To prevent and cure different human diseases recently, considerable attention has been paid to eco-friendly and bio-friendly plants[9]. The rich knowledge base of countries like India and China in medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs. Data analysis has shown that more and more people are consulting the herbal medicine practitioners. World Health Organization has also identified the importance of herbal medicines. Approximately 60-70% patients living in rural areas are dependent on herbal medicine. Several authors have reported favorable results with herbal drugs (mostly in the form of extracts) either in animal or in human studies[10]. Medicinal plants are the main source of pharmaceuticals and healthcare products[11]. The use of medicinal plants is a source of extraction and development of several drugs from these plants[12]. Plants consist of many secondary metabolites such as alkaloids, phenolic compounds, etc which possesses antimicrobial properties. In developing countries the practice of complementary and alternative medicine is now on the increase. Several clinical and pre-clinical studies have provided the scientific basis for the efficacy of many plants used in folk medicine to cure diseases[13,14]. There is a need for a permanent search and development of new drugs, though there are many antibiotic and antifungal agents are resistant or multi resistant strains are available[15]. Therefore there is a need for the search of newer antibiotic sources. Plants are cheap and safe alternative sources of antimicrobials[16-18]. *Moringa oleifera* Lam. (Family Moringaceae) is well – known for its various medicinal properties which are widely used for treating bacterial infection, fungal infection, anti-inflammation, sexually-transmitted diseases, malnutrition

*Author for Correspondence: naynikapatel24179@gmail.com

Table 1: Antimicrobial activity in crude n-Hexane extract of selected plant species.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive						Gram negative					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Moringa oliefera	L	CH	-	-	-	-	-	-	-	-	-	-	-	-
		HH	-	-	-	-	-	-	-	-	-	-	-	-
		FH	8	5	2	6	6	3	11	-	-	8	7	-
	S	CH	6	-	-	-	-	-	-	-	-	-	1	-
		HH	1	10	-	2	-	-	-	1	-	8	6	-
		FH	-	-	-	-	-	-	7	-	-	4	-	2
	FT-IM	CH	-	-	-	-	-	-	-	-	-	-	-	-
		HH	-	-	-	-	-	-	8	-	-	-	-	-
		FH	5	6	-	2	8	-	7	5	-	7	8	-
	FT-Y	CH	-	-	-	-	-	-	-	-	-	-	-	-
		HH	-	-	-	-	-	4	2	-	-	-	-	7
		FH	6	4	7	-	-	-	6	5	-	-	-	-
	FT-M	CH	-	-	-	-	-	-	8	1	1	-	4	7
		HH	7	3	6	3	9	-	6	-	-	3	6	-
		FH	3	7	7	7	8	-	7	5	1	4	4	7
	FT-R	CH	1	-	-	-	-	-	4	-	-	4	-	-
		HH	3	-	-	-	-	-	-	-	-	-	-	-
		FH	1	1	-	-	-	-	2	1	-	5	3	-
	Seed	CH	-	-	-	-	1	-	-	-	-	-	1	-
		HH	-	-	-	-	-	-	1	5	-	-	-	-
Ciprofloxacin (20 µg/ml)			11	10	12	11	14	9	7	14	8	22	10	9
Doxycycline (20 µg/ml)			14	12	9	5	11	8	15	19	11	20	13	4

BC-*Bacillus cereus* ;BS-*Bacillus subtilis* ; EC-*Escherichia coli* ;EN-*Enterococcus faecalis* ; KP-*Klebsiella pneumoniae* ;ML- *Micrococcus luteus*; PS-*Pseudomonas aeruginosa* ; SA- *Staphylococcus aureus*; SE-*Staphylococcus epidermidis*; SM-*Serratia marcescens* ; SP-*Salmonella paratyphi* ;ST-*Salmonella typhi ;* L-Leaf; S-Stem; F-Flower; FT-Fruit (IM-Immature; Y-Young; M-Mature; R- Ripen) ;
CH- Cold Hexane; HH-Hot Hexane; FH Fresh Hexane

and diarrhoea. Moringa species have long been recognized by folk medicine practitioners as having value in the treatment of tumors1[9]. Almost all the parts of this plant: root, bark, gum, leaf, pods, flowers, seeds and seeds oil have been used for the various ailments in the indigenous medicine[20]. It has been known to be anti-helminthic activity, antimicrobial activity, detoxifier, immune booster and anti –parasitic activity[21]. Antimicrobial activities of various *M. oleifera* morphological parts against some pathogenic microorganisms have been reported[22-26]. However, not so extensive work on its antimicrobial properties has been studied and more so, some of its morphological parts are unexplored. Hence, the present study was an attempt to examine the role of different part i.e. leaf, stem, flower and fruit extracts of *M. Oleifera* Lam. as a potential antimicrobial agent against some human pathogenic bacteria and also help to reduce the multiple drug resistance.

MATERIAL AND METHODS

Plant material

The fresh leaf, stem, flower and fruit of *M. oleifera* Lam. were collected from Karamsad, Gujarati, India, and identified by referring "Flora of Gujarat state"[27] and confirmed by Dr. A.S. Reddy (Plant Taxonomist) and Dr. Sandip Patel, Research student, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat.

Extract Preparation

The fresh leaf, stem, flower and fruit of *M. oleifera* Lam were collected and washed thoroughly with running tap water to remove dirt particles. All the materials were dried at room temperature and powdered with grinder. Extract was prepared by infusion extraction method given by[28]. For sequential extraction 50 gm of dry powdered or fresh material of each sample was soaked in 250 ml n-Hexane at room temperature for 24 hours. Extracts were filtered through Whatmans filter paper no.1 and the filtrates were centrifuged at 3000 rpm for 10 minutes to remove solid debris. The supernatant was collected and concentrated by solvent recovering assembly (J-sil, India) and dried completely at room temperature and stored it in a refrigerator until further use.

The filtrate collected on filter paper was completely dried and resuspended sequentially in to each of 250 ml ethyl acetate, methanol and distilled water at room temperature for 24 hours. The extract was filtered and the filtrate was centrifuged at 3000 rpm for 10 minutes and the supernatants were collected. All the fractions were stored in a refrigerator until further use.

