Agrosearch (2013) 13 No.1: 95 - 113

Http://dx.doi.org/10.4314/agrosh.v13i1.10

THE ANTIMICROBIAL EFFECTS OF THE LEAF EXTRACTS OF *Moringa oleifera* ON SELECTED CLINICAL BACTERIAL ISOLATES

*Adetitun, D.O., Araoye, H. K., Akinyanju, J. A. and Anibijuwon, I. I.
Department of Microbiology, University of Ilorin, Ilorin, Nigeria.
*Correspondence: adetitun.do@gmail.com, +2348036910988

ABSTRACT

Antibiotic susceptibility pattern of two batches of clinical isolates consisting of eight organisms were collected from the Department of Medical Microbiology, University of Ilorin Teaching Hospital (UITH). The isolates collected for the first batch were from wound infections and include Staphylococcus aureus, Non-haemolytic Streptococcus, Escherichia coli, Proteus sp, Pseudomonas sp, Klebsiella pneumonia, Vibrio cholera and Salmonella typhi. The organisms in the second batch include Staphylococcus aureus, Streptococcus thermophilus, Escherichia coli, Pseudomonas sp, Klebsiella pneumonia, Bacillus anthracis, Salmonella typhi and Vibrio cholera. The test showed that all the test organisms were susceptible to Ofloxacin, Ciprofloxacin, Pefloxacin and Gentamycin with reasonable zones of inhibition. Cold Methanoic Extract, 95% Ethanoic Extract, Absolute Ethanoic Extract and Distilled Water Extract of Moringa oleifera were assayed for in vitro antibacterial activity using agar diffusion method. The first set of organisms produced zones of inhibition ranging from 1mm-6.5mm. The second set of organisms produced zones of inhibition ranging from 2mm-21mm. The result showed that the potency of the extracts depend on the solvent used for the extraction and method of extraction. Cold Methanoic Extraction (CME) was found to be most effective. The extract has its highest activity at pH 4.29. The extracts were found to be bacteriostratic but not bacteriocidal.

Keywords: *Moringa oleifera*, antimicrobial effect, leaf extractsand clinical isolates

INTRODUCTION

Moringa oleifera belongs to the order Brassicales and family Moringaceae. The word *Moringa* probably came from davidian language Tamil and commonly referred to as “shojne” in Bengali. It is an exceptionally nutritious vegetable tree with a variety of potential uses. The tree is slender, with drooping branches that grow to approximately 10m in height. The *“Moringa”* tree is grown mainly in semi arid, Tropical and sub tropical areas. It grows best in dry sandy soil; it tolerates poor soil including coastal areas. The immature green pods called “drumstick” are probably the most valued and widely used part of the tree. The seeds are sometimes removed from matured pods and eaten like peas or roasted like nuts. The extracts from the seeds is used as a flocculant in low-cost form of water treatment.tep by step extraction and treatment procedure to produce “90.00%” to “99.99%” bacterial reduction. The seeds are also considered an excellent biofuel source for making biodiesel. The flowers are edible when cooked and are said to taste like mushrooms. The

roots are shredded and used as condiment in the same way as horseradish; however they contain the alkaloid spirochin, a potentially fatal nerve-paralyzing agent. The leaves are highly nutritious being a significant source of beta-carotene, vitamin c, protein, iron and potassium. The leaves are also commonly crushed into powder and used in soups and sauces. The tree is also a good source of calcium and phosphorous (Olsen, 1987).

Specific components of *Moringa* preparations that have been reported to have hypotensive, anticancer and antibacterial activity include 4-(4'-0-acetyi-a-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin and pterygospermin. *Moringa* preparations have been cited in the scientific literature as having antibiotic, antitrypanosome, antiulcer, antinflammatory and hypoglycermic as well as having considerable efficiency in water purification by flocculation, sedimemtation, antibiosis and even reduction of *Schistoma cercariae* titer (Fahey, 2005).

The *"Moringa"* tree is considered one of the world's most useful trees, as almost every part of*Moringa* tree can be used as food or has some other beneficial properties (Fuglie, 2000). *Moringa* trees have been used to combat malnutrition, especially among infants and nursing mothers (Jed*et al.*, 2005). According to ethnobotanical studies its roots are useful in dyspepsia, anorexia, verminosis, diarrhea, colic, flatulence, paralysis, inflammations, amenorrhea, dysmenorrheal fever, strangury, vesicle and renal calculi. It is used in cough, asthma, bronchitis, pectoral diseases, splenomegaly, epilepsy and cardiopathy. Leaves are anti-inflammatory, anodyne, antihelmic, ophthalmic and rich in Vitamin A and C (Vastuguna *et al.,* 1989). The method often used to test the efficacy of commercially manufactured antibiotics against microorganism is the Kirby-Bauer method (Willey *et al., 2*008).

The objectives of this work were to determine the susceptibility of selected organisms to the leaf extracts of *Moringa oleifera, t*o determine the effectiveness of extraction solvent and their different concentrations on the selected microorganisms and to compare the susceptibility of selected organisms to some antibiotics.

