

Antibacterial activity of *Moringa oleifera* on Multi-drug resistant isolates from Wound infections in Abeokuta, South – West Nigeria

Shobayo BI¹, Ojo DA¹, Olaboopo AO¹, Agboola DA², Omemu AM³, Akingbade OA⁴

¹ Department of Microbiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

² Department of Biological Sciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

³ Department of Food Service and Tourism, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

⁴ School of Medical Laboratory Science, Lagos University Teaching Hospital, Lagos State, Nigeria

bodeshobayo@gmail.com, daojo3@yahoo.com; Tel: +2347032565917

Abstract: Microbial resistance to antibiotics is a global problem. Hence, scientific investigation of the efficacy of medicinal plants as alternative to antibiotics in the treatment of microbial infections has become necessary. Efficacy of extracts of *Moringa oleifera* was investigated as antimicrobial agent against one gram positive and four gram negative bacteria isolated from wound infections. The plant leaves were extracted with methanol, ethanol and aqueous solvents and their antimicrobial susceptibility profile was determined using disc diffusion method. Prior to the determination of Minimum Inhibitory Concentration (MIC), 20, 40, 80 and 100 mg concentrations of the leaves extracts were phytochemically screened for the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides and phenols. Inhibition by *M. oleifera* leaf extracts was noticed against all the tested bacteria except *P. aeruginosa*. Phytochemical analysis indicated the presence of tannins, flavonoids, terpenoids and saponins in all extracts of *M. oleifera*. Minimum Inhibitory Concentration (MIC) range of 7.34mg ml⁻¹ to 58.75 mg ml⁻¹ was noticed with the minimum value observed against *S. aureus*. The study reveals that *M. oleifera* had broad spectrum of activity on all the tested bacteria except *P. aeruginosa*.

[Shobayo BI, Ojo D, Agboola D, Akingbade O. **Antibacterial activity of *Moringa oleifera* on Multi-drug resistant isolates from Wound infections in Abeokuta, South – West Nigeria.** *N Y Sci J* 2014;7(12):15-19]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 3

Keywords: Antibacterial activity, *Moringa oleifera*, Multi-drug, Resistant, Wound

1. Introduction

Moringa oleifera is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in height, with open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark (Roloff et al., 2009). The moringa plant (*Moringa oleifera*) has been the object of much research due to its multiple uses and well-known bactericidal potential (Ghebremichael et al., 2005). Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo et al., 2008). Such plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Till today plant materials continues to play a major role in primary health care as therapeutic remedies in many developing countries (Jonathan and Fasidi, 2003).

Wound infection is one of the health problems that are caused and aggravated by the invasion of pathogenic organisms in different parts of the body. It has been shown that wound infection is universal and the bacterial type varies with geographical location, resident flora of the skin, clothing at the site of wound, time between wound and examination (Anupurba et al., 2010). In general,

a wound can be considered infected if purulent materials drain from it, even without confirmation of positive cultures. Also, many wounds are colonized by bacteria, whether infected or not. Infected wounds may not yield pathogens by culture owing to the fastidious nature of some pathogens, or if the patient has received an antimicrobial therapy (Nwachukwu et al., 2009). Previous studies from different parts of the country showed that *Pseudomonas species*, *Staphylococcus aureus*, *Klebsiella species*, *Escherichia coli*, and *Proteus* are the most common pathogens isolated from wound (Akingbade et al., 2012).

In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms has been developed due to indiscriminate use of synthetic drugs in the treatment of infectious diseases. This drives the need to screen medicinal plants for novel bioactive compounds as a basis for pharmacological studies since the bioactive principles obtained from the plants are biodegradable, safe and have fewer side effects (Prusti et al., 2008). This work investigated the role of aqueous, methanol and ethanol extracts of *Moringa oleifera* as potential antimicrobial agents against pathogenic bacteria causing wound infections.

