Original Article

Antimicrobial Activities and Phytochemical Analysis of *Moringa oleifera* Leaves on *Staphylococus aureus* and *Streptococcus* species

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ABSTRACT

Objective: This study is aimed at determining appropriate solvent that is effective among the aqueous, ethanol, and petroleum ether extracts of *Moringa oleifera* leaves respectively, examined for their antimicrobial activities against selected clinical organisms including *Staphylococcus aureus* and *Streptococcus* species.
Methods: Leaves of Moringa plant were collected in a clean bag within the town of Akungba-Akoko, Ondo State, Nigeria. About 200g of the plant prepared in powdered form were separately soaked in 400ml of 95% Ethanol, Distilled water and Petroleum ether in a 500ml reagent bottle and stoppered. This was allowed to stand for 14 days to permit full extraction of the active ingredients. The extracts were tested on the microbial isolates cultured on Mueller Hinton agarusing an agar-disc diffusion method. Plant filtrates were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides. The minerals components were also determined using standard biochemical methods.
Results: This study showed that petroleum extract of *Moringa oleifera* is significantly more active against the growth of *Streptococcus* species with the 0.6g/ml concentration giving the highest measurement of zone of inhibition. Phytochemical screening shows that the plant sources contain some compounds like tannin, phenol, alkaloid, flavonoids, oxalate, saponin and phytate part which forms their bioactive components. While the minerals component from this sources (per 100g) are sodium(Na), potassium(K), calcium(C), magnesium(Mg), zinc(Zn), iron(Fe), lead(Pb), copper(Cu), manganese(Mn) and phosphorus(P).
Conclusion: This study helps to determine the local and pharmaceutical value of *Moringa oleifera* leaves for treatment of

some diseases including clinical organisms such as *Staphylococus aureus* and *Streptococcus* species. The data obtained in this study will be useful in discovery of new antibiotic for therapeutic purposes especially in overcoming some resistant strains problems.

Keywords: Antimicrobial; Phytochemical; *Moringa oleifera* Leaves; *Staphylococus aureus*; *Streptococcus* species.

INTRODUCTION

The continuous search for valuable medicinal plants is in progress scientifically in order to combat the surge of antimicrobial resistance. *Moringa oleifera* which is native to South Asia and now found throughout the tropics plays a major role here. Ayurvedicsystem of medicine associates the effectiveness of *Moringa* leaves with the cure or prevention of about 300 diseases[1].The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed[2]..Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies[3, 4]). The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them[5,6]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy[7].

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of living host or by interfering in the metabolism of microbes infecting them. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favor of the host. So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them[5,6].

Moringa (*Moringa oleifera*) belongs to the family, Moringaceae and order**,** Brassicales. This medicinal plant is short, slender, deciduous, perennial tree, to about 10m tall; rather slender with drooping branches; branches and stem brittle, with corky bark; leaves feathery, pale green, compound, tripinnate, 30-60cm long, with many small leaflets, 1.3-2cm long, 0.6-0.3cm wide. Its pods are pendulous, brown, triangular, splitting lengthwise into 3parts when dry, 30-120cm long, 1.8cm wide, containing about 20seeds embedded in the pith, pod tapering at both ends, 9-ribbed; seeds dark brown, with 3 papery wings[8]. Native to India, Arabia, and possibly Africa and the East Indies; widely cultivated and naturalized in tropical Africa, tropical America, Sri Lanka, India, Mexico, Malabar, Malaysia and the Philippine Islands[7]. Moringa plant parts have substantial anti-inflammatory activity. For instance, the root extracts exhibits significant anti-inflammatory activity in carrageenan induced rat paw oedema[9,10].

The crude methanol extract of the root inhibits carrageenan- induced rat paw oedema in a dose dependent manner after oral administration. Moreover, n-butanol extract of the seeds of *Moringa* shows anti-inflammatory activity against ovalbumin-induced airway inflammation in guinea pigs[11]. Amelioration of inflammation associated chronic diseases can be possible with the potent anti-inflammatory activity of Moringa bioactive compounds.

Moringa has diversifiedmedicinal value, which has long been recognized in the Ayurvedic and Unani system[12]. Nearly every part of this plant, including root, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil have been used for various ailments in the indigenous medicine[13] but recent research is also showing several active constituents that makes it widely accepted for use in modern medicine. This study thus helps to evaluate the effectiveness of *Moringa oleifera* sources and their pharmaceutical values on selected human pathogens such as *Staphylococcus aureus* and *Streptococcus* species. Similarly, phytochemical properties of the medicinal plants studied were also determined.