MATERIALS AND METHODS

Collection of Test Organisms

Pure culture of selected organisms were obtained from the medical microbiology laboratory of University of Ilorin Teaching Hospital (U.I.T.H) and preserved by inoculating into sterile agar slants and were kept in the refrigerator to serve as stock culture. Organisms collected were *Staphylococcus aureus,* Non-Haemolytic *Streptococcus, Escherichia coli, Klebsiella pneumonia, Proteus* sp, *Pseudomona*s sp, *Streptococcus thermophilus, Bacillus anthracis, Vibrio cholera and Salmonella typhi.* Biochemical tests were used to confirm the purity of the organisms.

Culture Media

The media used include Nutrient agar, Mueller Hinton Agar and Nutrient broth. They were prepared according to the manufacturer's specifications.

Collection of Plant Material

Fresh plant leaves of *Moringa oleifera* were collectedfrom Plant Biology Department, University of Ilorin. Identification was carried out at the Herbarium unit of the PlantBiology Department. The leaves were air-dried. The dried leaves were ground into powder and stored in a tightly covered container.

Preparation of Plant Extracts

The extraction methods include 95% ethanoic extraction (95%EE), cold ethanoic extraction (CEE), cold methanoic extraction (CME) and extraction using distilled water (DWE).

95% Ethanoic Extraction

Fifty grams (50g) of the powdered plant material were soaked in 200ml of 95% ethanol in a conical flask. This was placed on a shaker for 120hrs. The resulting extract was passed through muslin cloth and further filtered through Whatman No 1 filter paper. The resulting filtrate was evaporated to dryness at 40°C in a water bath. The resulting residue was reconstituted again in 95% ethanol at stock concentration of 200mg/ml. the stock concentration was filtered with membrane filter before being stored in an amber bottle at room temperature (Awe and Omojasola, 2003).

Absolute Ethanoic Extraction (AEE)

Fifty grams of the powdered plant material were soaked in 200ml of absolute ethanol in a conical flask. This was placed on a shaker for about 120hrs (5days) at about 28± 2°C. The resulting extract was passed through Muslin cloth and filtered through a Whatman No 1 filter paper. The resulting extract was evaporated to dryness at 40°C in a water bath. The residue obtained was reconstituted again into 95% ethanol at stock concentration of 200mg/ml. The stock concentration was filtered with membrane filter before storage.

Cold Methanoic Extraction (CME)

Fifty grams of the plant material were soaked in 200ml of methanol in a conical flask. This was placed on a shaker for about 120hrs at about 28± 2°C. The resulting extract was passed through Muslin cloth and filtered through a Whatman No 1 filter paper. The resulting extract was evaporated to dryness at 40°C in a water bath. The residue obtained was reconstituted again in 95% ethanol at stock concentration of 200mg/ml. The stock concentration was filtered with membrane filter and stored in an amber bottle at room temperature.

Distilled Water Extraction (DWE)

Fifty grams of the plant material were soaked in 200ml of distilled water in a conical flask. This was placed on a shaker for about 120hrs. The resulting extract was passed through muslin cloth and filtered with Whatman No1 filter paper. The resulting extract was evaporated to dryness at 80°C in

a water bath. The resulting residue was reconstituted again in sterile distilled water (Mashiar *et al.*, 2009).

Assay of Plant Extracts

Each of the extracts was tested for growth or contaminants by plating them on nutrient agar and incubating at 37°C for 24hrs. Where no visible growth was observed, the extract was then assessed for antimicrobial activity.

Determination of pH of the Extract of *Moringa oleifera*

The pH of the extracts was determined by dipping a pH meter into conical flasks containing the extracts. The constant reading on the pH meter was taken as the pH of the antimicrobial substance in question.

Sensitivity Test of Extract of *Moringa oleifera* using Ditched Plate Method

Overnight broth culture of the test organisms were swabbed on sterile Mueller Hinton agar in Petri dishes using sterile cotton swab sticks. A sterile cork borer of size 6mm in diameter was used to make ditches on the plates. 1ml of the respective leaf extracts were then put into each appropriately labeled ditches using sterile pipettes. The inoculated plates were left on the table for 1hour for the extract to diffuse into the agar. The plates were then incubated at 37°C for 24hrs. After 24hrs, the zone of inhibition was measured. The diameter of the zone of inhibition around each well was measured to the nearest diameter along two axis of 90° to each other and the mean of the reading was calculated (Omotayo, 1998).

Determination of Effect of Varying Concentration of the Plant Extract on the Selected Organisms

Overnight broth culture of the test organisms were swabbed on the sterile Mueller Hinton agar in the Petri dishes using sterile cotton swabs. A sterile cork borer of size 6mm in diameter was used to make ditches on the plate. Stock concentration of the extract of *Moringa oleifera* (95%EE, AEE, CME and DWE) was diluted from 100mg/ml to 75mg/ml to 50mg/l and finally to 5mg/ml with extraction solvent. Each of these concentrations was introduced in to the ditches on the inoculated plates. The plates were left for 1hr for the extract to diffuse. The plates were subsequently incubated at 37°C for 24hrs. Zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The organisms that were susceptible to the extracts of *Moringa oleifera* were inoculated unto one of the sterile nutrient broths and incubated overnight at 37°C. 1ml of the extract was serially diluted with nutrient broth from 100mg/ml concentration to 75mg/ml to 50mg/ml to 25mg/ml and finally 5mg/ml. Each of the diluted extract was then inoculated with 0.3ml of the overnight broth culture of the test organisms. A control was setup which contains only nutrient broth and the extract. The inoculated and control tubes were incubated at 37°C for 24hrs after which they were observed for

turbidity. The lowest concentration that shows no turbidity was taken as the MIC (Willey *et al.,* 2008).

