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ANTIBACTERIAL ACTIVITY OF THE CRUDE EXTRACTS OF *MORINGA OLEIFERA* LEAF LAM (*MORINGACEAE*)

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ABSTRACT

Moringa oleifera plant has been widely accepted and used as herbal cure for many infectious and non-infectious medical conditions. This study was designed to evaluate the antibacterial activities of ethanolic extract of *Moringa Oleifera* leaf (EMOL) against three standard bacteria: a pathogenic bacterium (*Escherichia coli*, ATCC 25922) and two virulent ones (methicillin-resistant *Staphylococcus aureus*, MRSA, ATCC 25923 and *Acinetobacter bauamanii*, ATCC 19606). Various concentrations (1000, 500, 250, 125, 62.5 and 31.25) mg/ml of EMOL were prepared in 3% dimethylsulfoxide (DMSO) and screened for antibacterial activity using agar well diffusion method. The results of the zones of inhibition showed that EMOL at 1000mg/ml has antibacterial activity on *Staphylococcus aureus* (13.00mm), *Escherichia coli* (11.00mm) and *Acinetobacter bauamanii* (11.00mm). The minimum inhibitory concentrations (MIC) were 256 mg/ml, 512mg/ml and 256mg/ml for MRSA (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Acinetobacter bauamanii* (ATCC 19606) respectively. EMOL has significant antibacterial activity against pathogenic and virulent bacterial pathogens, and may be a potential source of another effective and useful antibacterial agent.

Keywords: Gastroprotection, Gastroduodenal ulcer, Indomethacin, *Nigella sativa*

INTRODUCTION

In recent times, treatment of a number of infections has been made difficult and complicated because of increasing resistance of microbial pathogens to previously potent drugs. Of particular interest among the microbial pathogens are the multi-drug resistant bacteria pathogen often termed 'super bugs'. Notable super bugs includes

methicillin-resistant-*Staphylococcus aureus* (MRSA), Vancomycin-resistant-*Staphylococcus aureus* (VRSA), Vancomycin-resistant-*Enterococcus faecalis*, extended spectrum beta-lactamase (ESBL) producing Gram negative bacilli, multi-drug resistant *Mycobacterium tuberculosis* among others.

The spectrum of diseases caused by the 'super bugs' vary and the consequences of their infections include: extended hospital stay, high cost of treatment, high morbidity and mortality (Arti Kapil, 2005). The super bugs have therefore impacted negatively on man thus necessitating the search for potent antibacterial agents or the "magic bullet".

Moringa oleifera is an acclaimed 'wonder plant' because of its several potential uses in medicine (Anwar, *et al* 2007, Bhatnagar *et al* 1961 and Fahey, 2005, Rathi *et al* 2006). It is widely accepted and used as herbal remedy for many infectious and non-infectious medical conditions (Anwar, *et al* 2007, Fahey 2005, Posmontier 2011); and may be potential source of a novel antimicrobial agent.

Scientific evidence for the antimicrobial potentials of *Moringa oleifera* is needed for establishing its antimicrobial activity. Most of the previous attempts at defining the antibacterial attributes of *Moringa oleifera* involved assays against clinical bacterial isolates of humans and not certified standard bacterial organisms (Pal *et al* 1995, Rajamanickam & Sudha 2013). The multi-drug resistant nature of the bacteria used in some of these assays were not defined given the global challenge of managing infections caused by the super bugs (Rahman *et al* 2009; Thilza *et al* 2010). Furthermore, many studies examined the activity of *Moringa oleifera* against bacterial organisms of plant and animal origin in addition to those from other environmental sources like water and sewage (Vieira *et al*, 2010; Lar *et al* 2011) probably because some of these organisms can be acquired by man and may result in human infections. Though these studies have indicated that *Moringa oleifera* has

antimicrobial properties, it will be beneficial to know the effect of *Moringa oleifera* against pathogenic, virulent, multi-drug resistant bacteria for which alternative potent therapeutic drugs are seriously desired. This study was therefore designed to evaluate the antibacterial activity of *Moringa oleifera* leaf against standard bacteria pathogens, some of which are virulent and multi-drug resistant.