MATERIALS AND METHODS

Source of plant materials

Leaves of Moringa plant were collected in a clean bag within the town of Akungba-Akoko, Ondo State, Nigeria. The plant parts were authenticated at the department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko.

Source of Microorganisms

The test organisms were selected based on their availability. Hence, two bacteria isolates were used for the test. The organisms were collected from the Microbiology laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The organisms were; *Staphylococcus aureus* and *Streptococcus* species. The bacteria were maintained on nutrient agar slant and stored in the refrigerator at 4^{o}C. The bacteria were sub cultured onto fresh media at regular interval until it was used for the test.

Extraction Methods

The leaves of the Moringa plant were washed with distilled water, dried in shade and then grinded to powder. About 200g of the powder were separately soaked in 400ml of 95% Ethanol, Distilled water and Petroleum ether in a 500ml reagent bottle and stoppered. This was allowed to stand for 14 days to permit full extraction of the active ingredients. The fluids were then filtered using Whatman No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in fridge prior to use. A 2.0µg/ml solution of each extract was prepared with DMSO (dimethyl sulfoxide) and fractionated into 0.6µg/ml, 0.4µg/ml and 0.2µg/ml concentrations needed for the bioassay.

Sterility Test of the Plant Extracts

The aqueous and the ethanolic extract were tested for growth or contamination. This was carried out by inoculating 1ml each of them on nutrient agar and incubated at 37^{o}C for 24hours. The plates were observed for growth. No growth in the extract after incubation indicates that the extracts were sterile. The extracts were then accessed for antimicrobial activity.

Antimicrobial Assay

The antimicrobial properties of the extracts were determined using the agar-disc diffusion method and the diffusion disc method. In the agar diffusion method, twenty-four hour old broth cultures of the test organisms were swabbed onto a sterile Mueller Hinton agar in petri dishes using

sterile cotton swab. A sterile cork borer of 6mm diameter was used to punch wells on the agar on each of the petri dishes. The holes were filled with 0.5ml of extracts. Control experiments were also carried out where the holes were filled with 0.5ml metronidazole. Each hole was labeled representing a particular concentration[14].

In the disc diffusion method, the petri dishes containing Mueller Hinton agar were seeded throughout with the twenty-four hours old test organisms. Diffusion discs are then impregnated with the same concentrations of extracts with the agar diffusion method which is 0.6µg/ml, 0.4µg/ml and 0.2µg/ml and also with 0.5ml of metronidazole which is used as the control. The discs are then evenly dispensed and lightly pressed onto the agar surface. The process was carried out for each extract and the inoculated petri dishes were left for few minutes for extract to diffuse into agar. The culture plates were incubated at 37^0C for 24 hours, after which the zones of inhibition were measured where obtainable[14].

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined against bacteria after the antimicrobial test have been performed. This shows the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. The isolates were cultured on Mueller Hinton agar and agar diffusion method was used for this purpose. Sterile cork borer of diameter 6mm was used to bore holes on the plates after seeding the plates with the bacterial strains being tested. It was left for about one hour at room temperature and subsequently incubated at 37^oC. Results were read after 24hours of incubation.

Qualitative Method of Analyses

Preliminary test / Preparation test

The *Moringa oleifera* leave filtrates used for this study were prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The sample source obtained were used for the phytochemical screeningfor flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides.

(i) Test for Alkaloids

Alkaloids was determined based on the modified method of Trease and Evans[15]whereby about 0.2gram of *Moringa oleifera* sample was warmed with 2% of H_2SO_4 for two minutes. It was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the present of Alkaloids**.**

(ii) Test for Tannins

The test was performed by following a standard procedure of Maxson and Rooney[16]. In doing this one milliliter of the filtrate was mixed with 2m1 of FeCl. A dark green color indicated a positive test for the tannins

(iii) Test for Saponins

One milliliter of *Moringa oleifera* leave filtrate were diluted with 2 ml of distilled water; the mixture were vigorously shaken and left to stand for 10min during which time, the development of foam on the surface of the mixture lasting for more than 10min, indicates the presence of saponins.

(iv) Test for Anthraquinones

A Borntranger test[17], was performed whereby one milliliter of the plant filtrate was shaken with 10ml of benzene; the mixture was filtered and 5 ml of 10 % (v/v) ammonia were added, then shaken and

observed. A pinkish solution indicates a positive test[17].

(v) Test for Anthocyanosides

One milliliter of the plant filtrate was mixed with 5 m1 of dilute HCI; a pale pink color indicates the positive test.

