POTENCY OF THE FRACTIONS OF *MORINGA OLEIFERA* LAM MORINGACEAE *STAPHYLOCOCCUS AUREUS*

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The aim of this study is to determine the comparative effect of the methanol leaf extract and partitioned fractions of *Moringa oleifera* on Methicillin-Resistant *Staphylococcus aureus* (MRSA). Phytochemical screening of the fractions of methanol leaf extract of plant sample was carried out by employing standard methods, and its various fractions (chloroform and aqueous), which were also subjected to antimicrobial activity against Methicillin Resistant *S. aureus* (MRSA) gotten from the nostrils of randomly selected healthy individuals. The crude extract was partitioned using two solvents, chloroform and aqueous. Antimicrobial activities of the chloroform and aqueous fractions against the test organisms were determined. Methicillin Resistant *S. aureus* (MRSA) isolate number 63 was found to be the most susceptible bacteria to the aqueous and chloroform fractions with diameter zone of inhibition of 6- 21 mm and 4- 7 mm, as well as 6-14 mm respectively. This study does not only reveal the antibacterial activity of *M. oleifera*, but also provides a scientific basis for the traditional use of the plants. Pure chemical compounds can be isolated from the plant and antimicrobial activity against other bacteria and fungi should be studied to use them as sources and templates for synthesis of new drugs to control infectious diseases.

Key words: *Moringa oleifera*, partitioning, *Staphylocuccus aureus*, phytochemical.

INTRODUCTION

Recently, there is evidence that medicinal plants can be an alternative for the treatment of non-fatal infectious diseases. They can also function as potential new sources, cheaper and better antibiotics, with high inhibitory properties of pathogenic strains. Many studies provide scientific data on plant prevalence for various infectious diseases (Amal and Nashwa, 2017). Large use and abuse of antibiotics in treating bacterial infections and in agriculture, livestock and poultry have led to the development and spread of resistant strains. Owing to this problem, companies are faced with a total health problem that is seriously related to the emergence of infectious bacteria that are resistant to some of the antibiotics that are prevalent. In addition to rising drug costs, this situation paves the way for an urgent increase in chronic infections in developing countries, which is why alternative treatments that are more efficient, safer and less toxic are required (Chekesa and Mekonnen, 2015).

The *Moringa oleifera* plant was born in South Asia, but is found in the tropics today and has been known to plays an important role in medicine. M. oleifera is a small or medium sized tree with a height of 5 to 10 m. *M. oleifera* are wild plants that grow on the plains, especially on fences and farms at home. It thrives under tropical islands and many near river and river sand. It grows well in humid tropics or hot dry areas and thus survives in poor soil, which hardly affects drought. It tolerates a variety of precipitation with a minimum annual rainfall of 250 mm and a maximum of more than 3000 mm and pH 5.0 to 9.0 (Upadhyay et al., 2015).

Ayurvedic medical systems combine the efficacy of Moringa for the treatment or prevent disease. Ajayi and Fadeyi (2015) observe the use of herbal medicines and medicinal plants in most developing countries as therapeutic tools to maintain good `health. In addition, the following is growing dependence on the use of medicinal

plants in the industrial community recruitment and development of drugs and chemotherapy drugs from these plants as well as from traditional herbal medicines. The healing properties of plants may be due to antioxidants, antimicrobial and antipyretic effects of phytochemicals (Deka and Nath, 2015). Bioactive compounds from medicinal plants play an important role in the regulation of host-microbial interactions that support the host. It is therefore important to identify bioactive compounds in plants to isolate them, to purify active ingredients in raw extracts with various methods of analysis and characterization. The healing properties of plants may be due to antioxidants, antimicrobial and antipyretic effects of phytochemicals. Moringa oleifera Lam (Moringaceae) is a very valuable plant, which is also a very popular addition for impressive water treatment capacity and high nutritional value (Ajayi and Fadeyi 2015).