MATERIALS AND METHODS

Identification and Preparation of Leaf Extract:

Moringa Oleifera leaf was collected from a *Moringa* farm in Ilorin, Kwara State and authenticated by a plant taxonomist Professor F.A Oladele of Department Plant Biology, University of Ilorin. The plant material was stored in the herbarium of the same department with a voucher number UIH/011/1011. The leaves were dried at room temperature for six weeks, pulverized and sieved to obtain the powder. Solvent extraction, performed at Prof S.A Ibiyemi laboratory, at the University of Ilorin, Ilorin, Nigeria, was as described by Sofowora, (1982) and Pavia *et al.* (1982). Four hundred gram (400g) of *Moringa oleifera* leaf powder was extracted in 2L of ethanol for 72 hours. The extract solution was filtered and concentrated using a rotary evaporator and subsequently freeze dried at Obafemi Awolowo University Central Research Laboratory, Ile-Ife, Nigeria. The percentage yield was 10.2%

Antibacterial Activity Assessment:

Antibacterial properties of ethanolic extract of *Moringa Oleifera* leaf (EMOL) was evaluated using three standard pathogenic bacteria: Methicillin -resistant

Staphylococcus aureus (MRSA) (ATCC 33591), *Escherichia coli* (ATCC 25922) and *Acinetobacter baumannii* (ATCC 19606). The organisms were cultured on Mueller-Hinton agar after overnight subculture in brain heart infusion broth. Five pure and discrete colonies of each of the standard organisms were inoculated separately into sterile physiologic saline solution contained in a Bijou bottle. The turbidity of the suspension then was adjusted to that of 0.5 McFarland turbidity standards and this was used as the inoculum.

Preparation of *Moringa oleifera* leaf extracts stock solution:

Ten thousand milligram (10, 000mg) of freeze dried EMOL was dissolved in 10ml of 3% DMSO to give a concentration of 1000mg/ml stock solution. Three percent (3%, w/v) DMSO was chosen for preparation of the stock solution having evaluated its activity at 0.1%, 1.0%, 3.0%, 5.0% and absolute concentration against the standard pathogenic bacteria used in this study. DMSO had no activity at $\leq 5\%$ concentration. EMOL stock solution of 1000mg/ml was serially diluted in saline to obtain extract concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.63mg/ml, 7.81mg/ml, 3.91mg/ml, and 1.95mg/ml.

***Antibacterial assay of Moringa oleifera* leaf extract by agar well diffusion method:**

Antibacterial activity of ethanolic extract of *Moringa oleifera* (EMOL) leaf against multidrug-resistant pathogens was evaluated by agar well diffusion method (WHO 1991). Briefly, a sterile swab stick was dipped into the inoculum and streaked on Muller-Hinton agar plate. A sterile cork borer (6mm diameter) was used to bore holes in the inoculated Muller-Hinton agar. Six holes per petri dish of 90mm diameter

were made and each hole was filled with 20 μ l of appropriate extract concentration. The inoculated culture plates were aerobically incubated at 37°C for 18-24 hours. The antimicrobial activity of the plant extract and the controls were measured as zone diameter of inhibition in millimetre using a calibrated ruler. Gentamicin (10 μ g) and DMSO (3%) were used as positive and negative controls respectively.

Minimum inhibitory concentration (MIC) of Moringa oleifera leaf extract

The MIC of the Moringa extract was determined by agar dilution technique (WHO, 1991; Andrews, 2001). Briefly, 1ml of the EMOL stock solution (1000mg/ml) was diluted in 97.6ml of physiologic saline to obtain extract concentrations of 10.245mg/ml. This was further diluted with various volumes of sterile distilled water in petri dishes under sterile condition and 19 mL of sterile molten Muller-Hinton agar (50°C) was added to attain desired EMOL concentrations. The admixture was swirled round and allowed to solidify. The plates were then spot inoculated with 1 μ l of each standard organism (10⁴CFU/spot) and incubated for 18hrs at 37°C. The plates were examined for bacterial growth and MIC was taken as the lowest concentration of EMOL that completely inhibited visible growth.

