COMPARATIVE ANTIBACTERIAL PROPERTY OF ETHANOLIC LEAF AND SEED EXTRACTS OF MORINGA OLEIFERA LAM.

Hemen T J.¹, Johnson J T.²*, Ujah O F², Udenze E C C³

¹Department of Biological Sciences, Faculty of Sciences University of Maiduguri, Maiduguri, P.M.B. 1069. Borno State Nigeria.
²Department of Biochemistry, College of Natural and Applied Sciences University of Mkar, Mkar, P.M.B. 017. Gboko, Benue State Nigeria.
³Department of Pharmacology, College of Medical Sciences University of Calabar, Calabar, P.M.B. 1115 Calabar, Cross River State.

ABSTRACT
The comparative antimicrobial property of Moringa Oleifera leaf and seed were evaluated in this study, the ethanolic leaf and seed extract of Moringa Oleifera was tested on 3 human pathogenic bacteria strains; Streptococcus spp, Staphylococcus aureus and Pseudomonas spp the results obtained reveals that both leaf and seed extract had antibacterial potentials against the bacterial strains used when compared with the control. However, leaf extract showed high inhibitory potenitals against Staphylococcus aureus (20.00mm) compared with the seed (10.10mm) and positive control (Ciprofloxacin), (13.00mm) moreso, the leaf extract also showed strong inhibitory powers against Streptococcus spp (16.00mm) and Pseudomonas spp (14.00mm) when compared with the seed (13.00mm) and (11.00mm) for Streptococcus spp and Pseudomonas spp respectively at P < 0.05. Sequel to the results above, the investigation suggest that the extracts of the leaf and seed have antibacterial potential but the ethanolic leaf extract appear more effective when compared with that of the seed.

Keywords: Antibacterial, Leaf, Seed, Moringa oleifera.

INTRODUCTION
Moringa oleifera is the most widely cultivated species of a monogeneric family, the monogaceae that is native to the sub-himalayan tracts of India, Paskistan, Banglades and Afghanistan. This rapid-growing tree (also known as thehor, mseradish tree, drumstick tree, benzolive tree, kelor, marango, molenge, moonga, mulangay, nebenday, saijhantree, sajna, orben oil tree), was utilized by ancient Romans, Greek and Egyptians; it is widely cultivated has become naturalized in many locations in the tropics. It is a perennial soft wood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. It is already an important crop in India, Ethiopia, the Philippines and the Sudan and is being grown in west, East and South Africa, tropical Asia, Latin America and the Caribbean, Florida and the pacific islands.
All part of *Moringa* trees are edible and have long been consumed by humans (Ross, 1999).

In the west, one of the best known uses of *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water, but seeds are also eaten green, roasted, powdered and steeped for tea or used in curries. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, vitamin c and carotenoids suitable for utilization in many of the so-called “developing” region of the world where undernourishment is a major problem (Tahiliani, 2000).

According to Lockett (2007) previous studies have reported that various part of *Moringa* roots, flower, bark and stems includes seed possess antimicrobial properties. Seeds of *Moringa Oleifera* are also known for *Moringa Oleifera* coagulation properties for treating water and wastewater due to flocculent protein/peptides. Seed extract of *Moringa Oleifera* have been found to have antimicrobial properties. Structural morphological study of microbes after treatment with antimicrobial agents it is an important parameter in understanding the mechanism of action of these agents. However, a little review is available on the mechanism microscopic study of *Moringa Oleifera*. Therefore, microscopic evaluation of the some fungal and bacterial strains was carried out after treatment with seed extracts of plant that demonstrated the antimicrobial effect of the optimizing medicinal plant extracts on the structural deformities of the microbes. Effect of temperature, pH, and different ionic concentrations on antimicrobial activity of *Moringa Oleifera* seed extracts was also investigated (Kebreab, 2007). The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune-compromised patients in the developing countries. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics reduced the specter of “untreatable” bacterial infections and add urgency to the search for new infection-fighting strategies (Zy et al .2005; Rajas et al; 2006). Been an important source of natural products for human health, the antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties Adriana et al; 2007. It is therefore very necessary that the search
for newer antibiotics become a continuous process. Sequel to this premise, plants has proved to be the cheapest and safer alternative source of antimicrobials (Dillard, and German, 2000); Sharif and Banik, 2006; Doughari et ál; 2007. Legend has it that Möringa’s effectiveness is known for treating more than 300 conditions and has been heavily utilized in folk medicine to treat a variety of health conditions. It has been targeted on the Discovery Channel as one of the best all natural supplements in the world (Shukla et ál; 1998). Möringa Oleifera lam (also known as Malunggay) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics. The Möringa plant provides a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value, Möringa Oleifera is very important for its medicinal value (Shukla et ál; 1998). Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia. This review focuses on the detailed phytochemical composition, medicinal uses, along with pharmacological properties of different parts of this multipurpose tree. India’s ancient tradition of ayurveda medicine sites 300 diseases that are treated with the leaves of the Möringa tree. (Adesokan et ál; 2000). More so, Möringa trees have been used to combat malnutrition, especially among infants and nursing mothers. A large number of reports on the nutritional qualities of Möringa now exist in both the scientific and the popular literature. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value (Trees for life, 2005). Health nutritionists claim that an ounce of malunggay has the same vitamin c content as seven oranges. An important function of vitamin c not known to many is its being an antioxidant. In fact, it has been recognized and accepted by the US Food and Drug Administration as one of the four dietary
antioxidants, the others being vitamin E, beta-carotenes and selenium. (A dietary oxidant is a substance in food that significantly decreases the adverse effects of harmful chemicals). Countless instances of life saving nutritional rescue that are attributed to *Moringa* (Udupa, et al; 1994). However, Various parts of the *Moringa* plant which are being used for health reasons. For one, the leaves of this plant proved to be a good source of calcium, iron, ascorbic acid and phosphorus. Its other parts such as the seeds, the young pods, and the flowers have been established to benefit individuals as far as antioxidant, anti diabetic, circulatory stimulations, and such other activities that are most beneficial to mankind, are concerned. There have been claims that *Moringa* can be used to lower blood pressure, aid the pains caused by rheumatism, headaches and migraines, as well as its being an antitumor plant. Malunggay is also used for purgative and antifungal purposes, as well. All these prove the claim that this plant is indeed multipurpose (Trees for life, 2005).