Determination of Minimum Bacteriocidal Concentration (MBC)

Sample from the tubes used in the MIC assays which did not show signs of turbidity after incubation were streaked out on solidified nutrient agar plates using sterile cotton swab and incubated at 37°C. The lowest concentration of the extract which shows no growth on plates after 24hrs of incubation indicates bacteriocidal effect and was taken as MBC (Alade and Itobi, 1993).The plates showing growth after 24hrs of incubation period was indicated to have bacteriostatic effect.

Antimicrobial Susceptibility Test of some Standardized (AntibioticsASTSA)

The antibiotic susceptibility testing by a standardized disc method as described by Bauer *et al.* (1965) was employed. The antibiotics used for this study had been prepared into kit containing multiple discs, each with small discs impregnated with different types of antibiotics. The antibiotics used and their corresponding concentrations are as follows:

*Augmentin	- 30µg	*Erythromycin	- 5µg
*Ceftriazone	- 30µg	*Nitrofurantan	- 200µg
*Gentamycin	- 10µg	*Cotrimaxazole	- 25µg
*Ofloxacin	- 5µg	*Amoxicillin	- 25µg
*Ciprofloxacin	- 10µg	*Tetracycline	- 30µg
*Pefloxacin	- 5µg	*Streptomycin	- 10µg
*Chloramphenicol	- 30µg		

The plate diffusion technique was used for the antibiotic sensitivity test. Overnight broth cultures of the organisms were swabbed on sterile Mueller Hinton agar plates using sterile swab sticks. The plates were allowed to solidify. The multiple antibiotic discs were then placed on the agar surface and pressed using sterile forceps to ensure complete contact with agar. All the plates were incubated at 37°C for 24hrs. The zones of inhibition generated by the antibiotics were measured to the nearest millimeters (mm) and interpreted as sensitive(S), moderately sensitive (MS), and resistant(R) (Willey *et al.,* 2008).

RESULTS

Antibiotic Susceptibility Pattern (ASP)

All the bacterial isolates were found to be sensitive to one or more of the antibiotics used for the test. All the Gram positive organisms were found to be sensitive to Ofloxacin, Perfloxacin and Ciprofloxacin and they were all resistant to Amoxicillin, Gentamycin, Chloramphenicol, Streptomycin Ceftriazone and Cotrimaxazole.The Gram negative organisms were all sensitive to Ofloxacin, Ciprofloxacin, Tetracycline and Pefloxacin and they were resistant toAugumetin and

Ceftriazone. The patterns of inhibition of the antibiotics are showed in tables 1-4.

Table 1: Inhibition Zone of Antibiotics on Gram positive Organisms from the First Batch of Clinical Isolates (mm)

Organisms	OFL (mm)	STR (mm)	GEN (mm)	PEF (mm)	COT (mm)	CPX (mm)
Staphylococcus aureus	22	12	14	23	15	20
Non-heamolytic *Streptococcus*	12	-	-	9	-	-

KEY:
GEN= Gentamycin OFL= Ofloxacin PEF= Pefloxacin
CPX= Ciprofloxacin STR= Streptomycin COT= Cotrimaxazole
> 11mm =sensitive. 7mm-10mm =moderately sensitive. 2 m m - 6 m m = resistant.

Table 2: Inhibition Zone of Antibiotics on Gram negative Organisms from the First Batch of Clinical Isolates (mm)

Organisms	AUG (mm)	CRO (mm)	NIT (mm)	GEN (mm)	COT (mm)	OFL (mm)	AMX (mm)	CPX (mm)	TET (mm)	PFX (mm)
Proteus sp	-	-	-	13	-	12	-	3	-	4
Pseudomonas sp	-	-	-	-	-	18	-	20	9	16
Escherichia coli	-	-	-	-	-	11	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-

KEY:
AUG= Augmentin OFL= Ofloxacin CRO= CeftriazoneAMX= Amoxycillin
NIT= Nitrofurantan CPX= Ciprofloxcin GEN= Gentamycin TET= Tetracycline
COT= Cotrimaxazole PFX= Pefloxacin
> 11mm =sensitive. 7mm-10mm =moderately sensitive. 2mm-6mm= resistant.

Table 3: Inhibition Zones of Antibiotics on Gram positive Organisms from the Second Batch of Clinical Isolates (mm)

Organisms	AMX (mm)	OFL (mm)	STR (mm)	CHL (mm)	CEF (mm)	GEN (mm)	PEF (mm)	COT (mm)	CPX (mm)	ERY (mm)
Staphylococcus aureus	-	19	8	-	-	7	12	-	14	-
Streptococcs thermophilus	-	20	-	-	-	14	25	-	19	-
Bacillus anthracis	-	-	-	-	-	-	19	-	16	14

KEY:
AMX=Amoxycillin GEN= GentamycinOFL= Ofloxacin PEF= Pefloxacin
STR= Streptomycin COT= Cotrimaxazole CHL= Chloramphenicol CPX= Ciprofloxacin
CEF= Ceftriazone ERY= Erythromycin