2. Material and Methods

The fresh leaves of *Moringa oleifera* were collected from Abeokuta, Ogun State and identified in Biological Science Department (Botany) of Federal University of Agriculture, Abeokuta, Ogun State. Bacterial strains used in the study (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) were collected from Federal Medical Centre, Abeokuta and confirmed biochemically. The organisms were sub-cultured in nutrient broth and nutrient agar for use in experiment.

The plant leaves of *Moringa oleifera* were washed with running tap water and then shade dried at room temperature for 15 days. Ten grams of the dried leaves were weighed, crushed directly by grinder and dipped into 100ml distilled water in a conical flask. The flask was stoppered with rubber corks and left for 2 days with occasional shaking and filtered off using sterile filter paper (Whatman no. 1). The filtrates were evaporated under reduced pressure to get a thick residue which was treated as experimental drug for the present study. The standard extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test (Akueshi et al., 2002). Various concentrations (100, 80, 40 and 20mg/ml) of aqueous extracts were prepared accordingly from the stock. The same procedure as above was followed for the preparation of 100mg/ml, 80mg/ml, and 40mg/ml and 20mg/ml concentrations with 100% ethanol and methanol solvents.

Phytochemical analysis of the extracts was carried out based on the methods described by Talukdar et al., (2010). Qualitative test were conducted on the crude extracts to examine for the presence of the alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides and phenols.

Antibacterial activity of *Moringa oleifera* leaves in aqueous, methanol and ethanol solvents was tested against studied bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) using the disc diffusion method. Discs containing different concentrations (100, 80, 40 and 20 mg/ml) of dissolved plant extract was prepared using sterile Whatman filter paper No. 1 (6 mm in diameter). The discs were dried at 50°C. Overnight cultures of each of bacterial isolate was diluted with sterile normal saline to give inoculum size of 10^6 cfu/ml. Nutrient agar medium was prepared, sterilized, cooled and poured in to sterile petri dishes to a depth of 4 mm about 25 ml/plate to solidify. Pure cultures of the test organism were used to inoculate the petri dishes. This was done by spreading the inocula on the surface of the prepared nutrient agar plate using sterile cotton swabs which have been dipped in the diluted

suspension of the organism. The discs were then aseptically placed evenly on the surface of the inoculation and gently pressed down to ensure contact using a pair of forceps. Negative controls were prepared using the solvents only. Tetracycline (Tet), Ampicillin (Amp), Gentamycin (Gen), Ofloxacin (Oflo) and Ceftriazone (Cef) were used as positive controls to determine the sensitivity of bacterial strain. The plates were incubated at 37°C for 24h. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

MIC was determined by two-fold serial dilution method as described by Chandrasekaran and Venkatesalu (2004). The dose levels of 117.5 mg ml⁻¹ of the extracts were serially diluted separately to achieve various concentrations of 58.75, 29.37, 14.68, 7.34, 3.67, 1.83, 0.91 mg ml⁻¹ and 0.458, 0.229, 0.114µg ml⁻¹. Exactly 0.1 ml of varying concentrations of the extract samples was added to test tubes separately, containing 9 ml of standardized suspension of tested bacteria (10^8 cfu ml⁻¹). The test tubes were incubated at 37°C for 24 h. Controls were used with the test organisms, using distilled water instead of the plant extract. The least concentration of the samples with no visible growth was taken as the MIC.

The data was analyzed by simple arithmetic means of the various extracts and standard error was compared to the control. The results were expressed as the mean ± SD for triplicate experiments.

3. Results

Phytochemical analysis carried out on the dry leaves of *Moringa oleifera* using aqueous, methanol and ethanol revealed that flavonoids and saponins were the major phytochemicals present in the extracts as shown in Table 1.

