International Journal of Research and Review
www.ijrrjournal.com E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Antimicrobial Activities and Phytochemical Investigation of *Moringa Oleifera* Lam. Leaf Extracts

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ABSTRACT

Phytochemicals investigation and Antimicrobial activities of water and methanol extracts of *Moringa Oleifera* leaf were investigated in an attempt to evaluate its antimicrobial potentials. The inhibition was determined using Ditch method against four selected bacterial species and three fungal species. The phytochemical screening indicated the presence of alkaloids, flavonoids, glycoside, saponin, steroids, tannins and volatile oil. Both water and methanol extracts showed highest antifungal activity against *S. cerevisiae* with zones of inhibition of 9, 12 and 14 mm at the concentration of 60, 90 and 120 mg/ml respectively in water extract; and inhibition zones of 11, 15, 16 and 18 mm at the concentration of 30, 60, 90 and 120 mg/ml respectively in methanol leaves extract of *M. oleifera. A flavus* on the other hand was the resistant fungal species to the *M. Oleifera* extracts showing no visible zone of inhibition in water extract; and exhibiting inhibition in methanol with 7 and 9.5 mm zones of inhibition at 90 and 120 mg/ml respectively. The antifungal standard drug ketoconazole exhibited highest activity against *C. albicans* with 19 mm zone of inhibition, followed by *S. cerevisiae* 15 mm zone of inhibition. In methanol extracts against *Pseudomonas aeruginosa* shows the highest inhibition of 15.00 mm at the highest concentration (120mg/ml), while *Escherichia coli* and *Micrococcus species* had 14.00 mm and *Staphylococcus aureus* with least inhibition of 8.33mm at the lowest concentration (30mg/ml). The zones of inhibition of tetracycline on test bacteria showed that *P. aeruginosa* was the most susceptible with the diameter of 21 mm, followed by *S. aureus* with 20 mm. The results obtained provide a support for the utilization of this plant in traditional medicine and recommend its further investigation of it phytochemicals.

Keywords: *Moringa Oleifera*, antibacterial activity, Phytochemical Screening, Susceptibility Testing.

INTRODUCTION

Antimicrobial resistance to antibiotics has become a global public health challenge. Although the antibiotics and other chemotherapeutic agents have been of value in controlling many illnesses but they depend on judicious utilization to minimize the incidence of resistant strains. [1] For many decades, medicine had depended exclusively on plant parts such as leaves, flowers and barks. According to World Health Organization (WHO), over 80% of the global population depends on traditional medicines for their primary health care needs. The medicinal properties of plants lie in some chemical substances that produce a definite physiologic action on human body. The most important bioactive

compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. [2]

M. oleifera is one of the species of family Moringaceae, native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the pacific and Caribbean Islands. *M. oleifera* has been naturalized in many tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and "Mothers best friend". *M. oleifera* is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in its height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark. [3] *M. oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. *M. oleifera* parts are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia. [4] It has impressive range of medicinal uses with high nutritional value. *M. oleifera* is commonly used by local communities in North Western Nigeria for the treatment of disease such as skin infections, aphrodisiac, asthma, enlarge spleen or liver, diabetes, hypertension, tuberculosis and cancer. Different parts of this plan contain a profile of important minerals, and a good source of protein, vitamin, a carotene, amino acids and various phenolics. The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals. [5] In the tropics, it is used as forage for livestock, and in many countries, it is used as a micronutrient powder to treat various ailments. [6]

The leaf of this plant has diverse biological activities, including hypocholesterolemic, [7] antidiabetic, [8] hypertensive agent, [9] antitumor, [10] antifungal, [3] antibacterial. [11–13] The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of novel anti-infective agents from higher plants. [2] However, a very important step in the screening of the sanitizing and preservative activity of a plant material is to evaluate its antimicrobial properties. It is important to screen the phytochemical and antimicrobial properties of *M. oleifera* leaves on some selected microorganisms and also to verify its phytochemical constituent. The aim of the present study was to carry out phytochemical investigation and to evaluate the antimicrobial activity of water and methanolic extract of *M. oleifera*.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh leaves of *M. oleifera* were collected from Sokoto central market, Sokoto state, Nigeria. The species was identified and authenticated at the herbarium of the department of biological sciences, Usmanu Danfodiyo University Sokoto, where the voucher specimen was prepared and deposited. The leaves were air dried and pulverized into coarse powder using mortar and pestle. The powder sample were sieved through a sieve to get fine powder samples and stored in a polythene bag until when required for further uses.