Table 4: Inhibition Zone of Antibiotics on Gram negative Organisms from the Second Batch of Clinical Isolates (mm)

Organisms	AUG (mm)	CRO (mm)	NIT (mm)	GEN (mm)	COT (mm)	OFL (mm)	AMX (mm)	CPX (mm)	TET (mm)	PFX (mm)
Pseudomonas sp	-	-	-	-	10	14	18	18	22	15
Escherichia coli	-	-	-	-	-	17	-	-	-	-
Vibrio cholera	-	-	-	-	10	23	-	17	-	14
Salmonella sp	-	-	16	20	20	17	-	16	22	16
Klebsiella pneumoniae	-	-	-	-	-	24	-	15	-	-

KEY:
AUG= Augmentin OFL= Ofloxacin CRO= CeftriazoneAMX= Amoxycillin
NIT= Nitrofurantan CPX= Ciprofloxcin GEN= Gentamycin TET= Tetracycline
COT= Cotrimaxazole PFX= Pefloxacin

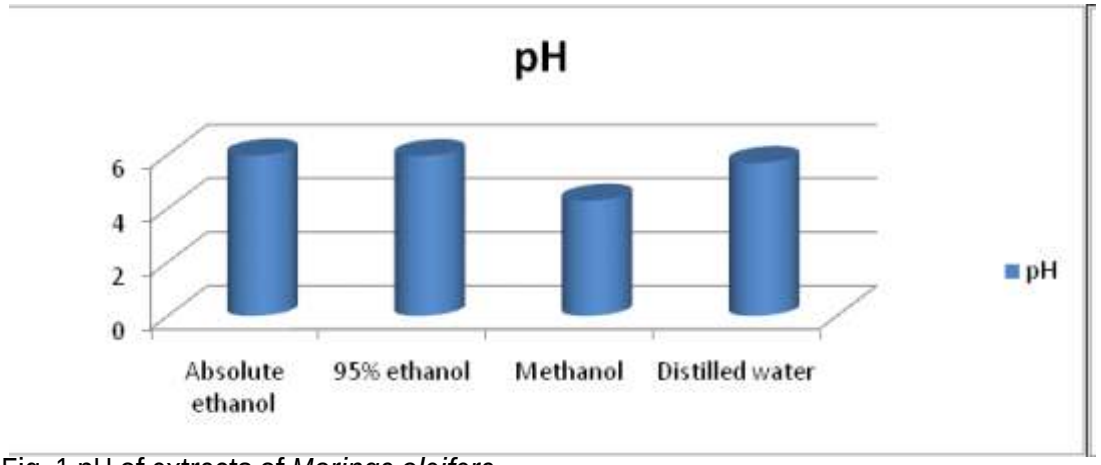

Fig. 1.pH of extracts of *Moringa oleifera*

Table 5: Phytochemical Screening of Extracts of *Moringa oleifera*

Phytochemicals	Results
Tannins	+
Saponins	+
Flavonoids	+
Phlobatannins	+
Anthraquinnones	-
Terpenoids	+
Alkaloids	+

KEY:
+ = present. - = absent.

Sensitivity of First Batch of Clinical Isolates to the Extracts of *Moringa oleifera*
The results of the zones of inhibition given by the extracts of *M. oleifera are* shown in Tables 6-9. Table 6 showed the pattern of inhibition by cold methanoic extract, table7 showed the pattern of inhibition by 95% ethanoic extraction, table 8 showed the pattern of inhibition by absolute ethanoic extraction while table 9 showed the pattern of inhibition by distilled water extraction.

Table 6: Pattern of Inhibition of the First Batch of Clinical Isolates collected from UITH to Different Concentrations of the Extracts of Dried Powdered Leaves of Cold Methanoic Extract (CME) of *Moringa oleifera*

S/N	Clinical isolates	Zones of Inhibition (mm) at varying Concentrations					
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	Control
1	*Staphylococcus aureus*	2	.-	2	1.5	2	-
2	Non-haemolytic *streptococcus*	2	1	3.5	-	4	-
3	*Pseudomonas* sp	-	-	-	-	-	-
4	*Escherichia coli*	2	6.5	4	2.5	2.5	-
5	*Klebsiella pneumoniae*	1.5	2.5	-	2.5	1.5	-
6	*Proteus* sp	-	-	-	-	-	-
7	*Salmonella typhi*	3.5	2	-	1.5	3	-
8	*Vibrio cholera*	-	1.5	-	2	1	-

Key: - = No zone of inhibition

Table 7: Pattern of Inhibition of the First Batch of Clinical Isolates todifferent concentrations of the Extracts of Dried Powdered Leaves of 95% Ethanoic Extract (95% EE) of *Moringa oleifera*

S/N	Clinical Isolates	Zones of Inhibition (mm) at Varying Concentrations					Control
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	
1	*Staphylococcus aureus*	-	-	1	1	1	-
2	Non-haemolytic *Streptococcus*	1.5	-	-	1	3.5	-
3	*Pseudomonas* sp	-	-	1	6	2	-
4	*Escherichia coli*	-	1.5	1	-	1	-
5	*Klebsiella pneumoniae*	-	1.5	-	-	-	-
6	*Proteus* sp	-	-	1	2	1	-
7	*Salmonella typhi*	-	-	-	-	3	-
8	*Vibrio cholera*	1	3	1	1.5	2.5	-