Organisms and chemicals

All the bacteria used in this study were of the American Type Culture Collection (ATCC) obtained from the University Boulevard, Manassas, VA 20110, USA. All the solvents (namely methanol, ethanol, butanol, chloroform, and ethyl acetate) and chemical reagents used in this study were of analytical grade and procured from SIGMA Chemicals Co. Dorset, UK; and BDH Chemicals Limited Poole, England, respectively.

RESULTS

Inhibitory activity of *Moringa oleifera* leaf Extract

The inhibitory activity of EMOL is shown in Table 1 as zones of growth inhibition against the tested bacteria. The inhibition zone were 13.0 mm for MRSA (ATCC 33591) and 11.0 mm for both *Escherichia coli* (ATCC 25922) and *Acinetobacter bauamanii* (ATCC 19606) at EMOL concentration of 1000mg/ml. Figures 1 – 3

are representative images showing the antimicrobial activity of different concentrations of EMOL, Gentamicin (10 µg, Positive control) and DMSO (3%, Negative Control) against MRSA (ATCC 33591), *Escherichia coli* (ATCC 25922) and *Acinetobacter bauamanii* (ATCC 19606) by the agar well diffusion technique.

Table 1: Inhibitory activity of *Moringa oleifera* leaf Extract

PATHOGENS	ZONE DIAMETER (mm) OF GROWTH INHIBITION						3% DMSO	Gentamicin control (10µg)
	VARIOUS CONCENTRATIONS OF EMOL							
	62.5 mg	125 mg	250 mg	500 mg	1000 mg			
Methicillin Resistant <i>Staphylococcus aureus</i> (33591)	0	0	0	10	13	0	23	
<i>Esherichia coli</i> (ATCC 25922)	0	0	0	7	11	0	24	
<i>Actinetobacter Bauamanii</i> (ATCC 19606)	0	0	0	9	11	0	10	

Minimum Inhibitory Concentration (MIC) of *Moringa oleifera* leaf extract

The minimum inhibitory concentrations (MIC) of EMOL were 256 mg/ml,

512mg/ml and 256mg/ml for MRSA (ATCC 33591), *Escherichia coli* (ATCC 25922) and *Acinetobacter bauamanii* (ATCC 19606) respectively

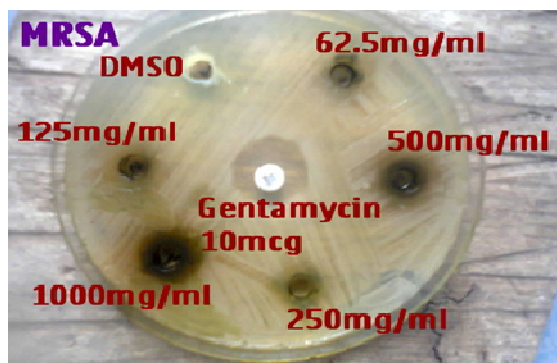


Figure 1: Antimicrobial activity of different concentrations of EMOL, Gentamicin (Positive control) and 3% DMSO (Negative Control) against Methicillin- resistant *Staphylococcus*