Aqueous extraction

Forty gram (40g) of the powdered seed was weighed using a weighing balance and transferred into a one liter beaker. Three hundred milliliter (300ml) of distilled water was added to the powder and allowed to stand for 48 hrs. This was then heated on a water bath (60^{o}C) and filtered while hot. Hot water was continuously added to the residue and subsequently filtered. The procedure was repeated three times and the filtrate was then evaporated to dryness on a water bath (60^{o}C). [14]

Ethanolic extraction

Forty grams (40g) of the seed powder was weighed using a weighing balance and transferred to a one liter beaker. Three hundred (300ml) of methanol was added to the powder and allowed to stand

for 48 hrs. The residue was then transferred to a soxhlet apparatus with ethanol for 48hrs and then evaporated to dryness on a water bath. [14]

Phytochemical Analysis

Phytochemical analysis of water and methanol extracts for qualitative detection of alkaloids, flavonoids, steroid,volatile oil, glycoside, tannins and saponins were carried out according to Patel *et al.*(2014) [3] as follows:

Alkaloids

Wagner's test-Drug solution + few drops of Wagner's reagent (dilute Iodine solution).

Dragendroff's test-Drug solution + Dragendroff's reagent (Potassium Bismuth Iodide).

Hager test-Drug solution + few drops of Hagers reagent (Saturated aq. Solution of Picric acid).

Mayer's Test- Drug solution + few drops of Mayer's reagent (K2HgI4).

Flavonoids

3ml of each extract was added to 10ml of distilled water and the solution was shaken. 1ml of 10% NaOH solution was added to the mixture.

Saponins

Frothing test - 3ml of each extracts and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously.

Steroids

Salkowski Test- 5 drops of concentrated H2SO4 were added to 1ml of each extract in a separate test tube.

Tannins

2ml of each extract in a separate test tube were boiled gently for 2min and allowed to cool. 3 drop of ferric chloride solution was added to each extract.

Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added.

Volatile oil

2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl

Antimicrobial Testing

The microorganisms used in the study *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus* spp, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisiae* were obtained from Microbiology Department, Usmanu Danfodiyo University Sokoto. The antimicrobial screening was carried out according to Onsare, Kaur, & Arora, (2013) [15] with slight modification. Different agar plates of suitable media were prepared and inoculated with 0.1ml of respective test organisms by spread plate method. Wells measuring 6.0 mm in diameter were cut out under aseptic conditions using a stainless steel borer.Each well was filled with 30, 60, 90 and 120 mg/ml extracts respectively. The inoculated Plates were allowed to congeal for 30 min to allow pre diffusion time and then incubated at 37^0C for 24hrs in case of bacteria and 25^0C for 24-48 in case of fungi. The plates were examined for evidence of zones of inhibition which appear as a clear area around the holes. [16] Sterile distilled water and methanol were used as negative control in each case. The diameter of such zone of inhibition was measured using a transparent meter ruler and the value was recorded and expressed to the nearest millimeter.

RESULTS AND DISCUSSION

The present study reveals that *M. oleifera* plant shows the presence of phytochemical constituents like alkaloids, flavonoids, glycosides, saponins, steroids, tannins and volatile oil in different solvent extracts as shown in Table 1. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs. [3] Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes. [17] Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal medicines and are under

investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions. [18] Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponinscause hemolysis of red blood cells. [3]

Table 1: Qualitative phytochemical screening of ethanol and aqueous leaf extract of Moringaoleifera

Solvents used	Alkaloids	Flavonoids	Glycoside	Saponin	Steroids	Tannins	Volatile oil
Water	+	+	-	+	+	+	+
Methanol	+	+	+	+	-	+	+

Result from the antifungal activity of *Moringa* leaves extract displayed varying degrees of antifungal activity against the screened species (Table 2). The inhibition of fungi at different concentration of water extract showed that *S. cerevisiae* was more susceptible showing zones of inhibition of 9, 12 and 14 mm at the concentration of 60, 90 and 120 mg/ml respectively. It was then followed by *C. albicans* with 6.5 and 10 mm inhibition zones at 90 and 120 mg/ml concentration respectively. There was no visible zone of inhibition of *A. flavus* even at the highest concentration of aqueous extract. The findings of the methanol extract of *Moringa* leaves followed the same trend with *S. cerevisiae* showing inhibition zones of 11, 15, 16 and 18 mm at the concentration of 30, 60, 90 and 120 mg/ml respectively. It was then followed by *C. albicans* with inhibition zones of 8, 9 and 14 mm at 60, 90 and 120 mg/ml concentration respectively. *A. flavus* showed the least susceptibility with 7 and 9.5 mm zones of inhibition at 90 and 120 mg/ml respectively. It has been showed that the highest zones of inhibition by both water and methanol leaves extracts were seen against *S. cerevisiae*. The result of this study was in agreement with previous study. [3] *A. flavus* was the most resisted fungal species in the present study, which in line with the findings from previous results by Oluduro, (2012). [19] The antifungal standard drug ketoconazole exhibited highest activity against *C. albicans* with 19 mm zone of inhibition, followed by *S. cerevisiae* 15 mm zone of inhibition.