Table 1. Qualitative phytochemical analyses of the extracts of *M.oleifera*

Phytochemical	Ethanol	Methanol	Aqueous
Phenols	-	-	-
Flavonoids	+	+	+
Tannins	+	-	+
Terpenoids	-	-	+
Akaloids	+	-	+
Glycosides	-	-	-
Saponins	+	+	+

(+) indicates presence while (-) indicates the absence of the components

Susceptibility patterns of the five bacteria isolates obtained from wound swabs to commonly used antibiotics are shown in Table 2. The highest zones of inhibition observed were 9mm and 8mm for *Staphylococcus aureus* and *Klebsiella pneumoniae* respectively against ceftriaxone and both were resistant to ampicillin and gentamycin. *Pseudomonas aeruginosa* was sensitive to gentamycin and was resistant to other antibiotics used in this study.

Table 2. Susceptibility pattern of the five bacteria isolates to commonly used antibiotics (mm)

Isolates	Tet	Amp	Gen	Oflo	Cef
<i>S. aureus</i>	8	R	R	R	6
<i>E. coli</i>	R	9	7	R	R
<i>Klebsiella sp</i>	R	R	R	7	9
<i>P. aeruginosa</i>	R	R	6	R	R
<i>P. mirabilis</i>	R	8	R	6	R

R = Resistant

Antimicrobial sensitivity profile of one hundred milligrams concentration (tested at a volume of 10 μ l/disc at a concentration of 100mg $^{-1}$) of the extracts in methanol, ethanol and aqueous solutions are shown in Table 3. *Escherichia coli* showed the highest zone of inhibition (sensitivity) of 14 \pm 0.16 mm in methanol while the least inhibitions were observed for *Klebsiella pneumoniae* and *Proteus mirabilis* at 8 \pm 0.10mm in the plant's aqueous solvent.

Table 3. Zones of inhibition of 100mg concentration of the extracts in three different solvents (mm)

Isolates	Methanol	Ethanol	Water
	100%	100%	100%
<i>S. aureus</i>	12 \pm 0.66	10 \pm 0.10	9 \pm 0.20
<i>E. coli</i>	14 \pm 0.16	9 \pm 0.52	11 \pm 0.9
<i>Klebsiella. sp</i>	10 \pm 0.32	12 \pm 0.08	8 \pm 0.10
<i>P. aeruginosa</i>	R	R	R
<i>P. mirabilis</i>	12 \pm 0.04	11 \pm 0.42	8 \pm 0.10

R = Resistant

Table 4. Zones of inhibition of 80mg concentration of the extracts in three different solvents (mm)

Isolates	Methanol	Ethanol	Water
	100%	100%	100%
<i>S. aureus</i>	6 \pm 0.11	8 \pm 0.24	
<i>E. coli</i>	5 \pm 0.22	7 \pm 0.15	
<i>Klebsiella. sp</i>	6 \pm 0.06	8 \pm 0.65	
<i>P. aeruginosa</i>	R	R	
<i>P. mirabilis</i>	7 \pm 0.12	6 \pm 0.45	

R = Resistant

At eighty milligrams concentration (tested at a volume of 10 μ l/disc at a concentration of 80mg $^{-1}$), antimicrobial sensitivity profile of of *Moringa oleifera* extracts had highest zone of inhibition (sensitivity) of 9 \pm 0.24mm to *Staphylococcus aureus* in methanol and the least zone of inhibition of 6 \pm 0.6 in aqueous was observed for *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* showed no zone of inhibition to methanol, ethanol and aqueous extracts of the plant (Table 4). At forty milligrams, only *Escherichia coli* and *Proteus mirabilis* showed sensitivity to the extracts while at twenty milligrams, no inhibition was noticed.

As shown in Table 5, the MIC of *Moringa oleifera* leaf extract revealed a strong inhibition against the tested bacteria (7.34mg ml $^{-1}$ to 117.5 mg ml $^{-1}$). Methanol, ethanol and aqueous extracts of the plant had inhibitory activity against all the tested bacteria. Their MIC values ranged from 7.34mg - 58.75 mg ml $^{-1}$, 14.68 mg ml $^{-1}$ - 117.5 mg ml $^{-1}$ and 14.68 mg ml $^{-1}$ - 29.37 mg ml $^{-1}$ respectively. No inhibition was detected against *Pseudomonas aeruginosa*.