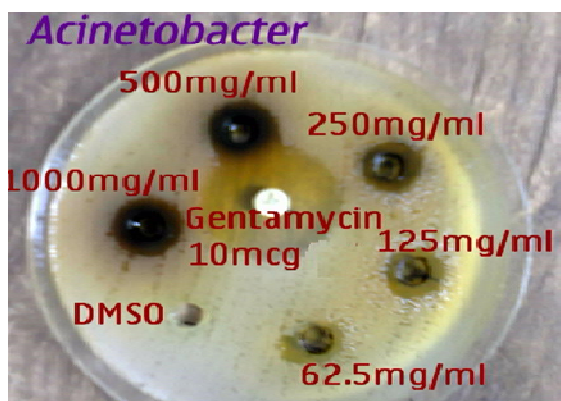


Figure 2: Antimicrobial activity of different concentrations of EMOL, Gentamicin (Positive control) and 3% DMSO (Negative Control) against *Acinetobacter baumannii*

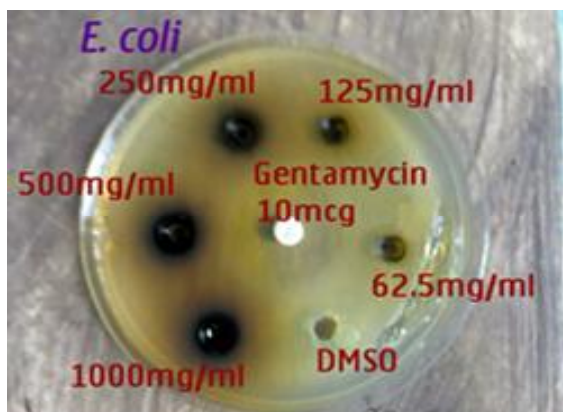


Figure 3: Antimicrobial activity of different concentrations of EMOL, Gentamicin (Positive control) and 3% DMSO (Negative Control) against *Escherichia coli*

DISCUSSION

The results obtained from this study showed that EMOL has antibacterial activity. As seen in Figures 1-3, the leaf extract showed antibacterial activities against Methicillin-resistant-*Staphylococcus aureus* (ATCC 33591), *Escherichia coli* (ATCC 25922), and *Acinetobacter baumannii* (ATCC 19606). Earlier reports like those of Rahman *et al* 2009; Bukar *et al* 2010; and Piexoto *et al* 2011 had all indicated that *Moringa oleifera* leaf has some antibacterial activity despite the use of the disc diffusion method which is different from the agar well diffusion technique of the present study. The difference in the techniques of assaying antibacterial properties of *Moringa oleifera* and the bacterial pathogens tested makes a detail comparison of these works difficult. However, Devendra *et al* 2011 examined *Moringa oleifera* seed instead of leaf for antibacterial activity using the agar well diffusion method applied in the present study and still demonstrated that *Moringa oleifera* has antibacterial properties. Of the tested organisms by Devendra *et al* 2011, only *Escherichia coli* is close enough for comparison with the present study, the difference in the strain type notwithstanding.

The diameter of the zone of inhibition for *Escherichia coli* (ATCC 25922) was 11.0mm in the present study as against 8.8mm of *Escherichia coli* (MTCC 443) in the report of Devendra *et al.* (2011) with the results of the compared studies indicating the antibacterial properties of *Moringa oleifera*. Karthy *et al* (2009) reported the antibacterial potential of the seed extracts of *Moringa oleifera* against MRSA and its ability to replace the β -lactam antibiotics which is seriously losing its effectiveness against MRSA. In the present study, *Moringa oleifera* leaf extract similarly demonstrated activity against MRSA.

The minimum inhibitory concentration of EMOL against the pathogenic organisms were found to be 256mg/ml for both MRSA (ATCC 33591) and *Acinetobacter baumannii* (ATCC 19606), and 512mg/ml for *Escherichia coli* (ATCC 25922). This finding is similar to that of Bukar *et al* 2010 who report 200mg/ml as the minimum concentration of *Moringa oleifera* extract with activity against food-borne bacterial pathogens. The slightly lower MIC as reported by Bukar *et al* 2010 may be attributed to the chloroform based *Moringa oleifera* extract used as against ethanolic extract of the present study. Furthermore, the bacterial organisms used for our study are well known virulent pathogens and expected to be more resistant compared to the food-borne pathogens used in the work of Bukar *et al* 2010.