The antimicrobial activity of leaves, seeds, root, and bark were evaluated against yeast bacteria, helminth and dermatophytes pathogenic to man. The fresh leaf juice and aqueous extract of seeds inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Chekesa and Mekonnen, 2015*)*. The seed extract exhibited significant antibacterial activity against pyodermia (skin infection), causing bacterium, *S. aureus* in experimental mice (Chekesa and Mekonnen, 2015*)*. The root chloroform fractions of an ethanol extract of the root bark have been discovered to be accountable for the antifungal and antibacterial activities. The bark extract possess antifungal properties (Packialakshmi and Archana, 2014).

S. aureus largely colonizes under the anterior nares. The other parts of the respiratory tract, the urinary tract, open wounds and intravenous catheters are equally possible sites for infection. Healthy persons might carry MRSA asymptomatically for a long period of time. Patients with compromised immune systems are at a considerably greater risk of secondary symptomatic infection (National Institute of Health, 2006).

Methicillin-resistant *S. aureus* is usually a bacterium that is accountable for quite a lot of difficult-to-treat human infections. It is also called oxacillin-resistant *S. aureus* (ORSA) (McDougal et al., 2003). MRSA is any strain of *S. aureus* that has emerged, through the course of natural selection, resistance to beta-lactam antibiotics, which include penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. The Strains that could not to resist these antibiotics are referred to as methicillin-susceptible *S. aureus* (MSSA). The development of such resistance does not allow the organism to be more intrinsically virulent than strains of *S. aureus* that do not have antibiotic resistance, but resistance as well does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous (Yan et al., 2013*)*.

In majority of the patients, MRSA can be detected by swabbing the nostrils and isolating the bacteria found inside the nostrils. In combination with extra sanitary measures for those in contact with infected patients, swab screening patients admitted to hospitals has been found to be effective in minimizing the spread of MRSA in hospitals in the United States, Denmark, Finland, and the Netherlands *(*Raygada et al., 2009).

There is rise in mortality partially due to infection in more seriously ill patients, there is no doubt that there is need for fresh approaches and new molecules to treat infections caused by pathogens that are resistant to virtually all the available antibiotics. The world is at a crucial point, which has not experienced the pre-antibiotic period, where, infections caused by several bacterial pathogens are untreatable. The aim of this study is evaluate antimicrobial properties of the methanol leaf extract of *M. oleifera* and fractions on MRSA bacterial strains.

MATERIALS AND METHODS

Bacteriological media

Nutrient Agar: Titan Biotech Ltd, India, Sabaround Dextrose Agar: Titan Biotech Ltd, India, Mannitol salt agar and Muller Hinton agar.

Collection and preparation of plant sample

The leaf of *M. oleifera* was collected from Abraka, Delta State and identified and authenticated with the voucher number *M. oleifera* – FHI 112301 by Mr. Odewo, Forest

Research Institute, Jericho Ibadan. The leave was air-dried and powdered; the powdered sample was then weighed and stored at room temperature (20±2°C).

Extraction of the Plant Sample

Eighty percent methanol solvent was used to extract the powdered leave sample of *M. oleifera*. Five hundred grams (500 g) of the powdered sample was subjected to cold maceration method of extraction by soaking it in 1500 ml of methanol for 5 days after which the extract was filtered with muslin cloth and concentrated by evaporating excess solvent present in it on a rotary evaporator. The resulting concentrate was stored in porcelain dish and weighed; thereafter it was stored in a refrigerator at 4°C until required for use in the experiment (CLSI, 2015).

Partitioning of crude methanol extract of *M. oleifera* leave sample

Methanol leaves extract of *M. oleifera* (50 g) one after the other was extracted with solvents of increasing polarity (chloroform and water). The methanol extracted was dissolved with 250 ml distilled water, and then 250 ml of chloroform was added to the mixture. The whole mixture was shaken vigorously in a separatory funnel and then allowed to stand. On the standing, the mixture separated into two layers. The upper layer was the aqueous phase, while the lower layer was the chloroform phase. The chloroform and aqueous layers were eluted out of the separatory funnel and stored for further antibacterial analysis (CLSI, 2015).