The crude hot plant extracts were also prepared by soxhlet extraction method. About 20gm powdered plant material was uniformly packed into a thimble and

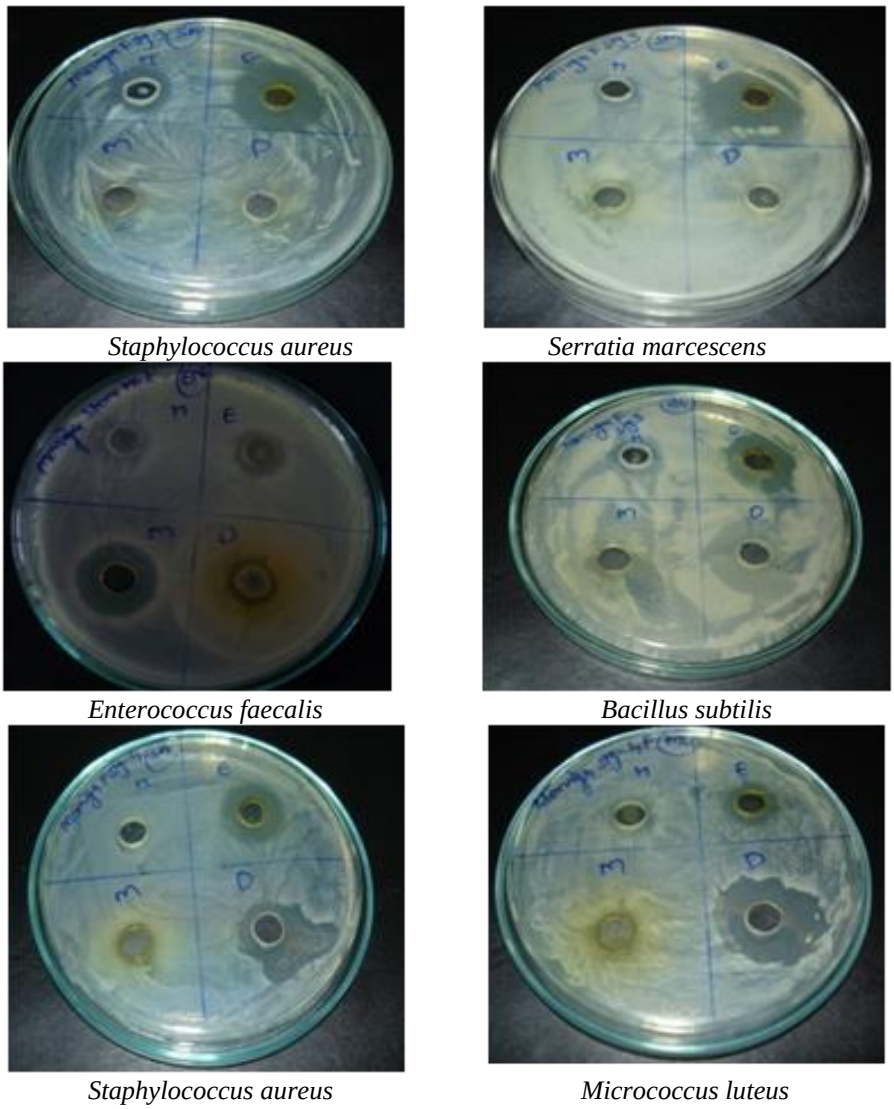
extracted sequentially with 250ml of different solvents separately i.e. ethyl acetate, methanol and distilled water. The extraction process continued for 24 hrs or till the solvent in siphon tube of an extractor became colourless. The extract was then transferred to evaporating plate for drying completely at room temperature and stored it in a refrigerator until further use.

Selected microorganisms

12 bacterial strains and 7 fungal strains used in the study, among these were six Gram-positive namely *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (Isolated), *Staphylococcus epidermidis* (ATCC 155), *Micrococcus luteus* (ATCC 4698), *Enterococcus faecalis* (Isolated) and six Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Salmonella typhi* (NCTC8394), *Salmonella paratyphi* (MTCC 735*), Pseudomonas aeruginosa* (ATCC 25668), *Klebsiella pneumoniae* (ATCC 15380), *Serratia marcescens* (Isolated) and fungal strains is *Aspergillus niger* (MTCC40211), *Candida albicans* (MTCC 183), *Trichoderma harzianum* (Isolated), *Fusarium oxysporum* (Isolated), *Aspergillus flavus* (MTCC4613), *Aspergillus parasiticus* (MTCC411) and *Alternaria burnsii* (ITCC 4285). All the tested strains are reference strains, and were collected from MTCC (Microbial type culture collection, Chandigarh), ATCC (American type culture collection, Manassas, Virginia) and NCTC (National collection of type culture). The bacterial and fungal cultures were grown on nutrient agar medium (Hi Media, pH 7.4) at 37ºC and potato dextrose agar medium (Hi Media, pH 5.6) at 27ºC respectively. Both the cultures were maintained at 4°C.

Antibacterial assay

In the present study, the antibacterial activities of leaf, stem, flower and fruit crude extracts prepared in different solvents were screened by agar well diffusion method[29]. An inoculum size of 1×10 CFU/ml of bacteria which compared with 0.5 McFarland turbidity in a refrigerator for 30 minutes for pre-diffusion of plant extract and turbidity standards was used [30]. Each extract of 100 µl (stock solution 100 mg/ml) was added in a previously marked sterile nutrient agar petriplates and the wells were



Staphylococcus aureus *Serratia marcescens*

Enterococcus faecalis *Bacillus subtilis*

Staphylococcus aureus *Micrococcus luteus*

Figure 1*:* Antimicrobial activity of n-Hexane, Ethyl acetate, Methanol and D/W extract of different parts of *Moringa oleifera.*

Table 2: Antimicrobial activity in crude Ethyl acetate extract of selected plant species.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive						Gram negative					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Moringa oliefera	L	CEA	-	-	-	-	-	-	-	-	-	-	-	-
		HEA	-	-	-	-	-	-	-	-	-	-	-	-
		FEA	6	6	-	-	7	7	10	5	-	9	-	-
	S	CEA	-	-	-	-	-	-	-	6	-	2	-	-
		HEA	-	1	7	-	-	-	4	4	-	4	-	1
		FEA	4	-	-	-	-	-	9	7	2	-	-	4
	F	FEA	1	9	1	-	-	-	6	11	-	6	-	12
	FT-IM	CEA	-	-	-	-	-	-	-	-	-	-	-	-
		HEA	4	7	-	-	9	2	8	8	-	8	-	4
		FEA	5	-	-	-	-	-	7	-	-	-	-	-
	FT-Y	CEA	-	-	-	-	-	-	-	-	-	-	-	-
		HEA	-	-	-	-	-	-	-	-	-	-	-	-
		FEA	5	5	9	-	10	1	7	-	-	4	-	8
	FT-M	CEA	-	-	-	-	10	-	10	1	-	-	-	-
		HEA	6	8	4	-	-	2	7	4	-	4	-	1
		FEA	12	11	15	12	16	10	19	14	12	22	-	15
	FT-R	CEA	7	-	1	-	-	-	7	6	-	-	-	-
		HEA	-	-	-	-	-	-	-	-	-	-	-	-
		FEA	11	11	8	5	12	9	11	11	6	12	-	9
	Seed	CEA	-	6	8	7	7	8	7	4	3	10	6	-
		HEA	7	3	7	-	-	6	7	8	-	6	-	6
Ciprofloxacin (20 µg/ml)			11	10	12	11	14	9	7	14	8	22	10	9
Doxycycline (20 µg/ml)			14	12	9	5	11	8	15	19	11	20	13	4

