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# Comparative Antimicrobial Effects of Ethanolic and Aqueous Extracts of the Stem and Root of *Moringa oleifera* on some Clinical Isolates (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans)*

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***Abstract:*** ***Moringa oleifera Lam*. is a multiuse tropical tree that is used for food, medicine. and several other purposes. Almost all the parts of this plant: root, bark, gum, leaf, fruit, flowers, seed and seed oil have been used for various ailments in indigenous medicine. Some part of the moringa plant is reported to possess a recombinant protein in it. The seed for instance is able to flocculate Gram-positive and Gram-negative bacteria cells. The seed could also act directly on microorganism by being bacteriostatic. Moringa contains antimicrobial peptides that could disrupt the cell membrane or inhibit essential enzymes in microorganism. In this study, the active agents in the leaves and roots were extracted with both ethanol and water. The extracts were then tested for antibacterial activity using the agar well diffusion method. MIC was determined by the tube dilution technique and their activity determined. The phytochemical ethanol extract was found to contain Spirochin, Moriginine, Catechol Tannins, Steroids and Triterpenoids, Saponins, Anthraquinones, Alkaloids, Reducing sugars, Pterygospermin in different concentrations. The Aqueous extract yielded Spirochin, Moriginine, Saponins, Anthraquinones, Alkaloids, Reducing sugars, Pterygospermin. The *Moringa oleifera* stem and root extracts demonstrated a strong antimicrobial property against all the tested clinical isolates (*Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli and Candida albicans)*. The plant extract also showed antimicrobial activity against all the test organisms, with the zones of inhibition ranging from 5-28 mm in diameter at varying concentrations. The fungi (*Candia albicans)* was more susceptible to the extracts with a zone of inhibition of 28 mm and remarkably better than ciprofloxacin, the positive control with a zone of inhibition of 25 mm on the *Candida albicans*. In this study, the root extracts of *Moringa oleifera* had a better effectiveness than the stem while exhibiting high antimicrobial potential on both fungi and bacteria.**

***Keywords:*** **Phytochemical Analysis, Antimicrobial Effects, *Moringa Oleifera,* Plant Extracts, Clinical isolates.**

## 1. INTRODUCTION

Moringa (*Moringa oleifera Lam*.) is a multipurpose tropical tree mainly used for food, medicinal, agricultural and several others.,. Moringa is able to withstand drought, is nutritious and fast-growing. The plant has since become one of the most economically viable crops in Africa (FAO, 2014; Radovich, 2009; Orwa *et al*., 2009; Bosch, 2004).

Moringa is a small to medium evergreen or deciduous tree that can grow to a height of 10-12 m. It has a spreading open crown, typically umbrella-shaped. The roots are deep. The bole of this plant is crooked, generally one-stemmed but sometimes forked from the base. The bark is corky and grey. The branches are fragile and drooping, with a feathery foliage. Young twigs and shoots are covered in short dense hairs, purplish or greenish white in colour. Moringa leaves are alternate, 7-60 cm long, tripinnately compound with each pinnate bearing 4-6 pairs of leaflets that are dark green, elliptical to obovate, and 1-2 cm in length. The inflorescences are 10-20 cm long, spreading panicles bearing many fragrant flowers. Moringa flowers are pentamerous, zygomorphic, 7-14 mm long and white to cream in colour.

The fruit is a typically 3-valve capsule, 10 to 60 cm in length, often referred to as a "pod" and looking like a drumstick (hence the name "drumstick tree"). The fruit is green when young and turns brown at maturity. The mature fruit splits open along each angle to expose the seeds. The capsule contains 15-20 rounded oily seeds, 1-1.5 cm in diameter surrounded by 3 papery wings, up to 2.5 cm long. Moringa seeds contain a large amount of oil (FAO, 2014; Radovich, 2009; Orwa *et al*., 2009; Bosch, 2004; Foidl *et al*., 2001).

Almost all the parts of this plant; root, bark, gum, leaf, fruit, flowers, seed and seed oil have been used for various ailments in indigenous medicine. Seeds of moringa are antipyretic, acrid and bitter and reported to show antimicrobial activity (Oliveira *et al*., 1999). Moringa seeds contain pterygospermin, a potent antibiotic and fungicide effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Orwa *et al*., 2009). Moringa leaves have been reported to be rich in β-carotene, protein, vitamin C, calcium and potassium. It is also a good source of natural antioxidants. (Kumar *et al.* 2010). Previous researchers reported that a recombinant protein in the seed is able to flocculate Gram-positive and Gram-negative bacteria cells. In this case, microorganisms can be removed by settling in the same manner as the removal of colloids in properly coagulated and flocculated water, On the other hand, the seeds may also act directly upon microorganisms and result in growth inhibition (Madsen *et al*., 1987; Casey, 1997; Broin *et al*., 2002).