Phytochemical analysis

The phytochemical analysis was carried out on the chloroform and aqueous fraction of *M. oleifera* methanol leave extract, following the methods described by Trease and Evans (1989) and Harbone (1998).

Ethical consideration

Before samples were collected, informed consent was obtained from participants, after approval was sought from the Delta State University Health Centre.

Bacterial Isolation

Seventy sterile swab sticks were used to swab the nostrils of eighty different individuals and mannitol salt agar was prepared according to manufacturer's specification and sterilized in an autoclave at a temperature of 121°C for 15 min. After sterilization, the mannitol salt agar was poured into eight petri dishes and allowed to solidify. The swab sticks were used to streak each petri dish and the petri dishes were incubated at 37°C for 24 h after which color changes were observed for each petri dish (CLSI, 2015).

Nutrient agar was prepared according to manufacturer's specification and transferred into bijou bottles which were autoclaved at 121 °C for 15 min at 1 atmospheric pressure after sterilization. It was kept in a slanted position and allowed to cool and solidify. After solidification, the different strains of *S. aureus* that were isolated were inoculated into different bijou bottles containing the slang nutrient agar and incubated at 37°C for 24 h (CLSI, 2015).

Determination of methicillin resistance *S. aureus* (MRSA) using cloxacillin

Mueller Hinton agar was prepared according to manufacturer's specification and sterilized in an autoclave. It was then allowed to cool and transferred into petri dishes and left to solidify. After solidification, different strains of *S. aureus* were spread on the surface of the agar plate. Discs containing with 5 µg of cloxacillin solution were placed on the surface of each culture plate and incubated at 37°C for 24 h. After incubation, zone of inhibitions were noted.

Sensitivity test of MRSA to *M. oleifera* plant chloroform and aqueous fractions

A two-fold serial dilution of the plant fractions was done (400, 200, 100, 50, 25 and 12.5 mg). Six holes were punched on the agar using 6 mm cork borer and each hole was labeled according to the different concentrations. The methanol plant extract was transferred into the culture plate according to their labeled concentrations and left for some time to diffuse, then it was incubated at 37°C for 24 h. After incubation, zones of inhibition for each concentration were observed (CLSI, 2015). The same was done for chloroform and aqueous fraction of *M. oleifera.*

RESULTS

The results of the phytochemical screening of the methanol leaf extracts of *M. oleifera* is presented in Table 1. The Table showed the result of phytochemical screening of chloroform and aqueous fractions of the methanol extract of *M. oleifera.* Nine bioactive constituent test were carried out on the chloroform and aqueous fraction of *M. oleifera* methanol leaf extract, the result revealed the presence of six plant bioactive constituents for chloroform fraction (saponin, tannin, phenol, alkaloid, steroid and terpenoid), while flavonoid and cardiac glycoside were absent in the chloroform fraction. Seven constituent were found to be present in the aqueous fraction of the methanol extract of *M. oleifera* among which are saponin, tannin, phenol, flavonoid, alkaloid, steroid and terpenoid with cardiac glycoside reported as being absent.

Table 1. Result of phytochemical screening of chloroform and aqueous fractions.

Plant constituents	Chloroform	Aqueous
Saponin	Present	Present
Tannin	Present	Present
Phenol	Present	Present
Flavonoid	Absent	Present
Alkaloids	Present	Present
Steroid	Present	Present
Terpenoid	Present	Present
Cardiac glycoside	Absent	Absent

Seventy nasal isolates were collected, out of which 12 *S. aureus* were identified after standard biochemical tests. Methicillin resistant *S. aureus* was determined and 11 out of 12 were found to be MRSA, as shown in Table 2.

Table 2. Isolation of *S. aureus* from the (nostril) nose swap.

Isolate	Results
6	+
8	+
9	+
21	-
33	+
38	+
45	+
48	+
61	+
63	+
65	+
69	+

Key:+ = Resistant, - = Susceptible.