BC-*Bacillus cereus* ;BS-*Bacillus subtilis* ; EC-*Escherichia coli* ;EN-*Enterococcus faecalis* ; KP-*Klebsiella pneumoniae* ;ML- *Micrococcus luteus*; PS-*Pseudomonas aeruginosa* ; SA- *Staphylococcus aureus*; SE-*Staphylococcus epidermidis*; SM-*Serratia marcescens* ; SP-*Salmonella paratyphi* ;ST-*Salmonella typhi*, L-Leaf; S-Stem; Fruit (IM-Immature; Y-Young; M-Mature; R- Ripen)

CEA- Cold ethyl acetate; HEA- Hot ethyl acetate; FEA-Fresh ethyl acetate;

punched with sterile cork borer and filled with each plant extract. Plates were placed then incubated at 37ºC for 24 hours. After incubation all the plates were examined and zone of inhibition (excluding well diameter in mm) was measured as a property of antimicrobial activity. Antibiotic such as Ciprofloxacin and Doxycycline (20µg/ml) as a positive control and 100% DMSO and solvents i.e. hexane, ethyl acetate and methanol as a negative controls.

Antifungal Activity

The fungal spores were harvested in sterile distilled water from seven days old culture for determination of antifungal activity. The fungal spores count was counted using haemocytometer under aseptic condition, in laminar air flow the potato dextrose agar medium pour into presterilized petriplate and inoculated by fungal strain respectively and kept for 10-15 minutes for solidifying. Each extract of 100 µl (stock solution 100 mg/ml) was added in a previously marked sterile Potato dextrose agar petriplates and the wells were punched with sterile cork borer and filled with each plant extract. Plates were placed then incubated at 27ºC for 48 hours. After incubation all the plates were examined and zone of inhibition (excluding well diameter in mm) was measured as a property of antifungal activity. Antibiotic such as Fluconazole and Ketacozole (20µg/ml) as a positive control and 100% DMSO and solvents i.e. hexane, ethyl acetate and methanol as a negative controls.

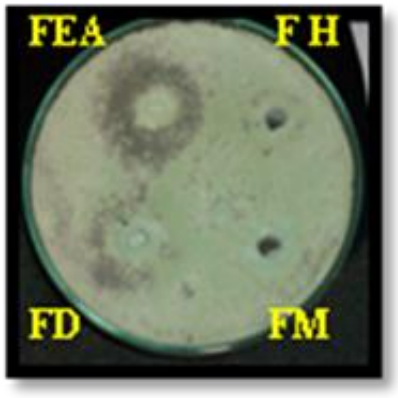


Figure 2: *Moringa Oliefera* fresh stem extract against *Tichoderma harzianum.*

Minimum inhibitory concentration (MIC)

In the present study, minimum inhibitory concentration (MIC) was evaluated by serial broth dilution method for the plant extracts showing more than 7mm 30ml of inhibition. Density of bacterial suspension was maintained uniformly throughout the experiment at $1x10^8$ CFU/ml by comparing with 0.5 Mc Farland turbidity

Table 3: Antimicrobial activity in crude Methanol extract of selected plant species.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive					Gram negative						
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Moringa oliefera	L	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	-	2	-	-	-	-	-	5	-	-	-	-
		FM	7	-	-	-	-	3	1	-	-	7	-	-
	S	CM	4	-	-	-	-	-	-	4	-	-	-	-
		HM	5	2	12	1	22	2	-	-	-	3	-	6
		FM	2	-	-	-	-	-	2	-	-	-	-	2
	F	FM	-	-	-	-	-	-	-	3	5	-	-	4
	FT-IM	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	2	-	-	-	-	-	4	-	-	-	-	-
	FT-Y	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	-		-	-	-	-	-	-	-	-	-	-
		FM	1	3	-	-	-	-	8	-	-	5	-	-
	FT-M	CM	5	-	7	4	7	-	6	8	-	-	-	-
		HM	5	6	10	-	-	4	5	5	-	-	-	-
		FM	-	-	6	-	-	1	-	-	-	-	-	-
	FT-R	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	8	1	-	-	-	1	5	4	-	2	-	5
		FM	2	2	-	7	3	-	2	5	-	3	-	-
	Seed	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	-	-	12	-	7	-	6	4	-	4	-	-
		HEA	7	3	7	-	-	6	7	8	-	6	-	6
Ciprofloxacin (20 µg/ml)			11	10	12	11	14	9	7	14	8	22	10	9
Doxycycline (20 µg/ml)			14	12	9	5	11	8	15	19	11	20	13	4

BC-*Bacillus cereus* ;BS-*Bacillus subtilis* ; EN-*Enterococcus faecalis* ;ML- *Micrococcus luteus*;SA- *Staphylococcus aureus*; SE-*Staphylococcus epidermidis*; EC-*Escherichia coli*; KP-*Klebsiella pneumoniae* ;PS-*Pseudomonas aeruginosa*; SM-*Serratia marcescens* ; SP-*Salmonella paratyphi;* ST-*Salmonella typhi*, L-Leaf; S-Stem; Fruit (IM-Immature; Y-Young; M-Mature; R- Ripen), CM-Cold methanol; HM-Hot methanol ; FM-Fresh