(vi) Test for Flavonoids

One milliliter of *Moringa oleifera* plant filtrate was mixed with 2 m1 of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1m1 of the plant filtrate were mixed with 2m1 of dilute NaOH; a golden yellow color indicated the presence of flavonoids[18].

(vii) Test for Reducing Sugars

The reducing sugar in the sample source was determined by measuring one milliliter of the plant filtrate unto which Fehling A and Fehling B was separately added; a brown color with Fehling B and a green color with Fehling A indicate the presence of reducing sugars.

(viii) Test for Cyanogenicglucosides

This test was intensified by weighing out 0.5g of the extract into 10ml sterile water to filter it and adding sodium picrate to the filtrate and heated to boil.

(ix) Test for Cardiac glucosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4.

Quantitative Method of Analyses

(i) Saponins

About 20grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20 % aqueous ethanol were added. The mixture was heated using a hot water bath. At about 55^{O}C, for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20ml of diethyl ether was added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60ml of n-butanol were added. The combined n-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material

(ii) Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weigh.

(iii) Cardiac glucosides

Legal test and the killer-kilianiwas adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4[15,19].

(iv) Tannins

Moringa oleifera leave was determined for its tannin component weighing about 500 mg of the sample source into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was

transferred into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract

(v) Alkaloids

The test was carried out by weighing five grams of the plant sample source into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was added. The reaction mixture were covered and allowed to stand for 4 hour. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass[15,19].

(vi) Phlobatannins

This is another plant component that was determined by weighingabout0.5grams of each plant extracts. This were dissolved in distilled water and filtered. The filtrate was boiled in 2% HCl, red precipitate show the present of phlobatannins.

RESULTS

This study shows the activity of some medicinal plants on specific aetiologic microbes, that is *Staphylococcus aureus*and *Streptococcus* species. Table 1 that the petroleum ether extracts of *Moringa oleifera* was significantly active against the growth of *Staphylococcus aureus* with the 0.2g/ml concentration giving the highest measurement of zone of inhibition (12.0mm). Ethanolic extract of *Moringa oleifera* also had antimicrobial effect on *Staphylococcus aureus* but not as much as that of the petroleum ether extract. Aqueous extract of *Moringa oleifera* had the least antimicrobial effect on *Staphylococcus aureus*. In Table 2, it was observed that the petroleum ether extract of *Moringa oleifera* was significantly active against the growth of *Streptococcus* species with the 0.6g/ml concentration giving the highest measurement of zone of inhibition (12.0mm). Ethanolic extract of *Moringa oleifera* also had antimicrobial effect on *Streptococcus* species but not as much as that of the petroleum ether extract. Aqueous extract of *Moringa oleifera* had the least antimicrobial effect on *Streptococcus* species. During the study, quantitative analysis of the phytochemical screening of *Moringa oleifera* was determined and it was shown that it has all phytochemical constituents except Steroids(Table 3).

In Table 4, Quantitative Analyses of Minerals elements shows the presence of Sodium (Na), Potasium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Phosphorus (P) except Lead (Pb)in the *Moringa oleifera*. *Moringa oleifera* sources have highest quantity of magnesium during the study. In table 5, Quantitative Analyses of Anti–nutrients present in *Moringa oleifera* was observed and measured in Percentage (%). It was shown that *Moringa oleifera* has all the anti-nutrients tested with flavonoid been the highest percentage component while saponin was not detected.

Table 6, shows the proximate percentage (%)nutrient composition of Ash, Moisture Content, Crude Protein, Fat, Fibre and Carbohydrate in *Moringa oleifera* with carbohydrate having the highest percentage component based on the proximate nutrients tested for this purpose.

DISCUSSION

Moringa oleifera is gaining more popularity as a valuable medicinal plant and

have previously been documented as sources of antibiotics[20]. 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[21]. 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[22]. 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[22].

Table 1 show that the petroleum extracts of *Moringa oleifera* is significantly active against the growth of *Staphylococcus aureus* with the 0.2g/ml concentration giving the highest measurement of zone of inhibition. Ethanolic extract of *Moringa oleifera* also has antimicrobial effect on *Staphylococcus aureus* but not as much as that of the petroleum ether extract. Aqueous extract of *Moringa oleifera* has the least antimicrobial effect on *Staphylococcus aureus*.