Table 8: Pattern of Inhibition of the First Batch of Clinical Isolates todifferent concentrations of the Extracts of Dried Powdered Leaves of Absolute Ethanoic Extract (AEE) of *Moringa oleifera*

S/N	Clinical Isolates	Zones of Inhibition (mm) at Varying Concentrations					Control
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	
1	*Staphylococcus aureus*	-	-	1	1	-	-
2	Non-haemolytic *Streptococcus*	-	-	-	-	-	-
3	*Pseudomonas* sp	-	-	-	-	1	-
4	*Klebsiella pneumoniae*	-	-	-	-	-	-
5	*Escherichia coli*	-	1	-	1	-	-
6	*Proteus* sp	-	-	-	-	-	-
7	*Salmonella typhi*	-	-	-	-	-	-
8	*Vibrio cholera*	1	-	-	2	-	-

Table 9:Pattern of Inhibition of the First Batch of Clinical Isolates todifferent concentrations of the Extracts of Dried
Powdered Leaves of Distilled Water Extract of *Moringa oleifera*

S/N	Clinical Isolates	Zones of Inhibition(mm) at Varying Concentrations					Control
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	
1	*Staphylococcus aureus*	-	-	-	-	-	-
2	Non-haemolytic *Streptococcus*	-	-	-	-	-	-
3	*Klebsiella pneumoniae*	-	-	-	-	-	-
4	*Pseudomonas* sp	-	-	-	-	-	-
5	*Escherichia coli*	-	-	-	-	-	-
6	*Proteus* sp	-	-	-	-	-	-
7	*Vibrio cholera*	-	-	-	-	-	-
8	*Salmonella typhi*	-	-	-	-	-	-

From table 6 it was observed that *Pseudomonas* sp and *Proteus* sp were not inhibited by the different concentrations of the extract. The other six organisms had zones of inhibition but the zones were not large enough. According to the standard table for measuring zones of inhibition, these values fall in the range that indicates that the organisms were resistant to the extract. From table 7, it was observed that 25mg/ml of the extract showed the highest zone of inhibition for

Pseudomas sp but the value still falls within the resistant range according to the standard table. The remaining organisms showed minute zones of inhibition.Table 8 showed that Non-haemolytic *Streptococcus, Klebsiella pneumonia, proteus* sp and *Salmonella typhi* were not inhibited by the extract; however the other organisms that showed zones of inhibition, had zone that fell in the resistance range. Table 9 showed that all the clinical isolates were resistant to the distilled water extract.

Another set of organisms were collected from the microbiology department of University of Ilorin Teaching Hospital and was also tested with the same extracts. The results obtained are shown in tables 10-13.

Table10: Pattern of inhibition of second batch of organisms collected from UITH to different concentration of extracts of dried powdered leaves of Cold Methanoic Extract (CME) of *Moringa oleifera*

S/N	Clinical Isolates	Zones of Inhibition (mm) at Varying Concentration					Control
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	
1	*Staphylococcus aureus*	-	-	-	-	-	-
2	*Streptococcus thermophilus*	-	-	-	-	-	-
3	*Escherchia coli*	6.5	-	2	-	-	-
4	*Klebsiella pneumoniae*	-	-	-	-	-	-
5	*Bacillus anthracis*	13	12	6	-	-	-
6	*Salmonella typhi*	21	13	-	-	-	-
7	*Pseudomonas* sp	-	9	-	-	-	-
8	*Vibrio cholera*	-	-	5	-	4	-

Key: - = No zone of inhibition

Table 11:Pattern of inhibition of second batch of organisms collected from UITH to different concentration of extracts of dried powdered leaves of 95% Ethanoic Extract (95%EE) of *Moringa oleifera.*

S/N	Isolates	Zones of Inhibition (mm) at Varying Concentrations					Control
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	
1	*Staphylococcus aureus*	-	-	-	-	-	-
2	*Streptococcus thermophilus*	-	-	-	-	-	-
3	*Klebsiella pneumoniae*	-	-	-	10	16	-
4	*Escherichia coli*	-	16	-	-	-	-
5	*Bacillus anthracis*	6	11	-	-	-	-
6	*Pseudomonas* sp	-	-	-	-	-	-
7	*Salmonella typhi*	-	-	-	-	-	-
8	*Vibrio cholera*	-	6	-	9	15	-

Key: - = No zone of inhibition

Table 12:Pattern of inhibition of second batch of organisms collected from UITH to different concentration of extracts of dried powdered leaves ofAbsolute Ethanoic Extract (AEE) of *Moringa oleifera.*

S/N	Isolates	Zones of Inhibition (mm) at Varying Concentrations					Control
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	
1	*Staphylococcus aureus*	-	-	-	-	-	-
2	*Streptococcus thermophilus*	-	-	15	14	-	-
3	*Escherichia coli*	-	13	-	-	6	-
4	*Vibrio cholera*	-	-	-	-	-	-
5	*Pseudomonas* sp	-	-	-	-	-	-
6	*Bacillus anthracis*	-	-	14	-	4	-
7	*Klebsiella pneumoniae*	-	-	12	10	4	-
8	*Salmonella typhi*	-	-	-	-	-	-