The antimicrobial activity of *Moringa oleifera* has been attributed to the presence of a number of phytochemicals but most importantly, the activity of the polypeptide 4 (α -L-rhamnosyloxy) benzylisothiocyanate (Eilert *et al*, 1981). Guevara *et al.*, 1999 had also reported that *Moringa oleifera* contain at least seven active ingredients with antitumor promoting activity and inhibitory effects on Epstein-Barr virus. Vinoth *et al* (2012) suggested that the antibacterial activity of *Moringa oleifera* seed extract was due to the bioactive polypeptides it contained. The mechanism of antibacterial action of the polypeptides isolated from *Moringa oleifera* seed was reported to be due to the induction of membrane destabilization and cytoplasmic leakage in susceptible microorganisms (Suarez *et al* 2003). Oluduro *et al* (2012) evaluated the antibacterial potential of the seed extract on orthopaedic wounds and attributed the observed effect to the presence of benzyl isothiocyanate and phenyl methanamine

isolated from the seeds in their study while Padla *et al* (2012) reported antimicrobial effect of two isothiocyanate derivatives isolated from the seed extract of *Moringa*. Isothiocyanate compounds appear responsible for both antimicrobial and other properties of *Moringa oleifera* going by the outcome of these studies and will need further evaluation. Studies earnest desired therefore will include evaluation of antibacterial properties of isothiocyanate against virulent bacteria and its toxic properties.

In conclusion, our study has supported other previous data which showed antibacterial effects of *Moringa oleifera*. In addition, this study point to a promising role for *Moringa* as an alternative source of drugs for effective treatment for infections caused by virulent multi-drug resistant bacteria such as MRSA and *Acinetobacter*. Further studies are underway in our laboratory to characterize the active constituents in EMOL responsible for the reported antibacterial activity for further analysis.

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REFERENCES

- Andrews JM (2001): Determination of minimum inhibitory concentrations. *Journal of antimicrobial chemotherapy* 48 (Suppl. S1): 5-16.
- Anwar F, Latif S, Ashraf M, Gilani AH (2007): *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Res* 21:17-25.
- Bhatnagar SS, Santapau H, Desai JD, Maniar AC, Ghadially NC, Solomon MJ, Yellore S, Rao TNS

(1961): Biological activity of Indian medicinal plants. Part 1 antibacterial, anti-tubercular and antifungal action. *Ind J Med Res.* 49: 799-805.

Bukar A, Uba A, Oyeyi TI (2010): Antimicrobial profile of *Moringa oleifera* Lam. Extracts against some food-borne microorganisms. *Bayero J Pure Appl Sci* 3 (1): 43-48.

Devendra B.N, Srinivas N, Prasad.Talluri V.S.S.L, Swarna Latha P (2011): Antimicrobial activity of *Moringa oleifera* lam., leaf extract, against selected bacterial and fungal strains. *International Journal of Pharma and Bio Sciences.* 2 (3); B13 –B18.

Eilert U, Wolters B and Nadrtdt A (1981): The antibiotic principle of seeds of *Moringa*

oleifera and *Moringa stennotopala*. *Planta Med.* 42:55 –51.

Fahey JW (2005): *Moringa oleifera*: A review of the Medical evidence for its nutritional, Therapeutic and prophylactic properties. Part 1. *Tree for life Journal* 1 (5): 1 –15
<http://www.TFLJournal.org/article.php/20051201124931586>. Accessed 12/04/2013.

Lar PM, Ojile EE, Dashe E and Oluoma JN (2011). Antibacterial activity of *Moringa oleifera* seed extracts on some gram negative bacterial isolates, *African Journal of Natural Sciences*, 14, 57 – 62.