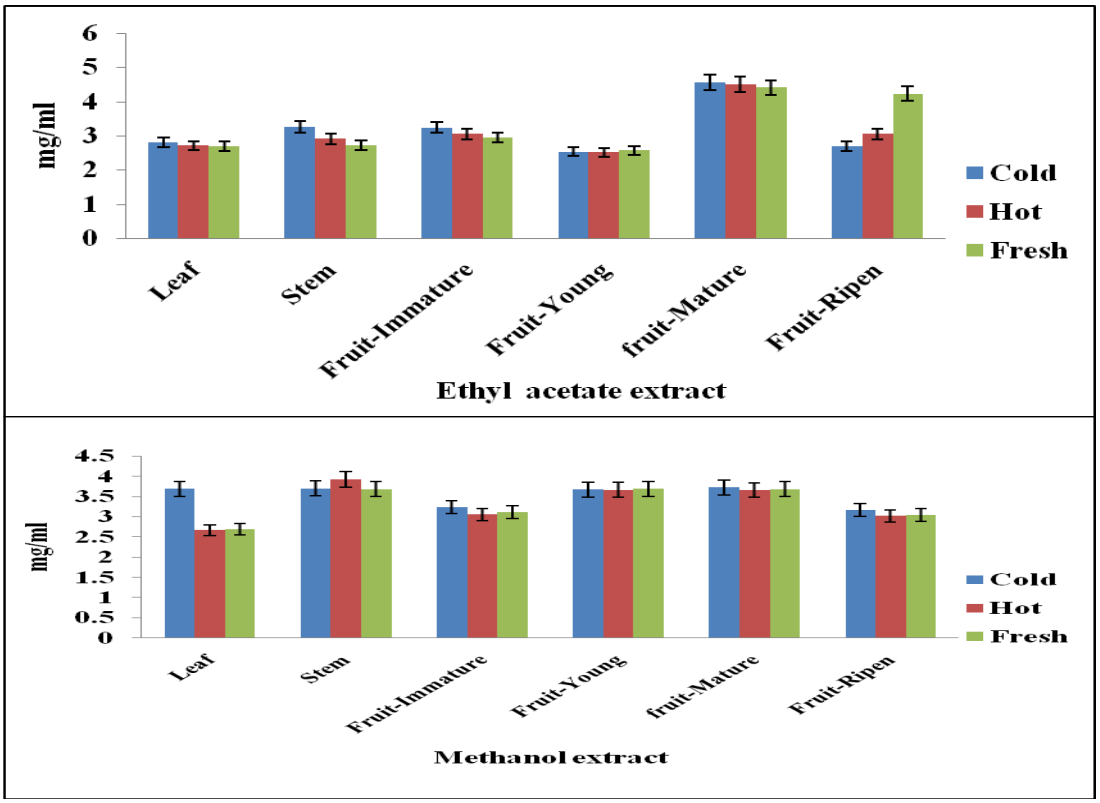


Figure 3: Total phenols content in ethyl acetate and methanolic extract in different parts of *Moringa oleifera.*

standards. 40µl of plant extract from stock solution (100mg/ml) was taken into the first dilution tube and added 960µl of nutrient broth and mixed well. 500µl of solution from first dilution tube was taken and added 500µl of nutrient broth into second tube, this step was repeated 5times and from last tube 500µl solution was discarded. Final volume was made upto 1ml by adding 500µl of test organism in each tube. The MIC was tested in the concentration range between 8mg/ml to 0.250mg/ml. Tubes were incubated at 37ºC for 24 hours in an incubator. 100µl (0.1%) 2,3,5 – triphenyl tetrazolium chloride solution as a growth indicator was incorporated in each tube to find out the bacterial inhibition and tubes were further incubated for 30 minutes at 37ºC. Bacterial growth was visualized when colorless 2, 3, 5-triphenyl tetrazolium chloride was converted into red color formazon in the presence of live bacteria. MIC assay was repeated thrice by using DMSO and nutrient broth as controls.

Quantitative phytochemical analysis of crude extract

The crude extracts of different plant parts (leaf, stem, flower, and fruit) were prepared in ethyl acetate; methanol and distilled water were evaluated for quantitative analysis of phenols and flavonoids by using standard procedures.

Total Phenol estimation

Each plant extract (0.2 ml) in test tube was taken separately and added 3 ml distilled water and then added 0.5 ml FCR. After 3min. of incubation, 2 ml of 20% Na_2CO_3 solution was added into each tube and mixed thoroughly. Reaction tubes were placed in boiling water bath for exactly 1 min, cooled and the absorbance was measured at 650 nm against a reagent blank using visible spectrophotometer. A standard curve was prepared using different concentrations of catechol. Total phenol was expressed as mg phenol in terms of catechol per gram of fresh tissue.

Total Flavonoids estimation

Aluminum chloride colorimetric method was used with some modification to determine flavonoids content. 1 ml of each plant extract was mixed with 3ml of methanol, 0.2ml of 10% Aluminium chloride, 0.2ml of 1M Potassium acetate and 5.6ml of D/W and kept at room temperature for 30minutes.The absorbance was measured at 420nm using UV-Visible spectrophotometer. Quercetin was used as standard 1mg/ml. All the tests were performed in triplicates. Flavonoid content was determined from the standard curve and expressed as Quercetin equivalent mg/gm of extracted compound[31].

RESULTS AND DISCUSSION

In-vitro antimicrobial activity

The present study of fresh, cold and hot extraction in n-hexane, ethyl acetate, methanol and distilled water showed zones of inhibition of bacterial growth at varying with extraction methods. The antibacterial activity against

Table 4: Antimicrobial activity in crude D/W extract of selected plant species.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive					Gram negative						
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Moringa oliefera	L	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	-	-	-	-	-
		FD	6	-	-	-	-	3	1	-	-	7	-	-
	S	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	4	-	-	-	-	-	-	-	-	1
		FD	6	-	-	9	7	-	2	2	-	7	-	2
	F	FD	12	-	13	6	-	4	1	-	5	-	-	3
	FT-IM	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	3	-	-	-	-	-	-	-	-	-	-	-
		FD	-	-	-	-	-	-	-	6	10	3	-	-
	FT-Y	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	-	-	-	-	-
	FT-M	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	4	5	-	27	-	1	3	-	-	-	-
		FD	-	-	6	-	-	-	-	-	-	-	-	-
	FT-R	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	4	-	-	-	-
		FD	-	7	-	18	-	-	-	-	-	1	-	1
	Seed	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	2	1	-	-	1	-	-	3	-	1
Ciprofloxacin (20 µg/ml)			11	10	12	11	14	9	7	14	8	22	10	9
Doxycycline (20 µg/ml)			14	12	9	5	11	8	15	19	11	20	13	4

BC-*Bacillus cereus* ;BS-*Bacillus subtilis* ; EN-*Enterococcus faecalis* ;ML- *Micrococcus luteus*;SA- *Staphylococcus aureus*; SE-*Staphylococcus epidermidis*; EC-*Escherichia coli*; KP-*Klebsiella pneumoniae* ;PS-*Pseudomonas aeruginosa*; SM-*Serratia marcescens*; SP-*Salmonella paratyphi* ; ST-*Salmonella typhi*, L-Leaf; S-Stem; Fruit (IM-Immature; Y-Young; M-Mature; R- Ripen), CD- Cold distilled water; HD- Hot distilled water; FD-Fresh distilled water

various plant part extracts varied against different test organisms. Among different parts hexane extracts only fresh leaf and hot stem and mature fruit extracts, ethyl acetate fresh part extracts under the study, hot methanol stem and mature fruit extracts, distilled water fresh flower, mature and ripen fruit fresh and hot extracts have shown considerable antibacterial activities to one or more organisms. Remaining extracts prepared in hexane, ethyl acetate, methanol or distilled water exhibited least or no activity against the selected test organisms (Table.1-4)

The ethyl acetate extract of fresh mature and ripen fruit showed stronger antibacterial activity against studied Gram- negative bacteria and Gram-positive bacteria and similarly fresh ethyl acetate stem extract display antifungal activity.