Key: - = No zone of inhibition

Table 13:Pattern of inhibition of second batch of organisms collected from UITH to different concentration of
extracts of dried powdered leaves of Distilled Water Extract of *Moringa oleifera*

S/N	Isolates	Zones of Inhibition (mm) at Varying Concentrations					Control
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	5mg/ml	
1	*Staphylococcus aureus*	-	-	-	-	-	-
2	*Streptococcus thermophilus*	-	-	-	-	-	-
3	*Escherichia coli*	-	-	-	-	-	-
4	*Pseudomonas* sp	-	-	-	-	-	-
5	*Vibrio cholera*	-	-	-	-	-	-
6	*Bacillus anthracis*	-	-	-	-	-	-
7	*Klebsiella pneumoniae*	-	-	-	-	-	-
8	*Salmonella typhi*	-	-	-	-	-	-

Key: - = No zone of inhibition

Table 10 depicted that *Salmonella typhi* and *Bacillus anthracis* were susceptible to the extract at100mg/ml and 75mg/ml. *Pseudomonas* sp was also susceptible to the extract at a concentration of 75mg/ml. *Staphhylococcus aureus, Streptococcus thermophilus* and *Klebsiella pneumoniae* showed no zones of inhibition against the extract.
From table 11, it was observed that *Staphylococcus aureus, Streptococcus thermophilus, Pseudomonas sp* and *Salmonella typhi* were resistant to the extract. While *Klebsiella pneumonia* and *Vibrio cholera* were susceptible to the extract at the 5mg/ml and 25mg/ml concentrations. *Bacillus anthracis* was also susceptible to the extract at 75mg/ml and 100mg/ml concentrations.
Table 12 showed that *Vibrio cholera, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas sp* were resistant to the extract. *Streptococcus thermophilus*wassusceptible to the 50mg/ml and 75mg/ml concentrations. From Table 13, it was observed that all the test organisms were resistant to the extract.

Table 14: Minimum Inhibitory Concentration (MIC) of dried powdered leaves of extracts *Moringa oleifera*

S/N	Isolates	Minimum Inhibitory Concentration in mg/ml		
		Methanol	95%ethanol	Absolute ethanol
1	*Staphylococcus aureus*	-	-	-
2	*Streptococcus thermophilus*	-	-	-0.15
3	*Escherichia coli*	-1.3	1.6	-0.4
4	*Klebsiella pneumoniae*	-	0.3	-0.09
5	*Pseudomonas* sp	-3.5	-	-
6	*Bacillus anthracis*	-1.7	1.5	-0.4
7	*Vibrio cholera*	-0.4	0.03	-
8	*Salmonella typhi*	0.8	-	-

Key: - = No turbidity

Table 15: Minimum Bacteriocidal Concentration (MBC) of dried powdered leaves extracts of *Moringa oleifera*

S/N	Isolates	Minimum Inhibitory Concentration		
		Methanol	95% ethanol	Absolute ethanol
1	*Staphylococcus aureus*	++++	++++	++++
2	*Pseudomonas* sp	+	++++	++++
3	*Bacillus anthracis*	++	+	++
4	*Escherichia coli*	++	+	++
5	*Klebsiella pneumoniae*	++++	+	++
6	*Salmonella typhi*	+	++++	++++
7	*Vibrio cholera*	++	+	++++
8	*Streptococcus thermophilus*	++++	++++	++

KEY:+ = minute growth++ = slightly heavy growth++++ = heavy growth

DISCUSSION

In this study, two sets of clinical isolates were collected from the Department of Medical Microbiology, University of Ilorin Teaching Hospital (UITH). The first batch of clinical bacterial isolates include *Staphylococcus aureus,* Non-haemolytic *Streptococcus, Escherichia coli, Pseudomonas* sp, *Proteus* sp, *Klebsiella pneumonia, Vibrio cholera* and *Salmonella typhi.* The second batch of clinical isolates includes*Staphylococcus aureus, Streptococcus thermophilus, Bacillus anthracis, Pseudomonas* sp, *Escherichia coli, Klebsiella pneumoniae, Vibrio cholera* and *Salmonella typhi.* The antibiotics used for the study had been prepared into kit containing ten different antibiotics for Gram positive organisms which include Amoxycillin 25µg, Ofloxacin 5µg, Streptomycin 10µg,

Chloramphenicol 30µg, Ceftriazone 30µg, Gentamycin 10µg, Pefloxacin 5µg, Cotrimaxazole 25µg, Ciprofloxacin 10µg, and Erythromycin 5µg produced by FONDOZ laboratory. Another set of kit containing ten different antibiotics for Gram negative organisms which include Augmentin 30µg, Nitrofurantan 20µg, Ceftriazone 30µg. Gentamycin 10µg, Cotrimaxazole 25µg, Ofloxacin 5µg, Amoxicillin 25µg, Ciprofloxacin 10µg, Tetracycline 30µg, Pefloxacin 5µg.

