

Antimicrobial Potential of Leaf juice and Extracts of *Moringa oleifera* lam against some Human pathogenic Bacteria.

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Abstract: The antibacterial activity of ethanol, hot and cold water extracts of both fresh and dried leaves as well as the juice extracts of *Moringa oleifera* Lam. were obtained by standard disc diffusion methods against some human pathogenic bacteria; *Staphylococcus aureus*, *Streptococcus faecalis*, *E. coli* and *Pseudomonas aeruginosa*. The ethanol extracts of fresh leaves and dried leaves displayed a potential broad-spectrum activity against all the tested organisms, *Staphylococcus aureus*, *Streptococcus spp*, *E. coli* and *Pseudomonas aeruginosa*. Also, cold water extracts of fresh and dried leaves as well as hot water extracts of fresh and dried leaves inhibited the growth of the tested organisms with the exception of *E. coli* and *Pseudomonas aeruginosa* which were not inhibited by cold and hot water extract of fresh and dried leaves. The result of this study revealed that, the antibacterial activity of the extract could be enhanced if the components are purified. The plant therefore holds a promise as a potential source of new drugs for treating infections caused by these clinical pathogens.

Keywords: Antimicrobial, leaf juice, *Moringa oleifera*, Pathogenic bacteria.

I. Introduction

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries (Al-Bari *et al.*, 2006). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Rojas *et al.*, 2006). For a longtime, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies world-wide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana *et al.*, 2007). Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds etc.

The practice of complementary and alternative medicine is now on the increase in developing countries in response to world health organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections (Dilhuydy, 2003). However, despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver, 1993).

It is therefore very necessary that the search for newer antibiotic source be a continuous process. Plants are the cheapest and safer alternative sources of antimicrobials (Sharif and Banik, 2006; Doughari *et al.*, 2007). *Moringa oleifera* Lam. is the most widely cultivated species of a monogeneric family, the moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan (Fahey, 2005), is widely used for treating bacterial infections, fungal infection, anti inflammation, sexually-transmitted diseases, malnutrition and diarrhea. *Moringa oleifera* species is cultivated and use as vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots) for cooking and cosmetic oil (seeds) and as a medicinal plant (all plant organs) (Rebecca *et al.*, 2006). The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferon and many other phytochemicals. Various parts of the plant such as the leaves, roots, seeds, bark, fruits, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor (Makonnen *et al.*, 1997), Antipyretic, antiepileptic, antiinflammatory, antiulcer (Pal *et al.*, 1995a). Other important medicinal properties of the plant include antispasmodic (Caceres *et al.*, 1992), diuretic (Morton, 1991), antihypertensive (Dahot, 1998) cholesterol lowering (Mehta *et al.*, 2003), antioxidant, anti diabetic, hepatoprotective (Ruckmani *et al.*, 1998), antibacterial and antifungal activities (Nickon *et al.*, 2003). In addition, *M. oleifera* seeds posses water purifying powers (Muyibi and Evison, 1995a, Kawo, 2007) by flocculating bacterial cells (kawo, 2007). *M. oleifera* seeds can also be used as a less expensive bioabsorbent for the removal of heavy metals (Sharma *et al.*, 2006).

The present study is to investigate the role of juice, aqueous and ethanolic extracts of *Moringa oleifera* leaves as potential antimicrobial agent against some human pathogenic bacteria.

II. Materials And Methods

2.1 Collection and Identification of Plant Sample

The fresh leaves of *Moringa oleifera* Lam. were collected from Federal College of Agriculture, Ishiagu Ebonyi State. The plant specimen were identified and authenticated by a taxonomist in the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana.

2.2 Preparation of the Extracts

2.2.1 Fresh Leaf juice Extract

Twenty – five grams (25g) of fresh leaves of *M. oleifera* Lam. were weighed out and crushed directly by grinder without adding any solvent, and leaf juice was collected in a clean airtight bottle, after which it was stored for antibacterial activity test.

2.2.2 Cold aqueous Extracts of Fresh and dried Leaves

Twenty – five grams (25g) of fresh leaves of *M. oleifera* Lam. were weighed out and crushed directly by grinder and was suspended into 100ml of cold distilled water in a conical flask and was covered with rubber cork and left for 7 days with occasional shaking. It was filtered off on the 7th day using sterile filter paper (Whatman no. 1) into another clean conical flask; it was subjected to water bath evaporation where the aqueous solvent was evaporated at its boiling temperature of 100°C. The standard extracts obtained was then stored in the refrigerator at 4°C for antibacterial activity test. (Akueshi *et al.*, 2002).

In another case, the well air-dried fresh leaves was ground and 25g was weighed out and was dipped into 100ml cold distilled water in a conical flask stoppered with rubber corks and was left for 7 days with occasional shaking. The other steps were the same as followed in case of cold water extracts of fresh leaves.

2.2.3 Hot aqueous Extracts of Fresh and dried leaves

Here, the same step was used as in cold water treatment with 30-minutes boiling while plant material was dipped in distilled water.