Findings of a study in India, which were used as the basis of many news reports on malunggay as a wonder plant, states that *Moringa* contains anticancer compounds (phytochemicals) that help stop the growth of cancer cells. *Moringa* is said to be effective in treating ovarian cancer, among a host of other diseases like arthritis, anemia, heart complications, kidney problems, scurvy, asthma, and digestive disorders, ulcer, gastritis, diarrhea, colitis, dysentery (Trees for life, 2005). *Moringa* has been observed by scientists to contain unique compounds and enact mechanisms that help purge the liver of these toxins and even reverse the damage that they cause. Silymarin is a flavonoid or specialized molecule found in *Moringa* that has been shown to help reduce the effects of hepatoxins on the liver, improving its resiliency. It also protects the liver from the toxic effects of rare earth metal salts such as praseodymium, cerium, and indium, which are known to causes the body to get rid of these poisonous compounds in a more rapid manner. This same mechanism also helps make *Moringa* effective against several known poisonous compounds such as amanitin and phalloidin.

**MATERIAL AND METHOD**

**Collection of Plant Material**

The dried leaves and seed of *M Oleifera* were collected from university of Agriculture campus, Makurdi, Benue state and were identified in the Botany department with no
DACB32494 and voucher specimen was deposited in the herbarium. The leaves were shade dried at room temperature and then crushed using grinder into powder. 135.5g of the leaf powder were weighed out and soaked into 700ml of 90% ethanol solvent into a conical flask stopper with rubber cork and left for 48hrs in the refrigerator at 4°C. The extract was filtered off using sterile filter paper into a clean conical flask and subjected to water bath evaporation where the extract obtained were then stored in a refrigerator at 4°C for antimicrobial test (Akueshi et al; 2002). However, the seed were crushed into powder, 4.2g of the seed powder were weighed out and the seed Ethanolic solution was immediately soaked with Buffer 5.0 into a conical flask the seed Ethanolic solution was concentrated with the addition of Ammonia sulphate (NH₄SO₄) which was gotten from the addition of 80% Ammonia with 100% concentrated sulphuric acid. However, the seed Buffer solution in concentration with NH₄SO₄ was distributed into 6 test tubes following centrifugation at 10,000xg, 4°C for 10min, the supernatant were collected, NH₄SO₄) was added to seed Buffer extract to enhance precipitation. The standard seed Buffer extract were obtained following Dialyses and was refrigerated for 24hrs at 4°C for antimicrobial test.

Test Microorganisms

Three (3) bacterial strains were used in the study, among were Staphylococcus aureus, Pseudomonas specie and Streptococcus specie. All the typed strains were collected from the department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu Industrial Area, Abuja and confirmed at the microbiology laboratory in university of Mkar, Mkar, Gboko, Benue state.

Antibacterial Assay

Antibacterial activity of two different samples, sample 1: dried leaves ethanol extract and 2: dried seeds Ethanolic extracts were individually tested against studied bacterial. In vitro antibacterial test was carried out using disc diffusion method. Standardized suspension of tested bacteria (10⁸ CFU ml) was spread on 9 different Petri dishes containing nutrient agar as growth media. The dishes were impregnated with dried leaf extract and dried seed extract 3 by 3 plates containing the tested Bacteria suspension in which 1 plate were used to test for the seed extract, 1 plate were used to test for the leaf extract and one plate were also used to test for the standard drug as control for each strain.
of tested Bacteria. Ceprofloxacin (antibiotic) was used as positive control to determine the sensitivity of bacterial strains following air drying, the plates were incubated at 37°C for 24hrs. Antibacterial activity was evaluated in (cm) which was converted to (mm) by measuring the zone of inhibition against the tested bacteria through visible observations.