The experiment carried out using antibiotics was done in duplicates; the mean of the two values of zone of inhibition of each antibiotic was calculated and recorded in tables 1-4. From table 1 containing results for zones of inhibition of Gram positive organisms in first batch of clinical isolates collected from the Department Microbiology of UITH, it was observed that *Staphylococcus aureus* showed multiple resistance to antibiotics such as Amoxycillin, Ceftriazone and Erythromycin. *Staphylococcus aureus* was however susceptible to Ofloxacin, Streptomycin, Gentamycin, Perfloxacin, Cotrimaxazole and Ciprofloxacin. Pefloxacin showed the highest activity against *Staphylococcus aureus*, followed by Ofloxacin and Ciprofloxacin. The organism was only moderately susceptible to Streptomycin, Gentamycin and Cotrimaxazole. Therefore, it can be suggested that the above named antibiotics that the organisms were susceptible to can be used in the treatment of infections by these organisms.

From the result on table 2 which contains the Gram negative organisms in the first batch of clinical isolates collected from the department of microbiology of UITH, it was observed that *Proteus* sp was susceptible to Gentamycin and Ofloxacin. Ciprofloxacin and Pefloxacin also showed zones of inhibition but the values fell within the resistant range (3mm and 4mm respectively). *Pseudomonas* sp was susceptible to Ofloxacin, Ciprofloxacin and Pefloxacin. *Pseudomonas* sp was moderately sensitive to Tetracycline.

Table 3 showed the result of zones of inhibition of Gram positive organisms in the second batch of organisms collected from the department of Microbiology, UITH. *Staphylococcus aureus* was susceptible to Ciprofloxacin, Pefloxacin and Ofloxacin, and was moderately sensitive to Streptomycin and Gentamycin. Table 4 contains result for zone of inhibition of Gram negative organisms in the second batch of organisms collected from Microbiology Department of UITH, it was observed that *Pseudomonas* sp was sensitive to Cotrimoxazole, Ofloxacin, Amoxycillin, Ciprofloxacin, Tetracycline and Pefloxacin. Tetracycline was most effective against the organism while Cotrimoxazole was the least effective. *Escherichia coli* were sensitive to only Ofloxacin and resistant to the remaining nine antibiotics.

It was observed from table 6 that *Pseudomonas* sp and *proteus* sp showed no zone of inhibition against the extract. *Escherichia coli* had its highest zone of inhibition of 6.5mm at the 75mg/ml concentration of the extract. Non haemolytic *Streptococcus* showed highest

zone of inhibition at the 5mg/ml concentration. *Salmonella typhi* had its highest zone of inhibition at 100mg/ml concentration which is the highest concentration of the extract. From these observations, it could be concluded that according to this study, even though some zones of inhibition were recorded for most of the organisms the values were not large enough to consider the first batch of clinical isolates susceptible to the different concentrations of the Cold Methanoic Extract of the dried leave powder of *Moringa oleifera.*

From table 7, it was observed that all the clinical isolates showed zones of inhibition against one or more of the concentrations of the 95% Ethanoic Extract of the dried leave powder of *Moringa oleifera* with organisms like *Salmonella typhi* and Non-haemolytic *Streptococcus* having their highest zones of inhibition at the least concentration of the extract. However, the values of the zones of inhibition were not large enough to be used to conclude that the organisms were susceptible to this extract.

Results on table 8 showed that Non-haemolytic *Streptococcus*, *Klebsiella pneumoniae, Proteus* sp and *Salmonella typhi* showed no zones of inhibition to all concentrations of the Absolute Ethanoic Extract of dried powdered leaves of *Moringa oleifera*. The remaining four clinical organisms showed zones of inhibitions ranging from 0.5mm to 2mm. The organisms showed zones of inhibition to only one or two of the different concentrations of the extract. The values however cannot be used to conclude that the organisms were susceptible to the extracts.

From table 9, it was observed that the first batch of clinical isolates showed no zone of inhibition to the Distilled Water Extract of dried powdered leaves of *Moringa oleifera*. This may be due to the fact that accessibility of methanol and ethanol to the bioactive component of the leave is greater than that of water. This is consistent with the work of other investigators (Brain and Turner, 1975, Mashiar *et al.,* (2009).

Table 10-13 showed result of zone of inhibition of the second batch of organisms collected from the Department of Microbiology, UITH. These organisms showed higher zones of inhibition whose ranges fell in the susceptible zone according to standard tables. This result might be due to the fact that these organisms have not developed resistance techniques unlike the first batch of clinical isolates which were resistant to most of the commercial antibiotics and all the extracts of dried powdered *Moringa oleifera* leaves. However, it was observed from table 10 that some of these organisms were resistant to Cold Methanoic Extract of the *Moringa oleifera* leaves. *Staphylococcus aureus, Streptococcus thermophilus* and *Klebsiella pneumonia, Salmonella typhi* and *Bacillus anthracis* showed highest zones of inhibition at the highest concentration, the values ranged between 12mm to 21mm which fell between ranges that can be used to conclude that these organisms were susceptible to this extract. *Pseudomonas* sp showed zone of

inhibition against only 75mg/ml concentration. *Vibrio cholera* showed zones of inhibition against the 5mg/ml and 50mg/ml concentration, the values were too minute to be used to conclude that *Vibrio cholera* wassusceptible to the Cold Methanoic Extract of the leave.*Escherichia coli* showed zones of inhibition of 4mm and 5mm at the 5mg/ml and 50mg/ml concentrations respectively but the values fell in the resistance range.