2.2.4 Ethanol (95%) Extracts of Fresh and dried Leaves

Here also, the same procedure was followed as in cold water treatment.

2.3 Source and Maintenance of test Organisms

Pure culture of *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *P. aeruginosa* clinically isolated from Rapha Medical Laboratory, Afikpo were used as test organisms. Pure cultures of the isolates were maintained in appropriate media for future use.

2.4 Standardization of inoculums

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4°C and sub cultured onto nutrient broth using a sterilized wire loop. The density of the suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of barium sulphate solution (Cheesbrough, 2002)

2.5 Antibacterial Assay

Antibacterial activity of the seven different samples; fresh leaf juice, cold water extract of fresh leaves, hot water extract of fresh leaves, cold water extracts of dried leaves, hot water extracts of dried leaves, ethanol extracts of fresh leaves and ethanol extracts of dried leaves were individually tested against the studied organisms.

In vitro, antibacterial test was then carried out by disc diffusion method (Bauer, 1996; Barry, 1980) using 25µl of standardized suspension of tested bacteria (10⁸cfu/ml) spread on Mueller-Hilton agar plates. The dried and sterile disc (8mm in diameter) impregnated with 0.1ml of standard plant extracts were placed on the seeded agar plates with the aid of sterile forceps. The plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring in millimeter (mm) the zones of inhibition against the tested bacteria (Bauer, 1996).

2.6 Minimum Inhibitory Concentration (MIC)

MIC of the different samples of the extracts of *M. oleifera* Lam was determined by two-fold serial dilution method. Five different concentrations of the samples were prepared, 25, 20, 15, 10 and 5 mg/ml. The above mentioned concentrations of the extracts were introduced into different wells bored on the sterile medium

each containing test organisms and were inoculated for 24 h at 37°C. The lowest concentration of the crude extract that showed zone of inhibition is the Minimum Inhibitory Concentration. Sterile water was used as control.

III. Results

3.1 Antibacterial Assay

The antibacterial activity of the fresh leaf juice, cold and hot water extract of fresh and dried leaves, and ethanol extracts of fresh and dried leaves of *Moringa oleifera* are presented in TABLES 1 and 2

Fresh leaf juice of *M. oleifera* which was sensitive to three of the tested pathogens; *Streptococcus faecalis*, *Staphylococcus aureus* and *P. aeruginosa*, with varying degree of inhibition. Their individual diameter zones of inhibition were, 15, 5 and 4 mm respectively.

Also, all the pathogens were sensitive to ethanol extracts of fresh and dried leaves with their respective diameter zones of inhibition as presented in the TABLE 1, with the exception of *E. coli* which showed no inhibition on the extract of the dried leaves.

Antibacterial activity of aqueous extracts of fresh leaves was equally demonstrated on cold water which displayed a potential effect on *S. aureus* and *Streptococcus faecalis* with diameter zone of inhibition as 10 and 8 mm. Also, hot aqueous extracts of fresh leaves was also shown to be sensitive on *S. aureus* and *Streptococcus faecalis* with their respective diameter zones of inhibition as 8 and 9 mm respectively. There was no inhibition of *E. coli* and *P. aeruginosa* from this extracts (TABLE 2).

The cold aqueous extracts of dried leaves exhibited antibacterial effect on *S. aureus* and *Streptococcus faecalis*, their diameter zones of inhibition were 9 and 7 mm respectively. This extracts was unable to inhibit the growth of *E. coli* and *P. aeruginosa*. Also, hot aqueous extracts of dried leaves displayed similar antibacterial effect on *S. aureus* and *Streptococcus faecalis*, and has no inhibition effect on *E. coli* and *P. aeruginosa*.

Table 1. Antibacterial activity of fresh leaf juice and ethanol extract of fresh and dried leaves of *m. oleifera* lam against some human pathogenic bacteria

Bacteria	Zones of inhibition diameter (mm)		
	Ethanol extract of		
	Fresh Leaf Juice	Fresh leaves	Dried leaves
<i>S. aureus</i>	5	22	11
<i>S. faecalis</i>	15	11	20
<i>E. coli</i>	N1	4	N1
<i>P. aeruginosa</i>	4	5	4

Key: N1 = No Inhibition

Table 2. Antibacterial activity of aqueous extracts of fresh and dried leaves

Bacteria	Zones of inhibition(mm)			
	Aqueous extracts of			
	Fresh leaves		Dried leaves	
	Cold	Hot	Cold	Hot
<i>S. aureus</i>	10	8	9	9
<i>S. faecalis</i>	8	9	7	6
<i>E. coli</i>	N1	N1	N1	N1
<i>P. aeruginosa</i>	N1	N1	N1	N1

Key: N1 = No inhibition

3.2 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration of the different extracts against the tested bacterial pathogens is shown in TABLE 3 and 4 below. The fresh leaf juice became inactive at concentration less than 15 mg/ml. The highest minimum inhibition concentration was 25mg/ml for *Staphylococcus aureus*, while it is 15mg/ml for *P. aeruginosa* and *S. faecalis*. The ethanol extracts of both the fresh and dried leaves have inhibitory activity against three of the tested pathogen; *S. aureus*, *S. faecalis* and *P. aeruginosa* and their MIC values ranged from 15- 25mg/ml. The aqueous extract of both cold and hot water extract of fresh and dried leaves were also found to be susceptible to *S. aureus* and *S. faecalis* and their respective MIC values ranged from 20-25mg/ml.