In Table 2, it was observed that the petroleum extract of *Moringa oleifera* is significantly active against the growth of *Streptococcus* species with the 0.6g/ml concentration giving the highest measurement of zone of inhibition. Ethanolic extract of *Moringa oleifera* also has antimicrobial effect on *Streptococcus* species but not as much as that of the petroleum ether extract. Aqueous extract of *Moringa oleifera* has the least antimicrobial effect on *Streptococcus* species. Previous study reported the *Moringa oleifera* leaf extract the non-activity of the water extract against microbes investigated study is in agreement with previous works which showed that aqueous extracts of plants generally exhibited little or no anti-microbial activity[23].

In Table 3, Quantitative analysis of the phytochemical screening of *Moringa oleifera* was determined. This confirms the presence of Alkaloid, Cyanogenic Glucoside, Steroid, Anthraquinone, Phenol, Tannins, Saponins and Flavonoids. The exception is that Steroids is not detected in *Moringa oleifera*. Table 4 shows the quantitative analyses of minerals present in *Moringa oleifera*. Here, *Moringa oleifera* also composes all minerals tested with magnesium having the highest composition while lead was not detected. This findings on the medicinal plant active component is consistent with the observations of Kutar *et al.,*[24] and Abubacker and Sathya[25]. They demonstrated the efficacy of medicinal plants in eliminating some Bacterial pathogensfrom human body including the oral cavity based on the phytochemical property.

Quantitative Analyses of Anti–nutrients present in *Moringa oleifera* was observed and measured in Percentage (%) as shown in Table 5. It was shown that this plant source has all the anti-nutrients present with flavonoid having the highest percentage composition while saponin was not detected. In Table 6, Quantitative analyses of proximate nutrient composition of *Moringa oleifera* was observed and measured in Percentage (%). *Moringa oleifera* composed all the proximate nutrients such as Ash, Moisture Content, Crude Protein, Fat, Fibre and Carbohydrate tested with carbohydrate having the highest percentage composition.

CONCLUSION

The result of this study correlates with previous reports on the antiviral, antibacterial, antifungal, anthelmintic,

antimolluscal and anti-inflammatory properties of plants[26,27]. The discovery and availability of moringa for clinical use enhance its economic sustainability in developing countries as reported by Baba *et al.,*[28]. Thus, this study helps to validate the nutritional and medicinal value of *Moringa oleifera* in the Southwestern part of Nigeria and Africa as a whole, which is now widely adopted as herbal preparations and tea complements in various parts of the world. Its medicinal properties and active components can also make it valuable for various therapeutic purposes.

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Conflict interest

No conflict of interest.

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Table- 1: Antibacterial effect of Moringa(*Moringa oleifera*) leaveon *Staphylococcus aureus.*

Moringa oleifera	0.2g/ml	0.4g/ml	0.6g/ml	Control
Ethanolic extract (mm)	8.0	5.0	4.0	7.0
Aqueous extract (mm)	4.0	1.0	3.0	6.0
Petroleum ether extract (mm)	12.0	8.0	10.0	8.0

Table-2: Antibacterial effect of *Moringa oleifera*extracts on *Streptococcus* species

Moringa oleifera	0.2g/ml	0.4g/ml	0.6g/ml	Control
Ethanolic extract (mm)	9.0	7.0	8.0	4.0
Aqueous extract (mm)	4.0	4.0	7.0	6.0
Petroleum ether extract (mm)	7.0	8.0	12.0	5.0

Table- 3: Qualitative Analysis of the Phytochemical Screening of Medicinal plants

Samples	Alkaloid	C. Glucoside	Steroid	Anthraquinone	Phenol	Tannins	Saponins	Flavonoids
Moringa oleifera	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve

Keys: +ve= Presence of constituents, -ve =Absence of constituents,±ve = Slightly present.

Table- 4: Quantitative Analyses of Minerals Present in Plant Extract (mg/100g)

Plant sample used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn	P
Moringa oleifera	11.68	19.54	12.75	22.66	20.51	7.50	ND	0.01	17.31	10.21

Key: ND- Not Detected

Table- 5: Quantitative Analyses of Anti –nutrients present in Plant Extracts Result in Percentage (%)

Parameters	*Moringa oleifera*
Tannin	3.00
Phenol	2.25
Phylate	3.89
Oxalate	3.90
Saponin	ND
Flavonoids	4.00
Alkaloids	2.90

Key: ND- Not Detected

Table- 6: Quantitative analyses of proximate nutrient composition of plant extracts

S/N	Ash	Moisture Content	Crude Protein	Fat	Fibre	Carbohydrate
Moringa oleifera	11.78	9.00	12.67	6.34	12.45	45.92