Table 3 shows that diameter zone of inhibition of chloroform fraction of methanol extract of *M. oleifera*. The result revealed that chloroform fraction had good activity on the MRSA isolates at various concentrations except for MRSA isolates of 6, 38 and 65, which showed no zone of inhibition of the fraction for all concentration. The high activity was seen at 500 mg/ml for all test organisms, recording zones of inhibition of 17, 9, 4, 5, 21, 8 and 7 mm for 8, 9, 4, 48, 21, 8, 33 and 61 test organisms respectively; while the least activity was seen at 100 mg/ml for 48 test organism with diameter zone of inhibition of 2 mm.

Result of antibacterial activity of aqueous fraction of the plant extract is shown in Table 4. The result revealed that aqueous fraction only showed good activity against MRSA strain 63 with highest activity at 400 mg/ml and a diameter zone of 14 mm for inhibition; while 50 and 12.5 mg/ml had diameter zones of inhibition of 6 mm, thus having the least activity against the test organism. The fraction was susceptible to other organisms as they recorded no zone of inhibition for other test organisms.

DISCUSSION

The emergence of antibiotic resistant bacterial

Table 3. Diameter zone of inhibition of chloroform fraction.

Organisms	Concentration (mg/ml)					
	400	200	100	50	25	12.5
6	-	-	-	-	-	-
8	17 mm	7 mm	5 mm	5 mm	7.6 mm	6 mm
9	9 mm	4 mm	4 mm	-	-	-
33	7 mm	-	-	-	-	-
38	-	-	-	-	-	-
45	4 mm	-	-	-	-	-
48	5 mm	3 mm	2 mm	-	-	-
61	7 mm	-	-	-	-	-
63	21 mm	20 mm	6 mm	5 mm	5 mm	7 mm
65	-	-	-	-	-	-
69	8 mm	6 mm	6 mm	4 mm	-	-

Key:- = no inhibition.

Table 4. Diameter zone of inhibition of aqueous fraction.

Organisms	Concentration(mg/ml)					
	400	200	100	50	25	12.5
6	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
33	-	-	-	-	-	-
38	-	-	-	-	-	-
45	-	-	-	-	-	-
48	-	-	-	-	-	-
61	-	-	-	-	-	-
63	14 mm	12 mm	8 mm	6 mm	7 mm	6 mm
65	-	-	-	-	-	-
69	-	-	-	-	-	-

Key:- = no inhibition.

strains is an important issue that creates problems in the treatment of infectious diseases and makes the search of an alternative therapy a must (Moyo et al., 2012; Uprety et al., 2012). *M. oleifera* is gaining more popularity as a valuable medicinal plant and has previously been documented as a source of antibiotics (Upretyet al., 2012). Medicinal plants possess curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolite found in one or more part of the plant (Patilet al., 2009). This present study showed the presence of secondary plant metabolites on chloroform and aqueous fraction of the methanol extract of *M. oleifera*, with the chloroform fraction having six metabolites (tannin, saponin, phenol, alkaloid, steroid and terpenoid); while the aqueous fraction showed the presence of seven metabolites which include tannin, phenol, saponin, alkaloid, flavonoids, steroid and terpenoid (Table 1). This finding is in line with that reported by Ajayi and Fadeyi (2015), who reported the presence of phenol, tannin, alkaloid, flavonoid and steroid. The finding is consistent with the report of Kuthar et al. (2015) who demonstrated the efficacy of medicinal plants in eliminating some bacterial pathogens from human body. Similar study carried out on the seed aqueous extract of *M. oleifera* revealed the presence of carbohydrate, reducing sugars, steroid, glycosides, flavonoids, saponins, amino acids and protein, fixed oils and fats and alkaloids in moderate concentration (Packialakshmi and Archana, 2014).