Table 5. Minimum inhibitory concentration (MIC) of *Moringa oleifera* leaf extracts against test organisms (MIC in mg ml $^{-1}$)

Bacteria	Methanol extracts	Ethanol extracts	Aqueous extracts
<i>S. aureus</i>	7.34	14.68	29.37
<i>E. coli</i>	14.68	14.68	29.37
<i>P. aeruginosa</i>	58.75	117.5	ND
<i>P. mirabilis</i>	29.37	29.37	29.37

ND: no detection

4. Discussions

The phytochemical analysis carried out on the dry leaves of *Moringa oleifera* using three solvents showed the presence of some bioactive compounds in the plant. Ethanol extracts of *M. oleifera* revealed the presence of flavonoids, tannins, alkaloids and saponins compounds. Bukar and Loyeyi, (2010), also in their research, revealed the presence of flavonoids and saponins in *Moringa oleifera* ethanol leaf extract. This is consistent with the result from this study, but tannins and alkaloids were also detected in this present study which was not reported by Bukar and Loyeyi, (2010).

The result from this present research showed that *M. oleifera* leaf ethanol (MLE) extract, *M. oleifera* leaf methanol (MLM) and *M. oleifera* leaf aqueous (MLQ) extract had broadest spectrum of activity on the test bacteria. The results showed a better activity of the extract against *S. aureus*, *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* than

ceftriaxone, tetracycline, ampicillin, gentamycin and ofloxacin antibiotics but *M. oleifera* leaf extract was not active against *P.aeruginosa*. In a work conducted, by Napolean *et al.* (2009), *S. aureus* and *E. coli* were reported to be sensitive to MLE. This result is consistent with the present study. The sensitivity of *E. coli* strains to *M. oleifera* extracts as observed in this study is consistent with the findings of Jabeen *et al.* (2008), in which *M. oleifera* seed extracts were reported to produce halos of bacterial inhibition measuring up to 20.5 cm. *P. aeruginosa* was also resistant to all the percent grades of *M. oleifera* leaf extract in methanol and ethanol solvents, and also in the aqueous solvent. Napolean *et al.* (2009), reported *P. aeruginosa* to be resistant to all concentrations of methanol used except the highest concentrations of 200mg/ml MLE that was sensitive. Gentamycin was the only antibiotic that was sensitive to *P.aeruginosa* in this present study and this agrees with a research carried out in south west Nigeria by Akingbade *et al.* (2012), who recorded a high susceptibility activity of gentamycin against *P. aeruginosa* obtained from wound infections.

The MIC values of *Moringa oleifera* leaf extract against four of the tested bacteria revealed significant inhibition which ranged from 7.34mg ml⁻¹ to 117.5 mg ml⁻¹. No activity was detected against *Pseudomonas aeruginosa*. Methanol extracts of the plants had MIC values ranging from 7.34mg - 58.75 mg ml⁻¹. MIC values of ethanol extracts ranged from 14.68 mg ml⁻¹ - 117.5 mg ml⁻¹ and aqueous extracts revealed MIC values of 14.68 mg ml⁻¹ - 29.37 mg ml⁻¹. The strong inhibitory activity of *Moringa oleifera* leaf extract as shown by the MIC values may be attributed to the presence of some bioactive compounds in the plant. Secondary metabolites of various chemical types present in the plant species are known to possess antimicrobial activity. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms (Tsuchiya *et al.*, 1996). Phenols and phenolic compounds are known to be toxic to microorganisms (Mason and Wasserman, 1987). The ability of tannins to inactivate microbial adhesins, enzymes and cell envelope transport proteins makes them antimicrobial active (Ya, *et. al.*, 1998). Saponin compound has since shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Trease and Evans, 1989).

In the present study all concentrations of dissolved plant extracts of *M. oleifera* showed varying degrees of inhibition against the bacterial strains. Hundred (100) mg/ml concentrations showed higher zone of inhibition as compared with other concentrations used in this study and with the

standard drugs tested with the bacteria. Attempts should be made to conduct *in vivo* studies with the extracts so as to confirm the present *in vitro* findings as the diameter of the zone of inhibition is not only affected by sensitivity of the microorganisms alone but concentration of the extract in the discs is used and it's rate of diffusion in the media as well.