Moringa contains antimicrobial peptides that are thought to act by disrupting the cell membrane or by inhibiting essential enzymes (Silvestro *et al*., 2000; Saurez *et al.*, 2003; Sutherland *et al*., 1990) Moringa seeds could inhibit the replication of bacteriophages. The antimicrobial effects of the seeds are attributed to the compound 4[α-L-rhamnosyloxy] benzyl isothiocyanate (Eilert *et al* 1981).

This study was therefore aimed at investigating the comparative efficacy of the ethanolic and Aqueous extracts of the stem and root on some clinical isolates.

## 2. MATERIALS AND METHODS

**Source of Moringa oleifera Stem and Roots**

A Good quality *M. Oleifera* tree was selected from a Moringa orchard in Ibadan, Oyo State, Nigeria. The stem (tree bark) and roots were carefully cut, dirt were removed manually by washing in clean water and were left to dry in ambient air and temperature for one week in the absence of direct sunlight.

**Preparation of Moringa Oleifera Stem and Root Extract**

After one week of drying a fairly constant weight was achieved, they were separately pulverised using a Warren blender that has been previously washed and rinsed with distilled water. 50 g each of the powder were weighed into a 500 ml conical flask and 250 ml of (96 %) ethanol was added, stirred and covered securely with cotton wool and aluminium foil and was left for 72 hrs at room temperature. (Kasolo *et al*., 2011).

At the end of the 72 hrs, the conical flask containing the stem-bark powder was shaken vigorously and then filtered through a No. 1 Whatman filter paper in Buchner funnel. The procedure was repeated for the second flask of root powder. The aliquots were collected and solvent was removed by simple evaporation in a water bath and they were labelled ESE and ERE for ethanolic stem and root extracts respectively (Group A).

Water extraction of the same stem-bark and root was carried out as follows; 50 g each of the dry powder were put in 500 ml boiled water (100 °C) and allowed to cool while being shaken at intervals. On cooling, they were filtered through a No. 1 Whatman filter paper in Buchner funnel. The filtrates were evaporated to dryness using low heat for 2 hrs to obtain a concentrated slurry and were labelled as ASE and ARE for aqueous stem extract and aqueous root extract respectively (Group B).

**Preparation of Cultures**

The cultures were prepared according to standard microbiological procedures as described below:

Pure clinical isolates strains of the three pathogenic bacteria (*Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli)* and fungi (*Candida albicans*) were obtained from the Igbinedion University Teaching Hospital (IUTH) and subsequently confirmed with phenotypic tests previously described by Barrow and Feltham (2003).

**Preparation of Seeded Plates**

Bacteria Seeded Plates (Group A)

Dilution of the three bacterial cultures were made by pipetting 0.1 ml from each culture into corresponding test tubes containing 9.9 ml of sterile distilled water. 20 ml of freshly prepared nutrient agar was poured into 3 different petri dishes (for each sub cultured organism) and allowed to set. 0.2 ml was pipetted from each test tube containing dilutions of the 3 microorganisms and introduced into the already set agar plates. (To prevent cross contamination of the plates, different pipette was used to dilute and to introduce the various dilutions into the set agar plates). The plate was agitated gently to ensure that the culture spreads evenly over the agar surface. Using a sterile cork borer, 6 wells were bored equidistance to one another in the seeded agar plates

Fungal seeded plates (Group A)

Dilution of the fungal culture was made by pipetting 0.1 ml from the sub-culture into a different test tube containing 9.9 ml of sterile distilled water. 20 ml of freshly prepared Sabouraud Dextrose Agar (SDA) was poured into a different petri dish for the fungal sub-culture and allowed to set. 0.2 ml of the fungal sub-culture was pipetted and introduced into the already set agar plate. The plate was agitated gently to ensure that the agar spreads evenly over the agar surface. Using a sterile cork borer, 6 wells were bored equidistance to one another in each seeded agar plate.

Group B Seeded Plates

The procedures for group A above were repeated to obtain a second group labelled as B group.

**Antimicrobial Screening**

Both the aqueous and ethanolic extracts of the stem and roots were standardised to 500 mg/ml and 250 mg/ml (for group the two groups A and B) and were subjected to antimicrobial screening by the Agar Diffusion Test method.