There is continuous and urgent need for discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because of alarming increase in the incidence of new and re-emerging infectious diseases (Parekh and Chanda, 2008). Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs (Parekh and Chanda, 2008). During this study, 12 clinical isolates previously identified as *S. aureus* were obtained from clinical samples collected from different individuals in the Delta State University, Abraka. The results of the antimicrobial susceptibility test revealed that 99% of the isolates were found to be resistant to Cloxicillin antibiotics and this reflects the emergence of new strains of *S. aureus* which are resistant to classes of antibiotics that it was sensitive to before, while only one of the isolates was susceptible to the antibiotic (Table 3.2). This result is comparable to that of Nwankwo and Nasiru (2011).

Antibacterial activity of chloroform and aqueous fraction (Tables 3 and 4) of the leave extracts showed good activity, especially for the chloroform fraction with activities seen at 400, 200, 100, 50 and 25 mg/ml on almost all test organisms, except for MRSA bacterial isolates of 6, 38 and 65; whereas aqueous fraction had activity only on 63 MRSA bacterial strain with the highest activity at 400 mg/ml having zone of inhibition of 14 mm. Aqueous fraction with concentration at 50 and 12.5 mg/ml recorded the least activities with 6 mm zone of inhibition respectively. Similar study carried out by El-Awady et al. (2015) showed that *M. oleifera* and *M. peregina* had activities at 8 and 16 mg/ml respectively, the concentration of antibacterial activity was quite low when compared to this present study.

The antibacterial activity of the chloroform and aqueous fractions of *M. oleifera* methanol leave extract agrees with the findings of many authors on the antimicrobial activities of this plant (Caceres et al., 1991). The antimicrobial activities demonstrated by the fractions in this study are quite remarkable, particularly as standard antibiotics are in the purified and concentrated form (Wilke et al., 2005). The result showed that chloroform fraction of the methanol extract of *M. oleifera* had good antibacterial property when compared to the aqueous fractions with the highest diameter inhibition zone; probably the plant active constituents were more soluble in the chloroform organic phase than the aqueous phase.

Conclusion

From the above results, it can be concluded that traditional medicinal plant, *M. oleifera*, possesses diverse antibacterial activity in its different parts against Methicillin Resistance *S. aureus* (MRSA) bacterial isolates. It can be concluded that chloroform fraction of the methanol extract of *M. oleifera* had good antibacterial property when compared to the aqueous fractions with the highest diameter inhibition zone. This means that chloroform fraction is more effective against test Methicillin Resistance *S. aureus* (MRSA) bacterial than aqueous fractions. Thus, *M. oleifera* is a promising natural antibacterial agent with potential applications in pharmaceutical industry as a source and template for the synthesis of new drugs to control infectious diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ajayi A. O. and Fadeyi, T. E. (2015). Antimicrobial Activities and Phytochemical Analysis of Moringa oleifera Leaves on Staphylococus aureus and Streptococcus species. American Journal of Phytomedicine and Clinical Therapeutics, 3(1): 643-663.

Amal, S. O. and Nashwa, A. A. (2017). Antibacterial effect of the ethanol leaves extract of *Moringa oleifera* and Camellia sinensis against multi drug resistant bacteria. International Journal of Pharmacology, 13: 156-165.

Caceres, A., Cabrera, O., Morales, O., Mollinedo, P. and Mendia, P. (1991). Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology, 33: 213–216.

Chekesa, B. and Mekonnen, Y. (2015). Antibacterial Activity of Moringa stenopetala against some human pathogenic bacterial strains. Science, Technology and Arts Research Journal, 4(2): 190-198.

Clinical and Laboratory Standard Institute (CLSI). (2015). Performance standards for antimicrobial disk susceptibility tests; Approved standard- 12th Edith CLSI document M02-A12. Wayne, PA: Clinical and Laboratory Standards Institute.

Deka, K. and Nath, N. (2015). Traditional hepatoprotective herbal medicine of Bongaigaon district, Assam (N.E. India). American Journal of Ethnomedicine. 2: 5.