Ethyl acetate mature fresh fruits extract displayed better activity (Fig. 1) against SM (22mm), good activity against EC (10mm) (SA (16mm), EN and ST (15mm) whereas moderate activity against KP (14mm), ML, BC, PS (12mm), BS (11mm) and SE (10mm) whereas Hot distilled water mature fruits extract showed better activity against SA (27mm) whereas cold ethyl acetate mature fruit extract showed moderate activity against SA, EC (10mm). The ethyl acetate fresh ripen fruits extract exhibited moderate activity against SM, SA (12mm), BS, KP, BC (11mm), EC (11mm) , SE, ST (9mm) and EN (8mm) while least to no activity in other test organisms and Distilled water fresh ripen fruit extract showed good activity against ML (18) whereas the cold seed ethyl acetate extract demonstrated moderate activity against SM (10mm) and fresh ethyl acetate flower extract showed moderate activity against ST (12mm), KP (11mm) similarly distilled water fresh flower extract demonstrated moderate activity against selected bacterial strains EN (13mm) and BC (12mm).

However, ethyl acetate hot mature fruit and seed extract, young fresh fruits extract and methanolic hot ripen fruits showed least to no inhibitory action against the tested bacteria. Methanolic hot stem extract exhibited better activity against SA (22mm), moderate activity against EN (12mm). Fresh leaf n-Hexane and ethyl acetate extract and stem n-Hexane hot, fresh ethyl acetate extract and fresh distilled water showed moderate activity in selected bacterial strains but no inhibitory effect of hot and cold

Table 5: Antifungal activity of selected crude ethyl acetate extract.

Plant Name	Part used	Extract	AN	AB	APF	AF	CA	FO	TH
	Leaf	CEA	-	-	-	-	8	-	-
		CEA	-	-	-	-	-	-	4
	Stem	HEA	-	-	-	-	-	-	-
Moringa oleifera		FEA	-						16
		CEA	-	-	-	-	-	-	
	Seed	HEA	-	-	-	-	-	-	5
		FEA	-	-	-	-	-	-	-
Ketacozole	-	-	9	-	10	10	20	12	10
Fluconazole	-	-	20	-	17	19	21	18	16

AN: *Aspergillus niger* APF:*Aspergillus paracitic*
CA: *Candida Albicans* AF:*Aspergillus flavus*
TH: *Trichoderma harzanium* AB:*Alternata burnsi* FO:*Fusarium oxysporum*
J- Juice; LT-Latex; L-Leaf; S-Stem; W-Whole plant ; INF –Inflorescence
CEA- Cold ethyl acetate; HEA- Hot ethyl acetate; FEA-Fresh ethyl acetate;
DMSO- Dimethyl sulphoxide

Table 6: Minimum inhibitory concentration of effective n-Hexane plant extract.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive						Gram negative					
			BC	BS	SA	ML	SE	EN	EC	KP	PS	ST	SP	SM
Moringa oliefera	L	FH	>8	-	-	-	-	-	8	-	-	-	-	>8
		FH	-	-	-	-	-	-	>8	-	-	-	-	-
	S	CH	-	-	-	-	-	-	-	-	-	-	-	-
		HH	-	>8	-	-	-	-	-	-	-	-	-	>8
	F	FH	-	-	-	-	-	-	-	-	-	-	-	-
		FH	-	-	>8	-	-	-	>8	-	-	-	>8	>8
	FT-IM	CH	-	-	-	-	-	-	-	-	-	-	-	-
		HH	-	-	-	-	-	-	>8	-	-	-	-	-
	FT-Y	FH	-	-	-	-	-	>8	-	-	-	-	-	-
		HH	-	-	-	-	-	-	-	-	-	>8	-	-
	FT-M	FH	-	>8	>8	>8	-	8	-	>8	-	-	-	-
		CH	-	-	-	-	-	-	>8	-	-	>8	-	-
		HH	>8	-	>8	-	-	-	-	-	-	-	-	-

Table 7: Minimum inhibitory concentration of effective Ethyl acetate plant extract.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	SM	KP
Moringa oliefera	L	CEA	-	-	-	-	-	-	-	-	-	-	-	-
		HEA	-	-	-	-	-	-	-	-	-	-	-	-
		FEA	>8	>8	>8	>8	-	-	>8	-	-	-	8	>8
	S	CEA	-	-	-	-	-	-	-	-	-	-	-	>8
		HEA	-	-	-	-	-	>8	-	-	-	-	-	-
		FEA	-	-	-	-	-	-	>8	-	-	-	-	>8
	FT-IM	CEA	-	-	-	-	-	-	-	-	-	-	-	-
		HEA	-	>8	8	-	-	-	2	-	-	-	8	8
		FEA	>8	-	-	-	-	-	4	-	-	-	-	-
	FT-Y	CEA	-	-	-	-	-	-	-	-	-	-	-	-
		HEA	-	-	-	-	-	-	-	-	-	-	-	-
		FEA	8	8	-	-	-	4	8	8	-	-	-	-
	FT-M	CEA	-	-	4	-	-	-	4	-	-	-	-	-
		HEA	8	8	-	-	-	-	8	-	-	-	-	-
		FEA	8	2	8	8	8	8	8	2		>8	2	2
	FT-R	CEA	>8	-	-	-	-	-	>8	-	-	-	-	>8
		HEA	-	-	-	-	-	-	-	-	-	-	-	-
		FEA	4	4	2	>8		>8	4	>8		>8	4	4
	Seed	CEA	-	-	>8	8	>8	4		-	-	-	4	-
		HEA	8	-	-	-	-	>8	>8		-	-	-	4

leaf ethyl acetate extract against selected bacterial strains. But no inhibitory effect of distilled water leaf, immature fruit, young fruit, mature fruit, stem and seed extracts was observed. The activity of the all extracts was compared with standard antibiotic Ciprofloxacin and Doxycycline[32].

Potential antibacterial activity of fresh leaf juice and ethanol extracts against four Gram negative bacteria *Shigella shiga, S. sonnei, PS*, and *Pseudomonas spp*. and six Gram-positive bacteria SA, BC, BS, BM, *Streptococcus-B- haemolytica*, *Sarcina lutea* and their respective MIC values. In this study *Serratia marcescens* appeared to be more susceptible to fresh leaf extract followed by EC, KP and BC.