Using one pasture pipette for each sample, 4 out of the 6 holes bored into each of the four seeded agar plates were filled as follows;

i. 500 mg/ml of ethanolic stem extract (500 ESE)
ii. 500 mg/ml of ethanolic root extract (500 ERE)
iii. 250 mg/ml of ethanolic stem extract (250 ESE)
iv. 250 mg/ml of ethanolic root extract (250 ERE)

The 5$^{th}$ and the 6$^{th}$ holes on the seeded agar plates were used for the positive and negative control by adding 50 µg/ml Ciprofloxacin (a second-generation broad-spectrum quinolone antibiotic) as the positive control and 50% Ethanol as the negative control. This procedure was replicated for both groups.

The plates were left on the work bench for an hour to allow the extracts and control drugs to diffuse into the agar. They were then later incubated at 37 $^{\circ}$C for 24 hours for bacteria and 30 $^{\circ}$C for 72 hours for the fungi (*Candida albicans*). and the zones of inhibitions were measured.

The antibacterial activity (Singariya *et al.*, 2012) was calculated with the formula below:

$$\% \, Activity = \frac{(Zone\ of\ inhibition\ \ of\ extract - Zone\ of\ inhibition\ of\ negative\ control)}{Zone\ of\ inhibition\ of\ positive\ control} \times 100 \qquad (1)$$

## 3. RESULTS

**Table 1: Qualitative Phytochemicals Screening of *Moringa oleifera* roots.**

| Phytochemical | Ethanol extract | Aqueous extract |
|---|---|---|
| Spirochin | ++ | + |
| Moriginine | ++ | + |
| Catechol Tannins | + | - |
| Coumarins | - | - |
| Steroids and Triterpenoids | +++ | - |
| Saponins | +++ | + |
| Anthraquinones | + | +++ |
| Alkaloids | +++ | ++ |
| Reducing sugars | + | + |
| Pterygospermin | ++ | ++ |

Legend: -: not detected; +: present in low concentration; ++: present in moderate concentration; +++: present in high concentrations.

The table shows the qualitative result of the phytochemical screening of aqueous and ethanolic root and stem peel extracts of *M. oleifera.* (Table 1). the Ethanol extract had catechol tannins, steroids and triterpenoids, saponins, anthraquinones, pterygospermin, alkaloids and reducing sugars. The greatest response for ethanol was observed in steroids and triterpenoids, plus saponins while the aqueous extract yielded saponins, anthraquinones, pterygospermin, alkaloids and reducing sugars. The Aqueous extract had a better result for anthraquinones and alkaloids compared to the result for the ethanol extract.

**Table 2: Mean Zones of Inhibition of Ethanolic Extract of Moringa oleifera Root**

| Microorganism | Concentrations (mg/ml) | | | | Positive and Negative Controls | |
|---|---|---|---|---|---|---|
| | 500 ESE | 500 ERE | 250 ESE | 250 ERE | Ciprofloxacin 50 µg/ml | Ethanol 50% |
| *Klebsiella pneumonia* | 19 mm | 22 mm | 0 mm | 0 mm | 40 mm | 0 mm |
| *Pseudomonas aeruginosa* | 20 mm | 23 mm | 0 mm | 0 mm | 43 mm | 0 mm |
| *Escherichia coli* | 21 mm | 24 mm | 0 mm | 0 mm | 39 mm | 0 mm |
| *Candida albicans* | 25 mm | 28 mm | 15 mm | 15 mm | 25 mm | 0 mm |

Legend: Positive Control: Ciprofloxacin µg/ml; Negative Control: Ethanol 50%-; ESE: Ethanolic Stem Extract; ESE: Ethanolic Root Extract

**Table 3: Mean Zones of Inhibition of Ethanolic Extract of Moringa oleifera Stem**

| Microorganism | Concentrations mg/ml | | | | Positive and Negative Controls | |
|---|---|---|---|---|---|---|
| | 500 ASE | 500 ARE | 250 ASE | 250 ARE | Ciprofloxacin 50 (µg/ml) | Distilled Water |
| *Klebsiella pneumonia* | 15 mm | 18 mm | 0 mm | 0 mm | 40 mm | 0 mm |
| *Pseudomonas aeruginosa* | 10 mm | 18 mm | 0 mm | 0 mm | 43 mm | 0 mm |
| *Escherichia coli* | 23 mm | 27 mm | 5 mm | 10 mm | 39 mm | 0 mm |
| *Candida albicans* | 25 mm | 25 mm | 5 mm | 7 mm | 25 mm | 0 mm |