Corresponding Author:

Bode Irete Shobayo
Department of Microbiology
Federal University of Agriculture, Abeokuta
Ogun State, Nigeria
E-mail: bodeshobayo@gmail.com

References

1. Akingbade OA, Balogun SA, Ojo DA, Afolabi RO, Motayo BO, Okerentugba PO, Okonko IO. Plasmid Profile Analysis of Multidrug Resistant *Pseudomonas aeruginosa* isolated from Wound Infections in South West, Nigeria. World Applied Science Journal 2012; 20(6):766-775.
2. Akueshi CO, Kadiri CO, Akueshi EU, Agina SE, Ngurukwem C. Anti-microbial potential of *Hyptis suaveolens* poit (*Lamiaceae*). Nigerian Journal of Botany 2002; 15:37- 41.
3. Anupurba S, Bhattacharjee A, Garg A, Sen M. R. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from wound infections. Indian Journal of Dermatology 2010; 51:286-288.
4. Bukar A, Loyeyi TI. Antimicrobial profile of *Moringa oleifera* lam. Extracts against some food – borne microorganisms. Bayero Journal of Pure and Applied Sciences 2010; 3(1):43 – 48.
5. Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. Journal of Ethnopharmacology 2004; 91(1):105-8.
6. Ghebremichael KA, Gunaratna KR, Henriksson H, Brumer H, Dalhammar GA. Simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. Water Research 2005; 39:2338-44.
7. Jabeen R, Shahid M, Jamil A, Ashraf M. Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pakistan Journal of Botany 2008; 40:1349-58.
8. Jonathan SG, Fasidi IO. Antimicrobial Activities of *Lycoperdon pusillum* and *Lycoperdon giganteum*, Nigerian Edible Macro Fungi. African Journal of Biomedical Research 2003; 6:88-90.
9. Mason TL, Wasserman BP. Inactivation of red beet beta-glucan synthesis by native and

- oxidized phenolic compounds. *Phytochemistry* 1987; 26: 2197 – 2202.
10. Napolean P, Anitha J, Emilin RR. Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica* 2009; 3(1): 33 – 37.
 11. Nwachukwu NC, Orji FA, Okike UM. Antibiotic Susceptibility Patterns of Bacterial Isolates from Surgical Wounds in Abia State University Teaching Hospital (ABSUTH), Aba – Nigeria. *Research Journal of Medicine and Medical Sciences* 2009; 4(2):575-579.
 12. Okigbo RN, Eme UE, Ogbogu S. Biodiversity and conservation of medicinal and aromatic plants in Africa. *Biotechnology and Molecular Biology Reviews* 2008; 3: 127-134.
 13. Prusti A, Mishra SR, Sahoo S, Mishra SK. Antibacterial Activity of Some Indian Medicinal Plants. *Ethnobotanical Leaflets* 2008; 12: 227-230.
 14. Roloff A, Weisgerber H, Lang U, Stimm B. *Moringa oleifera*. Lam. Weinheim 2009; 1785.
 15. Talukdar AD, Choudhary MD, Chakraborty M, Dutta BK. Phytochemical screening and TLC profiling of plant extracts *Cyathea gigantea* and *Cyathea brunoniana*. *Biological and Environmental Sciences* 2010; 5(1):70-74.
 16. Trease GE, Evans WC. *Drugs of Biological Origin*. In: *Pharmacognosy*, 12th ed. United Kingdom, Balliere Tindall, 1989.
 17. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Linuma M. A prenylated flavanone from roots of *Amorpha fruticosa*. *Journal of Ethnopharmacology* 1996; 50:27.
 18. Ya C, Gaffney SH, Lilley TH, Haslam E. *Chemistry and significance of condensed tannins*. Plenum Press, New York, 1998; 553.

11/28/2014