In this study, fresh mature and ripen fruit ethyl acetate extract and methanolic hot stem extract exhibited higher antibacterial activity compared Ciprofloxacin and Doxycycline. But only the powder (dissolved in DMSO) from fresh extract exhibited the highest antibacterial activity against all the studied bacteria.

However, fresh ethyl acetate stem extract display good antifungal activity against *Trichoderma harzanium* (16mm) (Fig.2).

n-Hexane, methanol and distilled water did not show any inhibitory action against *Aspergillus niger*, *Candida albicans, Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Alternaria burnsii.* (Table 5).

Ethyl acetate fresh ripen fruit extract displayed low MIC values at 2mg/ml against SA, 4mg/ml against BC, BS, EC, SM and >8mg/ml against KP, SE, EN, ST and PS, while in cold ripen fruit extract showed MIC values at >8mg/ml against BC, EC and KP. Methanolic hot ripened fruit extract showed MIC value at 4mg/ml against BC and >8mg/ml against EC, ST. Distilled water ripen fruit extract displayed MIC value at 4mg/ml against ML, 8mg/ml against BS. The MIC value of cold ethyl acetate seed extract was observed at 4mg/ml against EN, SM, 8mg/ml against SE, >8mg/ml against ML, SA while in hot ethyl acetate seed extract MIC value was confirmed at 4mg/ml against KP, 8mg/ml against BC, EN and >8mg/ml against EC. Various other prepared extracts exhibited MIC values mostly at 8 or >8 mg/ml against the tested organisms (Tables 6-9). Onsare *et al.* reported husk, seed and pod extract showed antimicrobial activity against EN, SA, SE, EC, KP, PS and ST and also showed low MIC values. In this study, low MIC values were obtained in fresh mature and ripened fruit SA, BC, BS, EC and SM in the range of 2mg/ml and 4mg/ml and for other organisms more than 8mg/ml[33].

In this study, results of phytochemical analysis indicate that of the screened bioactive constituents phenol and flavonoids only was found to be present in the leaf, stem, mature, ripen, young, and immature fruits of *Moringa oleifera.* Demonstration of antimicrobial activity against both Gram-positive and Gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds[34,35].

The attributing factor for antibacterial activity is total phenol concentration in cold, hot and fresh ethyl acetate and methanol extracts of different parts of *Moringa oleifera* (Fig.3) and observed that cold, hot and fresh methanol extracts showed higher phenolic content in comparison with ethyl acetate cold, hot and fresh extracts but varied among the different part extracts. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites[36].

Estimated total flavonoids in cold, hot and fresh ethyl acetate and methanol different part extracts of *Moringa oleifera* (Fig.4) and observed that cold, hot and fresh methanol different part extracts showed higher flavonoids content in comparison with ethyl acetate cold, hot and

Table 8: Minimum inhibitory concentration of effective Methanol plant extract.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive					Gram negative						
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	SM	KP
Moringa oliefera	L	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	-	-	-	-	-	-	-	-	-	-	-	8
		FM	>8	-	-	-	-	-	-	-	-	-	>8	-
	S	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	>8	-	-	-	-	4	-	>8	-	-	-	-
		FM	-	-	-	-	-	-	-	-	-	-	-	-
	FT-IM	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	-	-	-	-	-	-	-	-	-	-	-	-
		FM	-	>8	-	-	-	-	>8	-	-	-	-	-
	FT-Y	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	-	-	-	-	-	-	-	-	-	-	-	-
		FM	-	-	-	-	-	-	8	-	-	-	>8	-
	FT-M	CM	8	-	8	-	-	>8	8	-	-	-	-	8
		HM	>8	>8	-	-	-	8	>8	-	-	-	-	>8
		FM	-	-	-	-	-	>8	-	-	-	-	-	-
	FT-R	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	4	-	-	-	-	-	>8	>8	-	-	-	-
		FM	-	-	-	-	-	-	-	-	-	-	-	>8
	Seed	CM	-	-	-	-	-	-	-	-	-	-	-	-
		HM	-	-	>8	-	-	-	>8	-	-	-	-	-

Table 9: Minimum inhibitory concentration of effective D/W plant extract.

Plant Name	Part used	Extract	Zone of Inhibition											
			Gram positive					Gram negative						
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	SM	KP
Moringa oliefera	L	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	-	-	-	-	-
		FD	>8	-	-	-	-	-	-	-	-	-	>8	-
	S	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	-	-	-	-	-
		FD	>8	-	8	-	8	-	-	-	-	-	8	-
	FT-IM	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	-	-	-	-	-
		FD	-	-	-	-	-	-	-	-	-	4	-	>8
	FT-Y	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	-	-	-	-	-
		FD	-	-	-	-	-	-	>8	-	-	-	>8	-
	FT-M	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	0.125	-	-	8	-	-	-	-	-	-
		FD	-	-	-	-	-	8	-	-	-	-	-	-
	FT-R	CD	-	-	-	-	-	-	-	-	-	-	-	-
		HD	-	-	-	-	-	-	-	-	-	-	-	-
		FD	-	8	8	-	4	-	-	-	-	-	-	-

fresh different part extracts. Amongst various parts of *Moringa oleifera*, Mature, ripen, young, and immature fruits showed similar or the same range flavonoids content followed by leaf and stem cold, hot and fresh ethyl acetate and methanol extracts. With regard to the spectrum of antibacterial activity, results show that ethyl acetate extract of fresh mature and ripen fruit and stem have a broad spectrum of activity. This could be due to the presence of flavonoids and phenols which have been reported to have broad spectrum antibacterial activity. On the other hand n-hexane, methanol and distilled water extract showed a narrow spectrum of activity, the antimicrobial activity being restricted against selected bacteria and fungi.

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities[37]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, etc[38]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial

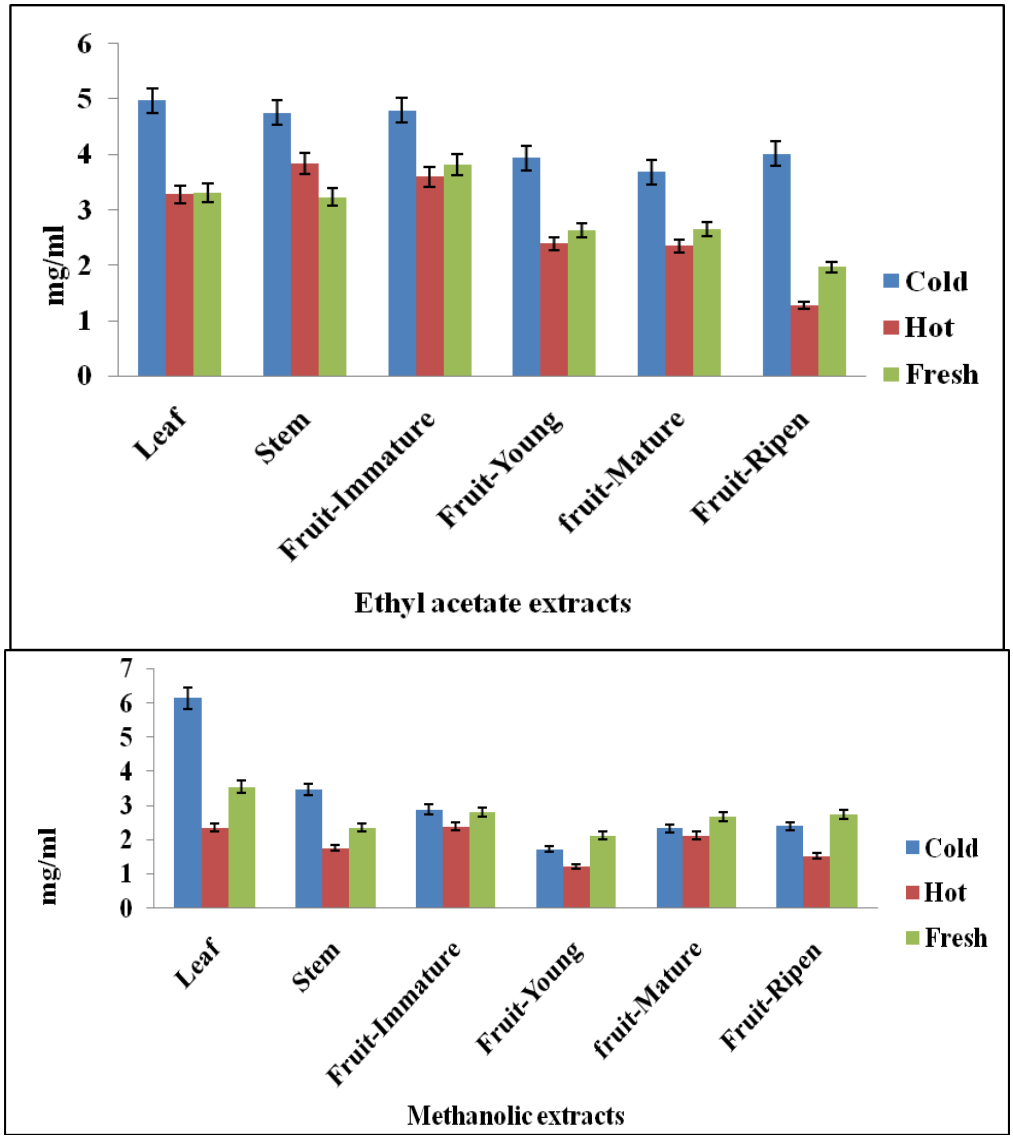


Figure 4: Total Flavonoids content in ethyl acetate and methanolic extract in different parts of *Moringa oleifera*.

substances against wide array of microorganisms *In-vitro*. Their activity is may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall[39]. They also are effective antioxidant and show strong anticancer activities[40,42].

Differences in polarity among the various solvents are perhaps responsible for the differences in solubility of plant active principles, hence variation in degree of activity. As far as the degree of susceptibility is concerned results (diameter of inhibition zone) clearly indicate that gram positive and gram negative bacteria are more susceptible to fresh ethyl acetate mature and ripen fruit and stem extract compared to n-Hexane, methanol and D/W extract and a narrow inhibition zone was recorded against fungi implying that the activity of fresh ethyl acetate stem extract only showed inhibition against *Trichoderma harzanium* compared to other selected fungal strains. When compared to the activity of a standard antibiotic, Ciprofloxacin and Doxycycline (20 µg) against the tested bacteria an interesting finding is the activity of crude fresh ethyl acetate mature and ripen fruit against SM and SA a bacterial isolate compared to other selected microorganisms. Gram-negative bacteria have been found to be less susceptible to plant extracts in earlier studies done by other researchers[43, 44].

Similar reports on phytochemical composition of various medicinal plants were made earlier by many workers[45-49]. However, it is very essential to isolate the bioactive fractions from these major groups so that it can be used further in designing specific drugs.

CONCLUSION

The antimicrobial activity of *Moringa oleifera ethyl acetate* extracts observed in this study might be due to the, flavonoids and phenols detected to be present in the plant. But, their antibacterial activity remains to be proofed. In general the antimicrobial activities of the n-hexane, methanol and distilled water extract are found to be low compared to the standard antibiotics used in this study. But it is hoped that they might produce comparable effect after further purification and analysis of the active constituents. In conclusion, the plant extract displayed an

activity against gram negative and gram positive which could support the traditional claim of the society. So, based on the findings, the authors recommended further study to be conducted concerning the chemical compositions and the structure elucidation of the active component of the plant.