Legend: Positive Control: Ciprofloxacin µg/ml; Negative Control: Ethanol 50%-; ESE: Ethanolic Stem Extract; ESE: Ethanolic Root Extract

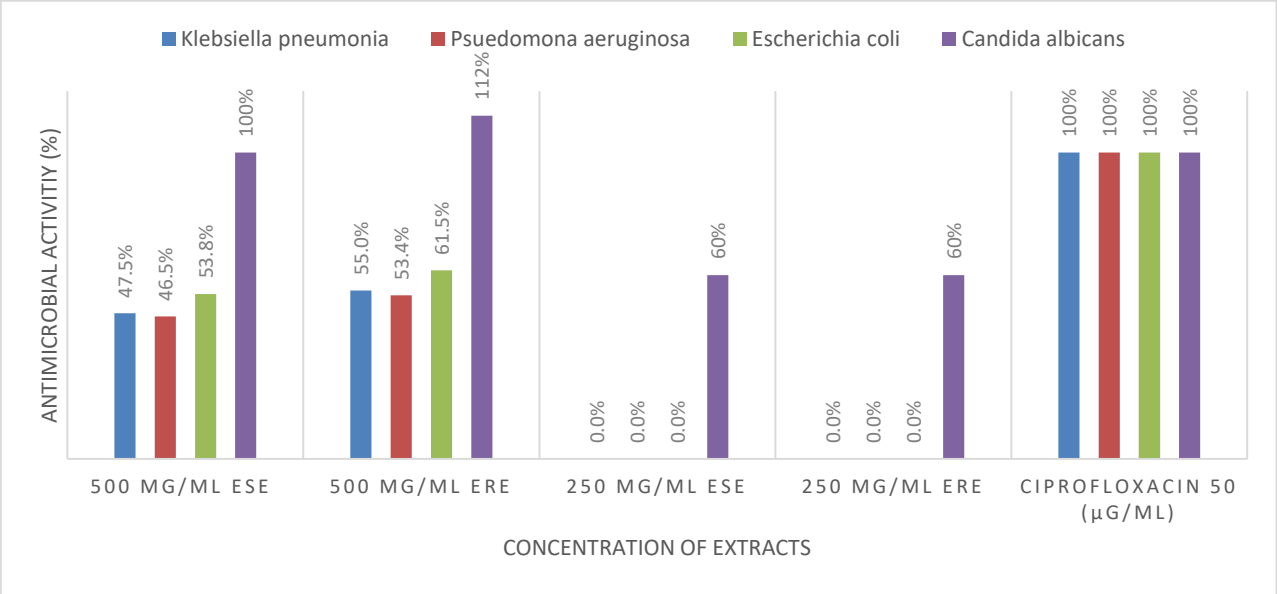


**Figure 1: Antimicrobial activity of the Ethanolic Extract of *Moringa oleifera* Root and Stem on *Klebsiella pneumonia Pseudomonas aeruginosa, Escherichia coli, Candida albicans***