Table 3. Minimum Inhibitory concentration of fresh leaf juice and ethanol extract of fresh and dried leaves of *M. oleifera* lam against some human pathogenic bacteria

Bacteria	Minimum Inhibitory Concentration(mg/ml)		
	Ethanol extract of		
	Fresh Leaf Juice	Fresh leaves	Dried leaves
<i>S. aureus</i>	25	25	25
<i>S. faecalis</i>	15	15	15
<i>E. coli</i>	N1	N1	N1
<i>P. aeruginosa</i>	15	15	15

Key: N1 = No Inhibition

Table 4. Minimum Inhibitory Concentration of aqueous extracts of fresh and dried leaves

Bacteria	Minimum Inhibitory Concentration(mg/ml)			
	Aqueous extracts of			
	Fresh leaves Cold	Hot	Dried leaves Cold	Hot
<i>S. aureus</i>	20	20	25	20
<i>S. faecalis</i>	25	25	25	25
<i>E. coli</i>	N1	N1	N1	N1
<i>P. aeruginosa</i>	N1	N1	N1	N1

Key: N1 = No inhibition

IV. Discussion

The result obtained from this work indicated that the fresh leaf juice and ethanol extracts of fresh and dried leaves have strong antibacterial activity on the organisms tested except on *E. coli* where it has little or no effect. Also the hot and cold aqueous extract of the fresh and dried leaves showed appreciable antibacterial activity on *S. aureus* and *S. faecalis*, while it has no effect on *E.coli* and *P. aeruginosa*.

The antibacterial activity of the crude plant extracts on the test organisms justifies the active principle observed in herbal preparations. Decoction of plant parts or boiling the plants parts in water is employed on several occasions. According to this research work, preparing an extracts with ethanol was shown to provide a

better antibacterial activity. This is because ethanol can extract the active ingredient of the plant more than aqueous extracts. This corroborates the work of Nair *et al.*, 2005 who had similar findings. Ethanol extracts of fresh leaves was sensitive to all the tested organisms used in this study namely; *Staphylococcus aureus*, *Streptococcus faecalis*, *E.coli* and *P. aeruginosa*. Fresh leaf juice and ethanol extracts of dried leaves (TABLE 1), also showed relatively higher inhibitory potency on three organisms tested except *E.coli*, this result is in agreement with Adesokan *et al.*, 2007 but contrary to Nari *et al.*, 2005 whose fresh juice, was sensitive to *E. coli*. Furthermore, *Staphylococcus aureus*, and *Streptococcus spp.*, has been reported to be less susceptible to plant extracts in earlier study conducted by other researchers (Kuhut *et al.*, 1994; Afolayan and Meyer, 1995). In this research, it was observed that *Staphylococcus aureus*, and *Streptococcus faecalis* were more sensitive to the ethanol extracts, fresh leaf juice and aqueous extracts. This is in line with the findings conducted by Doughari *et al.*, 2007. Also, in earlier research done by other researchers, hot water extracts could not inhibit any organisms, (Kuhut *et al.*, 1994 and Afolayan 1995). But in this study, hot water extract of fresh and dried leaves were able to inhibit two organisms: *Staphylococcus aureus*, and *Streptococcus faecalis*

These consequences suggest that *M. oleifera* Lam. Leaves used, contain bioactive substances whose antibacterial potentials are highly comparable with that of antibiotic used in sensitivity test against microorganism. The activity of plants against microorganisms may be indicative of the presence of broad-spectrum antibiotic compounds in that plant. Failure of some of the extracts to exert antibacterial effect on the test organism is not enough to conclude that the extract does not contain substances that can exert antibacterial activity against the test organism because the potency of the extract depends on the method used to obtain the extract (Unaeze., 1986). Research has also shown that the difference in antimicrobial properties of the plant extracts might be attributable to the age of the plant used, freshness of the plant material, physical factor (temperature, light or water), contamination by field microbes, and incorrect preparation of the plant etc. (Siddluraja and Becker, 2003; Vaghasiya and Chanda, 2007).

Today, most pathogenic organisms are becoming resistant to antibiotic (Chandarana *et al.*, 2005). *Moringa oleifera* leaves have been reported to be a good source of natural antioxidants such as ascorbic acid, phenolic and carotenoids (Dillard and German, 2000). To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus *M. oleifera* Lam. Leaves could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria.

V. Conclusion.

The result of this work indicated that extracts of this Plant possess inhibitory potential against the tested pathogens. The active ingredients of this plant could be enhanced if the component is purified. This plant therefore holds a promise as a potential source of new drug for treating infection caused by these clinical pathogens.

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