**Minimum Inhibitory Concentration (MIC)**

The MIC leave sample was determined by two fold serial dilution method (Chandrasekaran and Venkatesalu, 2004). The dose level of 117.5mg ml⁻¹ was used and was serially diluted to achieve mgml⁻¹, 58.50, 29.37, 28.52, mgml⁻¹ and concentration. Briefly 0.1ml concentration of dried leaf extracts were added into the test tubes separately containing 9ml of standardized suspension of tested bacteria (10⁸CFUml⁻¹). The test tubes were incubated at 37°C for 24hrs control was used with the tested bacteria using distilled water instead of the plant extract. The least concentration of the sample with no visible growth was taken as the MIC (Adesokan, et al., 2000). However, MIC of seed, Ethanol extract was determined by diluting Ethanol seed extract of Moringa Oleifera on Petri dishes containing bacteria suspension and were incubated for 24hrs at 37°C MIC was determined by streak method in reference to Antibacterial Assay of Ethanol extract, the following MIC were achieved 24.20, 16.70, 30.39.

**RESULTS**

**TABLE 1: EFFECT OF ETHANOLIC LEAF OF MORINGA OLEIFERA AGAINST SOME HUMAN PATHOGENIC BACTERIA.**

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Zone of Inhibition</th>
<th>Ethanol Leaf Extract</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI</td>
<td>MIC</td>
<td>DI</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>20.00</td>
<td>58.50</td>
<td>13.00</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>16.00</td>
<td>29.37</td>
<td>19.00</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>14.00</td>
<td>28.52</td>
<td>16.00</td>
</tr>
</tbody>
</table>

DI = Diameter of inhibition (mm), MIC = Minimum inhibitory Concentration (mg/ml).
### TABLE 2: EFFECT OF ETHANOLIC SEED EXTRACT OF MORINGA OLEIFERA AGAINST SOME HUMAN PATHOGENIC BACTERIA.

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Zone of Inhibition</th>
<th>Ethanol Leaf Extract</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DI</td>
<td>MIC</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10.00</td>
<td>24.20</td>
<td>13.00</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>13.00</td>
<td>16.70</td>
<td>19.00</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>11.00</td>
<td>30.39</td>
<td>16.00</td>
</tr>
</tbody>
</table>

DI = Diameter of inhibition (mm)

MIC = Minimum inhibitory Concentration (mg/ml).

### TABLE 3: ANTIMICROBIAL ACTIVITY OF LEAF AND SEED EXTRACT OF MORINGA OLEIFERA AGAINST SOME HUMAN BACTERIAL STRAINS.

<table>
<thead>
<tr>
<th>Selected organism</th>
<th>Zone of Inhibition</th>
<th>Ethanol leaf extract</th>
<th>Positive control</th>
<th>Seed extract</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DI</td>
<td>MIC</td>
<td>DI</td>
<td>MIC</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>20.00</td>
<td>58.50</td>
<td>13.00</td>
<td>26.21</td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>16.00</td>
<td>29.37</td>
<td>19.00</td>
<td>26.16</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>14.00</td>
<td>28.52</td>
<td>16.00</td>
<td>25.27</td>
<td></td>
</tr>
</tbody>
</table>

DI = Diameter of inhibition (mm)

MIC = Minimum inhibitory Concentration (mg/ml).

**DISCUSSION**

The present study was conducted to obtain preliminary information on the comparative study of antibacterial activity of leaf and seed extract of *Moringa oleifera* lam. The disc diffusion method was applied to use in this study. The comparism of the antimicrobial activity of leaf and seed extracts of *Moringa Oleifera*, zones of inhibition of ethanol leaf and seed extract on tested bacterial strains were compared. As such, ethanol leaf extract showed stronger antibacterial activity against tested bacterial than Ethanol seed extract. Leaf extract showed high inhibitory potentials against *Staphylococcus aureus* with the zone of inhibition of 20.00 (mm) compared to seed extract 10.10 (mm), leaf extract showed high inhibitory potentials against *Streptococcus spp* 16.00 (mm) compared to seed extract 13.00 (mm) also, leaf extract showed high inhibitory potentials against *Pseudomonas spp* 14.00 (mm) compared to seed extract 11.00 (mm).
Conclusively, from the finding above, the leaf extract has more antibacterial activity than seed extract and thus provides a better antibacterial activity which is in line with the results obtained by Nair et al. (2005).

REFERENCES


For Correspondence:
Johnson, J. T.
Email: theophjoe@yahoo.com