Table 2: Antifungal activity of both aqueous and methanol extract of *M. oleifera* leaves

Aqueous Methanol Diameter of zone of inhibition (mm)									Ketoconazole
	30 mg/ml	60 mg/ml	90 mg/ml	120 mg/ml	30 mg/ml	60 mg/ml	90 mg/ml	120 mg/ml	Control
A. flavus	-	-	-	-	-	-	7	9.5	12
C. albicans	-	-	6.5	10	-	8	9	14	19
S. cerevisiae	-	9	12	14	11	15	16	18	15

The inhibition of bacterial growth by different concentration of leaves water extracts is shown in figure 1. At the highest concentration (120 mg/ml), *E. coli* showed 17.66 mm and *P. aeruginosa* with 14.33mm. At 90 mg/ml, *E. coli* had 17.00mm, and 12.00mm in *M. spp*. At 60mg/ml, *S. aureus* showed 12.33mm, and *M. spp* with 10.33mm. At 30mg/ml *E. coli* had 10.33mm, and lastly *S. aureus* with 7.00mm.

In Figure 2; the diameter of inhibition of bacterial growth by different concentration of leaf of *M. oleifera* in methanol extracts, highest zone of inhibition 15.00mm was observed in *P. aeruginosa* at the highest concentration (120mg/ml) this was followed by *E. coli* and in 13.66mm for *S. aureus* as lowest inhibition. At 90mg/ml highest activity was observed in *P. aeruginosa* with 13.66mm and *S. aureus* as lowest inhibition with 11.66. At 60mg/ml *S. aureus* had 12.33mm, and the least activity was seen in *E. coli* with 10.66mm.

At 30mg/ml *P. aeruginosa* has the highest activity with 13.00 mm and the least activity was observed in *S. aureus* with 8.33 mm. The zones of inhibition of tetracycline on test bacteria showed that *P. aeruginosa* was the most susceptible with the diameter of 21 mm, followed by *S. aureus* with 20 mm. The difference in response was

possible due to the nature of the bacterial species. It noted that in water extract, *E. coli* was observed to be the most susceptible organism which is similar to that of [20] *P. aeruginosa* is least susceptible to water extract, which is in line with Aliero, Aliero, & Buhari, (2008) and Mainasara*et al.*(2012) [21,22] using *Scadoxus maltiflorus* and *Calotropis procera* respectively. Methanolic extracts exhibited highest activity against *P. aeruginosa* while least activity was observed in *S. aureus*, this also reported in Yesmin*et al.*,(2014) using *Calotropis procera*. [23]

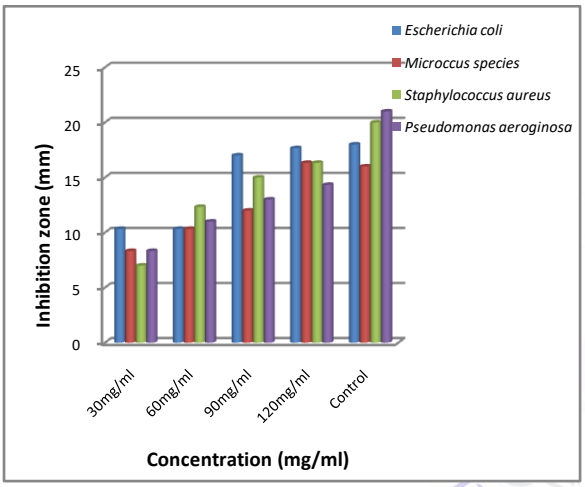

Figure 1: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of water extract of leaf of *M. oleifera*.

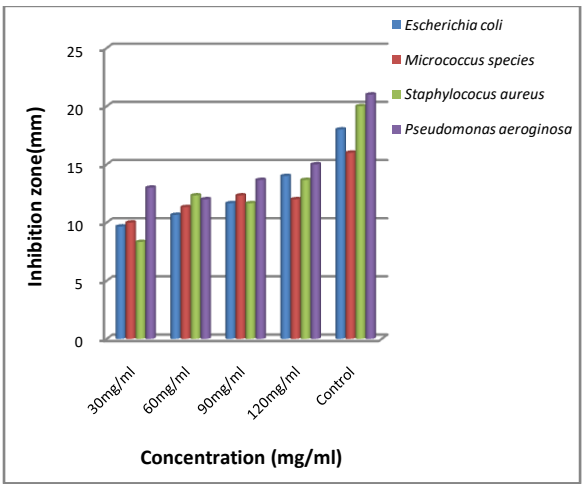

Figure 2: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of methanol extract of leaf of *M. oleifera*.

CONCLUSION

Antimicrobial activity was evaluated because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *M. Oleifera* showed good antifungal activity against several organisms like *Saccharomyces cerevisiae*, *Candida albicans*, *Escherichia coli*, and *P. aeruginosa* as supported by previous studies. The susceptibility of some microbial organisms to the leaf extract of *M. Oleifera* may be a pointer to its potential as a drug that can be used. The phytochemical constituents can be further investigated recommended in other to search for a novel herbal drug.

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How to cite this article: Sanusi SB, Mainasara MM, Maishanu HM et al. Antimicrobial activities and phytochemical investigation of *moringa oleifera* lam. leaf extracts. International Journal of Research and Review. 2017; 4(4):9-14.

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