El-Awady, M. A., Hassan, M. M., Abdel-Hameed, E. S. and Gaber, A. (2015). Comparison of the Antimicrobial Activities of the Leaves-Crude Extracts of Moringa peregrinaand Moringa oleifera in Saudi Arabia. International Journal of Current Microbiology and Applied Science, 4(12): 1-9.

Harbone, J.B. (1998). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, 2nd ed. Chapman and Hall: New York, pp. 182 – 190.

Kuthar, S. S, Digge V. G., Hogade M. G., Poul B. N, Jadge D. R., (2015). Screening of antibacterial activity of aqueous back extract of Bombaxceibaagainstsome Gram-positive and Gram-negative Bacteria. American Journal of Phytomedicine and Clinical Therapeutics, 3:7.

Moyo, B., Masika, P.J. and Muchenje V. (2012). Antimicrobial activities of Moringa oleifera Lam leaf extracts. African Journal of Biotechnology, 11: 2797-2802.

Nwankwo, E. O. and Nasiru, M. S. (2011). Antibiotic sensitivity pattern of *Staphylococcus aureus*from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria, Pan African Medical Journal, 8: 4.

Packialakshmi, N. and Archana, J. (2014). Phytochemical analysis and antibacterial activity of Moringa oleifera to treat different kinds of water samples. International Journal of Phytopharmacology, 5(3): 233-238.

MRSA Toxin Acquitted (2006). Study Clears Suspected Key to Severe Bacterial Illness. *NIH news release.* National Institute of Health. 2006-11-06.

McDougal, L. K., Steward, C. D., Killgore, G. E., Chaitram, J. M., McAllister, S. K. and Tenover, F. C. (2003). Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: establishing a national database. Journal of Clinical Microbiology. 41(11): 5113–5120.

Yan, M., Pamp, S. J., Fukuyama, J., Hwang, P. H., Cho, D. Y., Holmes, S. and Relman, D. A. (2013). Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and Staphylococcus aureus carriage. Cell Host Microbe 14(6): 631–640.

Raygada, J. L., and Levine, D. P. (2009). Managing CA-MRSA Infections: Current and Emerging Options Infections in Medicine 26: 2.

Parekh, J. and Chanda, S. V. (2008). Antibacterial activity of aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus species. Turkish Journal Biology, 32: 63-71.

Patil, S. B., Naikwade, M. N. S. and Magdum, C. S. (2009). Review on phytochemistry and pharmacological aspect of Euphorbia hirta Linn. JPRHC 1: 113-133.

Savithramma, N., Linga Rao, M., and Suhrulatha, D. (2011). "Screening of Medicinal Plants for Secondary Metabolites." Middle-East Journal of Science Research. 8: 579-84.

Sharma, P., Kumari, P., Srivastava, M. and Srivastava, S. (2006). Removal of

cadmium from aqueous system by shelled *Moringa oleifera*Lam. seed powder. Bioresources Technology. 97: 299–305.

Trease, G. E. and Evans, W. C. (1989). Textbook of Pharmacognosy. 12th Edn. Balliere, Tinadl, London.

Upadhyay, P., Yadav, M. K., Mishra, S., Sharma, P. and Purohit, S. (2015). *Moringa oleifera*: A review of the medical evidence for its nutritional and pharmacological properties. International Journal of Research in Pharmacy and Science, 5(2): 12–16.

Uprety, Y., H. Asselin, A. Dhakal, A., and Julien, N. (2012). Traditional use of medicinal plants in the boreal forest of Canada: Review and perspectives. Journal of Ethnobiology Ethnomedicine. 8. 10.1186/1746-4269-8-7.

Verpoorte, R. (1998). "Exploration of Nature's Chemodiversity: The Role of Secondary Metabolites as Leads in Drug Development." Drug Discovery Today 3(5): 232-238.

Wilke M. S., Lovering A. L., Strynadka N. C. (2005). Betalactam antibiotic resistance: a current structural perspective, Current Opinion in Microbiology. 8 : 525-533

Zija (2005) http://www.amustudio.com/zija/moringa/plant/*Moringa oleifera*.php.