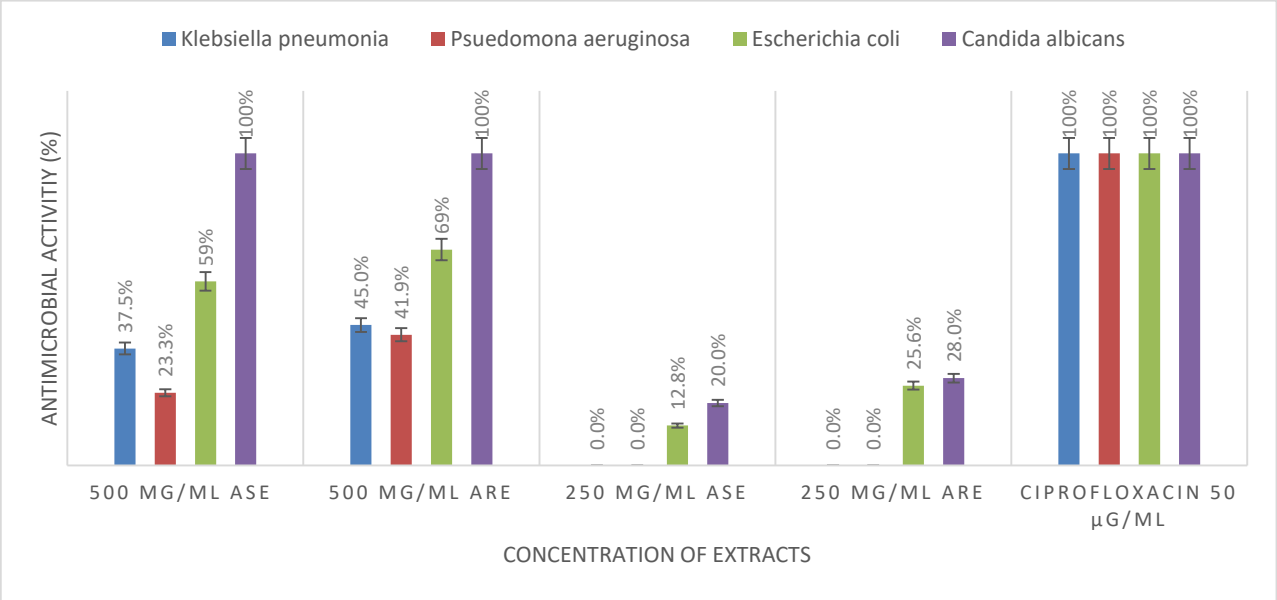


**Figure 2: Antimicrobial activity of the Aqueous Extract of *Moringa oleifera* Root and Stem *Klebsiella pneumonia Pseudomonas aeruginosa, Escherichia coli, Candida albicans***

## 4. DISCUSSION

Moringa oleifera stem and root extracts demonstrated a strong antimicrobial property against all the tested organisms and this is indicative of its antimicrobial properties. It also confirms its possibility as a broad-spectrum antibiotic. This is in total consonance with earlier reports of Pal *et al*., 1995 and Nikkon *et al*., 2003. The effect of the plant extract on the organisms varied from one microorganism to another and the type of solvent for extraction as well as the concentration used in each case. The fungi (*Candia albicans)* was more susceptible to the extracts with a zone of inhibition of 28 mm and comparatively better than ciprofloxacin that was used as the positive control with a zone of inhibition of 25 mm. Ciprofloxacin hydrochloride belongs to the second generation broad-spectrum quinolone antibiotic which functions by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV, enzymes necessary for the removal of supercoiling in bacterial DNA, thereby inhibiting cell division (Crumplin and Smith, 1976; Wang, 1985). Ciprofloxacin is used for the treatment of urinary tract infections, prostatitis (Hooper and Wolfson, 1991), shigellosis (Bennish *et al*., 1992), continuous ambulatory peritoneal dialysis infections (Ludlam *et al*., 1990), some diabetic foot infection (Peterson *et al*., 1989), typhoid fever, etc.

Whereas in general, all the test organisms (*Klebsiella pneumonia, Pseudomonas aeruginosa, and Candida albicans*) were more susceptible to ethanolic extract than the aqueous extract except in the case of Escherichia coli which showed greater susceptibility to aqueous extract. This may not be unconnected to the fact that the aqueous extract had a higher yield of Anthraquinones as showed in Table 1.

The activity of the plant extract was dose dependent and activity was directly proportional with concentration.

Higher activities were recorded in the root extracts than the in stem extract which may be due to the fact that the root contains pterygospermin, an active antibiotic principle, with powerful antibacterial and fungicidal effects (Rao *et al*., 2001) as well as the presence of 4- α-L-rhamnosyloxybenzyl isothiocyanate (Eilert *et al*., 1981) and aglycone of deoxy-niazimicine [N-benzyl, S-ethyl thioformate (Bhatnagar, 1961)

All the test organisms showed resistance to the 50% ethanol used as the negative control by growing all over the well without any zone of inhibition. This means that the use of ethanol in the extraction was not the factor responsible for the generally improved activity (high inhibition zones) of the ethanolic extract of *Moringa oleifera*, but rather the presence of the phytochemicals in the extracted solutions themselves. The ability of the extracts to inhibit the activities of common bacteria and fungus is an indication of the broad-spectrum antimicrobial potential of *Moringa oleifera.*

The activity of the extracts was computed to determine how they performed against standard antibiotic. The 500 mg/ml extracts had better antimicrobial activity showing impressive activity against all the clinical isolates for both the ethanolic and Aqueous extract.

## 5. CONCLUSION

The results of this study demonstrate the efficacy of Moringa as a potential broad-spectrum antibacterial agent with the roots extracts producing the most effective results. Its continous use in herbal formulations is justified by the results of this study however, there is need for an extensive research into its sub-acute and chronic toxicity capacity `.

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