From table 12, it was observed that *Staphylococcus aureus, Salmonella typhi, Vibrio cholera* and *Pseudomonas* sp showed no zones of inhibition against the Absolute Ethanoic Extract of dried powdered *Moringa oleifera* leaves. However, *Streptococus thermophilus, Escherichia coli, Bacillus anthracis* and *Klebsiella pneumoniae* showed zones of inhibition against the extract. The organisms showed zones of inhibition to concentrations ranging from 5mg/ml to 75mg/ml with values ranging from 4mm to 15mm.

Table 13 showed that the Distilled Water Extracts had no effect on the test organisms; this may be due to the fact during the extraction process the extract was subjected to a high temperature ($100^{?}$C).

This agrees with the work of Raheela *et al.,* 2008, the extract of *Moringa oleifera* was more effective under low temperature, or moderate temperature condition. But at high temperature the activity was lost.

Figure 1 showed that Cold Methanoic Extract of the powdered leave had the lowest pH value and this extract also showed zones of inhibition against more organisms than the other extracts. This agrees with Alade and Irobi (1993) which confirms that the antimicrobial activity of some plants significantly reduces with increase in pH.

Table 14 showed that the Minimum Inhibitory Concentrations (MIC) of the extracts ranged from -0.09 to 1.6. These MIC results correlate with reports that microorganisms vary widely in the degree of their susceptibility (Emeruwa, 1982; El Faraly *et al.*, 1983). Antimicrobial agents with low activity against an organism have high minimum inhibitory concentration while a highly active microbial agent gives low MIC. Table 15 showed the result of Minimum BacteriocidalConcentration (MBC), it was observed from the table that none of the organisms had MBC since growth was observed on all the MBC plates.

All the clinical isolates challenged with the different extracts of *Moringa oleifera* are common pathogens.

Public enlightenment campaign to educate people on the hazards of the misuse of antibiotics would be beneficial and should be encouraged. Individuals on drug prescription should be educated on the need to follow the prescribed doses strictly. Government should also pass a policy against the unnecessary use and mis-handling of antibiotics. It is suggested that *Moringa oleifera* leaves should be used as food supplement for all especially children in developing countries since the leaves contains antimicrobial substances that are effective against some pathogenic microorganisms.Further research

should also be done on the leaf extracts of *Moringa oleifera.*

REFERENCES

Alade, P. I. and Irobi, O. N. (1993). Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana.* ,*Journal of Ehtnopharmacology*39:171-174.

Awe, S. and Omojosola, P.F. (2003). Antimicrobial Screening of three medicinal plantsused for Diarrhea treatment in Ilorin Nigeria. *Nigeria Journal of Pure andApplied Science.*18:1375-1379.

Bauer, A.W., Kirby, W.M.M., Sherre, J.C. and Turch, M. (1965). Antibiotic Susceptibility Testing by a ,Standardized Single Disc Method. *Journal of Clinical Pathology*45(4):493-496.

Brain, K. R. and Turner, I. D. (1975).*Basic Medical Microbiology*.2nd Edition. LittleBrown and Co. Boston.Pp. 402.

El-Faraly, S., Cheatham, S. F. and Breelove, R. L. (1983). Antimicrobial Neolignans of Sassafras ,randalense root. *LIoydia*. 46(4) : 93-497.

Emeruwa, A. C. (1982). Antibacterial Substances from *Carica papaya* Fruit Extract. *LIoydia* 45(2):123-127.

Fahey, J.W. (2005). *Moringa oleifera*: A review of the medicinal evidence for its nutritional therapeutical, and prophylactic properties. *Tree of Life Journal*1:5.

Fuglie, L. J. (2000). New Uses of *Moringa* Studied in Nicaragua. *ECHO Development* Notes 68, June, 2000.

Jed, W. F. and Sc, D. (2005).*Moringa oleifera*: A Review of the Medicinal Evidence for its Nutritional, Therapeutic, and Prophylactic Properties. *Tree for Life Journal* 1:5.

Mashiar, M., Mominul, I., Sharma, A., Soriful, I., Atikur, R., Mizanur, R. and Alam, M. (2009).Antibacterial activity of leave juice extracts of *Moringa oleifera* against some Human pathogenic bacteria. *Journal National Science* 8(2):219-227.

Olsen, A. (1987). Low technology water purification by bentonite clay and *Moringaoleifera* seed flocculation as performed in Sudanese villages. Effects on *Schistosoma mansoni*cercariae. *Water Research* 21(5):517-522.

Omotayo, A.E. (1998), Antimicrobial activity of some anti malaria plants. *Proceedingsof NSM*1(1):31-41.

Raheela, J., Muhammad, S., Jamil, A. and Muhammad, A. (2008). Microscopic evaluation of the antimicrobial activity of seed extracts of M*oringa oleifera. Pak. J. Bot*. 40 (4):1349-1358.

Vastuguna, D., Yarra, V. And Subbarayudu, Y. (1989). *Rhajahmundry India.*9th edition. Mohan Publisher. 17.

Willey, J. M., Sherwoood, L. M., Woolverton, C. J. (2008). Antimicrobial Chemotherapy. *Prescott, Harley and Klein's Microbiology.*7th Edition.835-858.