Kapil A (2005): The challenge of antibiotic resistance: Need to contemplate. *Indian J Med Res.* 121: 83-91

Karthy ES, Ranjitha P, Mohankumar A (2009): Antimicrobial potential of plant seed extracts against multidrug resistant methicillin resistant *staphylococcus aureus* (MDR-MRSA). *Intern J Biol* 1(1):34-40.

Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T (1999). An anti-

tumour promoter from *Moringa oleifera* Lam. *Mutation Res* 40 (2):181-188.

Oluduro OA, Idowu TO, Aderiye BI, Famurewa O, Omoboye O.O (2012): Evaluation of antibacterial potential of crude extract of *Moringa oleifera* seed on orthopaedics wound isolates and characterization of Phenylmethanamine and Benzyl Isothiocyanate derivatives. *Res. J. of Medicinal Plants* 1-12.

Pal SK, Mukherjee Pulok K, Saha K, Pal M and Saha BP (1995). Antimicrobial action of the leaf extract of *Moringa oleifera* Lam. *Ancient Science of Life.* XIV (3): 197-199.

Padla EP, Solisa LT, Levidab RM, Shenc CC and Ragasa CY (2012). Antimicrobial Isothiocyanates from *Moringa oleifera*. *Z. Naturforsch.* 67 c; 557 – 564

Pavia DL, Lampman GM and Krutz SG (1982): Introduction to organic laboratory techniques, 2nd edition, Saunders Publication, USA.

Peixoto JFO, Silva GC, Costa RA, de Sousa Fontenelle JL, Vieira GHF, Filho AAF and dos Fernandes Vieira RHS (2011). In vitro antibacterial effect of aqueous and ethanolic moringa leaf extracts. *Asian Pacific J. of Tropical Medicine.* 201-204. doi: 10.1016/S1995-7645(11)60069-2

Posmontier (2011). The medicinal qualities of *Moringa oleifera*. *Holist Nurs Pract.* 25 (2): 80 - 87. doi: 10.1097/HNP.0b013e31820dbb27.

Rahman M. Mashiar, Sheikh MMI, Sharmin SA, Islam MS, Rahman MA, Rahman MM, Alam MF (2009): Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against Some Human Pathogenic Bacteria. *Journal of Natl Sci* (2009) Vol 8(2): 219-227

RAJAMANICKAM K and SUDHA SS (2013): *In-Vitro* Antimicrobial Activity And *In-Vivo* Toxicity Of *Moringa Oleifera* And *Allamanda Cathartica* Against Multiple Drug Resistant Clinical Pathogens, *International Journal of Pharma and Bio Sciences* 4(1): (B) 768 - 775

Rathi BS, Bodhankar SL, Baheti AM (2006). Evaluation of aqueous leaves extract of moringa *oleifera* Linn. for wound healing in albino rats. *Ind. J. Exp. Biol.* 44 (11): 898-901.

Sofowora (1982): Medicinal plant and traditional medicine in Africa. *John Wiley & sons.*

Suarez M, Entenza JM, Doerries C, Meyer E, Bourquin L, Sutherland J, Marison I, Moreillon P, Mermod N (2003): Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities. *Biotechnol Bioeng* 81 (1):13-20.

Thilza IB, Sanni S, Isah ZA, Sanni FS, Talle M and Joseph MB (2010). *In vitro* Antimicrobial activity of

water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases, *Academia Arena*, 2 (6): 80 – 82
<http://www.sciencepub.net/academia>

VIEIRA GHF, MOURAO JA, ANGELO AM, COSTA RA and VIEIRA RHSF (2010) Antibacterial effect (*in vitro*) of *Moringa oleifera* and *Annona muricata* against Gram positive and Gram negative

bacteria, *Rev. Inst. Med. Trop. Sao Paulo*, 52 (3): 129 - 32.

Vinoth, R.B Manivasagaperumal, Balamurugan S (2012). *International Journal of Research In Biological Sciences* 2(3): 98-102.

World Health Organization (1991) :Basic procedures in clinical bacteriology.WHO Geneva, p193.