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REFERENCES

1. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS and Subbaraju GV. ssessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science and Engineering 2005;* 2: 125-134.
2. Laloo RC, Kharlukhi L, Jeeva S and Mishra BP. Status of medicinal plants in the disturbed and the undisturbed sacred forests of Meghalaya, northeast India: population structure and regeneration efficacy of some important species. *Current Science* 2006; 90: 225-232.
3. Van Wyk BE and Wink M. Medicinal plants of the world: An illustrated scientific guide to important medicinal plants and their uses. South Africa: Briza Publications, 2004.
4. Mbuya LP, Msanga HP, Ruffo CK, Birnie A, Tengnäs B. Useful Trees and Shrubs for Tanzania: Identification, Propagation and Management for Agricultural and Pastoral Communities. Technical Handbook No 6. Regioal Soil Conservation/SIDA, Nairobi, 1994 p. 478.
5. Davis DK, Quraishi K, Sherman D, Sollod A and Stem C Ethnoveterinary medicine in Afghanistan: An overview on indigenous animal health care among Pashtun Koochi nomads. Journal of Arid Environments 1995; 31: 483-500.
6. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4(7): 685-688.
7. Kala CP. Local preference of ethno-botanical species in the Indian Himalaya. Implication for environmental conservation. Current Science 2007; 93(12): 1828-1834.
8. Sharief MU, Rao RR. Ethno-botanical studies of Shompens-A critically endangered and degenerating ethnic community in Great Nicobar Island. Current Sciences 2007; 93(11): 1623-1628.
9. Dubey NK, Kumar R, Tripathi P. Global promotion *of* herbal medicine: India's opportunity. Current Science 2004; 86:37-41.
10. Padma TV. India Ayurveda. *Nature, 2005,* 436:486.
11. Ivanova D, Gerova D, Chervenkov T and Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethanopharmacology 2005*; 96: 145-150.
12. Shrikumar S and Ravi TK .Approaches towards development and promotion of herbal drugs. *Pharmacology reviews* 2007; 1: 180-184.
13. Vijaya K and Ananthan S. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. *Journal of Alternative Complementary Medicine* 1997; 3:13-20.
14. Dilhuydy JM. Patients attraction to complementary and alternative medicine (CAM): a reality which physicians can neither ignore nor deny. Bulletin Cancer 2003; 90:623-628.
15. Silver LL. Discovery and development of new antibiotics: the problem of antibiotic resistance. Journal of Antimicrobial Agents and Chemotherapy. 1993; 37: 377-383.
16. Pretorius CJ and Watt E. Purification and identification of active components of *Carpobrotus edulis* L. Journal of Ethnopharmarcology 2001; 76:87-91.
17. Sharif MDM and Banik GR. Status and utilization of medicinal plants in Rangamati of Bangladesh. Res. Journal of Agriculture of Biological Sciences 2006; 2(6): 268-273.
18. Doughari JH, El-Mahmood AM. and Manzara S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. African. Journal of Microbiology 2007; 037- 041.
19. Ramachandran C, Peter KV and Gopalakrishnan PK. Drumstick (*Moringa oleifera*): A multipurpose Indian vegetable. Economic Botany, 1980; 34(3):276-283.
20. Odebiyi A, Sofowora AE. Phytochemical screenings of Nigerian medicinal plants parts. Lyodia 1999; 44: 234-246.
21. Thilza I, Sanni S, Zakari A, Muhammed T, Musa B. In vitro antimicrobial of water extract of *Moringa oleifera* leaf stalk on bacterial normally implicated in eye disease. Acad. Aren 2010; 2:80-83.
22. Caceres A, Cebreva O, Morales O, Miollinedo P, Mendia P. Pharmacological properties of *Moringa oleifera*: Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology 1999; 33:213-216.
23. Doughari JH, Pukuma MS, De N. Antibacterial effects of *Balanitesaegyptiaca* and *Moringa oleifera* Lam. on *Salmonella typhi*. African Journal of. Biotechnology 2007; 6(19):2212- 2215.
24. Kekuda N, Prashith MTR, Swathi D, Nayana KV, Meera BA and Rohini TR**.** Antibacterial and antifungal efficacy of steam distillate of *M. oleifera* Lam. Journal of Pharmaceutical Science 2010; 2:34-37.
25. Jamil A, Shahid M, Khan MM, Ashraf M. Screening of some medicinal plants for isolation of antifungal proteins and peptides. *Pakistan Journal of Botany* 2007; 39:211-221.
26. Nikkon F, Saud A, Rahman MH, Haque ME. *In vitro* antimicrobial activity of the compound isolated from

chloroform extract of *Moringa oleifera.* Pakistan Journal of Biological Science 2003; 6:1888-1890.
27. Shah GL. Flora of Gujarat State, Vol. 1& 2, Sardar Patel University Press, Vallabh Vidyanagar, 1978.
28. Houghtan PJ and Raman R. Laboratory handbook for the fractionation of Natural extracts. *Chapman and Hall*. London. 1998, 1-28.
29. Perez C, Pauli M and Bazaerque P. An antibiotic assay by the agar well method. Acta. *Biological Medicine Experimentalis 1990;* 15: 113-115.
30. Perilla MJ. Manual for the laboratory identification ad antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world.WHO (World Health Organization), 2003; 209-214.
31. Aiyegroro OA and Okoh AI. Preliminary Phytochemical screening and in vitro antioxidant activities of aqueous extract of Helichrysum longifolium DC. BMC *Complimentary and Alternate Medicine* 2010, 10: 21.
32. Rahman MM, Sheikh1 MMI, Sharmin SA, M. Soriful Islam, Rahman MA, Rahman MM 2, Alam MF. Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against Some Human Pathogenic Bacteria *CMU.J.Nat.Sc i 2009*, 8(2): 219-227.
33. Onsare JG, Kaur H. and Arora DS. Antimicrobial activity of *Moringa oleifera* from different locations against some human pathogens. *Academic Journal of Medicinal Plants* 2013, 1(5): 80-91.
34. ichewicz, R.H., Thorpe, P.A., 1996. The antimicrobial properties of Chile peppers (Capsicum species) and their uses in Mayan medicine. *J. Ethnopharm.* 52 (2), 61–70.
35. Srinivasan, D., Perumalsamy, L.P., Nathan, S., Sures, T., 2001.Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharm. 49, 217–222.
36. Singh, R., Singh, S.K. and Arora, S. (2007). Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food and Chemical Toxology,* 45: 1216-1223.
37. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd Edn. Spectrum Books limited, Ibadan, Nigeria 1993; 1-153.
38. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A and Bora U. Indian medicinal herbs as sources of antioxidants. *Food Research International* 2008. 41: 1-15.
39. Marjorie C. Plant products as antimicrobial agents.*Clinical Microbiology. Rev* 1996; 12: 564- 582.
40. Salah N, Miller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E, Evans C. Polyphenolic flavonoids asscavenger of aqueous phase radicals as chai breaking antioxidant.Arc. *Biochem. Broph* 1995; 2: 339-346.
41. Del-Rio A, Obdululio BG, Casfillo J, Main FG and Ortuno A. Uses and properties of citrus flavonoids. *African Journal of Biotechnology* 1997; 4(7): 685-688.
42. Okwu DE. Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global Journal of Pure and Applied Science 2001;* 7: 455-459.Kuhnt M, Probestle A, Rimpler H, Bauer R and Hei M. Biological and Pharmacological activities and further constituents of *Hyptis verticillata*. *Planta Medica* 1994; 61:227-232.
43. Afolayan AJ and Meyer. JJM. Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). Journal of Ethnopharmarcology 1995. 47: 109-111.
44. Chopra RN, Nayar SL and Chopra IC. Glossary of Indian Medicinal Plants Vol.I, Council of Scientific and Industrial Res, New Delhi,1956.
45. Del-Rio A, Obdululio BG, Casfillo J, Marin FG and Ortuno A. Uses and Properties of citrus flavonoids. *Journal of Agriculture and Food Chemistry* 1997; 45: 4505- 4515.
46. Obadoni BO and Ochuko PO. Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences 2001;* 8: 203-208.
47. Okwu DE. Phytochemicals and vitamin content of indigenous spices of Southeastern Nigeria. *J. Sustain. Agric. Environ 200;* 6(1): 30- 37.
48. Koche DK, Shirsat RP, Syed I and Bhadange DG. Phytochemical screening of eight folk medicinal plants from Akola District (MS) India. *International Journal of Pharma and Bioscience* 2010; 1(4): 256-261.