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In Vitro Antibacterial Activities of Different Extract of *Moringa oleifera* Leaf on Some Pathogenic Bacteria

K. V. Okolo [1], R. N. Umeji [2], A. C. Okafor [2], P. E. Anyiji [3]

[1] *Department of Pharmaceutical Biology and Traditional Medicine, University of Nnamdi Azikiwe, Awka, Nigeria*
[2] *Department of Microbiology, Renaissance University of Ugbawka, Enugu State, Nigeria*
[3] *Department of Medical Laboratory Technology, Anambra State College of Health, Obosi, Nigeria*

** Corresponding author:K. V. Okolo, Department of Pharmaceutical Biology and Traditional Medicine, University of Nnamdi Azikiwe, Awka, Nigeria. Tel: +80-37917595, E-mail: okolokv@gmail.com*

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Abstract

Introduction: Leaf extracts of *Moringa oleifera* (aqueous, diethyl ether, ethanol, and ethyl acetate) were tested on *Echerichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Salmonella typhi, Streptococcus pyogenes* and *Klebsiella aerogenes* using agar well diffusion method.
Methods: Antibiotics (control) used were tetracycline and ciprofloxacin at 250 mg/mL.
Results: Results obtained showed that 200 mg/mL of diethyl ether, ethanol, ethyl acetate and 100% aqueous extracts had more effect than 160 mg/mL of diethyl ether, ethanol, ethyl acetate and 80% aqueous respectively. The results showed that *Salmonella typhi* was more susceptible than other organisms and ethyl acetate extract was more effective than other extracts. The phytochemical screening indicated the presence of flavonoid, alkaloids, tannin, glycoside, saponin, and phenol.
Conclusions: The study showed that *Moringa oleifera* can be used as potential herbs for treatment of bacteria pathogens tested in this study and these activities could be attributed to the presence of these secondary metabolites.

INTRODUCTION

The use of plants and herbs extract in the treatment of human ailments is very ancient art, a practice that has been passed on for generations and scientists in Africa and other developing countries are conducting research into local plants abundant in the continent for their possible use in traditional medicine [1]. Research into traditional plants and herbs received further boost due to the increasing resistance to many orthodox medicine and thus a search for new organic molecules of plants with antimicrobial properties.
Moringa oleifera is fast growing, deciduous tree. It can reach a height of 10-12 m or 32-40 ft and the trunk can reach a diameter of 45 cm or 1.5 ft [2]. The tree has an open crown of dropping, fragile branches and the leaves build up feathery foliage of tripimate leaves. Moringa is a heat loving plant but does not tolerate freezing of frost. Moringa is particularly suitable for dry regions, as it can grow using rainwater without expensive irrigation techniques. The leaves are harvested every two weeks. The Moringa tree is a host to *Leveillula tavrica*, a powdery mildew which causes damage in papaya crop in south India [3]. Cultivation management should therefore be checked.

Moringa oleifera is one of the species of Moringa tree which is often referred to as 'the miracle tree' [4]. The tree is native to India [5], but it is currently found in many parts of the world. It belongs to the family Moringaceae. *Moringa oleifera* has been adopted as a national plant and it is being cultivated as a crop due to many claimed benefits offered by the plant. The plants is claimed to have many pharmaceutical benefits: antibacterial, anticancer, antiasthma, antihypertensive, immunity booster and antinflammatory. It improves fertility and reproductive health and nutritional supplement [6, 7]. It is a well-documented world renowned plant herb and also used in water purification and therefore helps in reducing the incidence of water borne diseases [8]. *Moringa oleifera* (lam.) leaves possess hypolipidaemic and antiathera sclerotic activities [9].
Bukar et al. [10] reported that *Moringa oleifera* ethanolic extract had the broadest spectrum of activity on the test bacteria. Doughari et al. [11] reported antibacterial activity from the aqueous, acetone and ethanolic extracts of the leaves of *Moringa oleifera*. Of the three solvents uses, ethanolic extracts of the plant demonstrated the highest activity, while the

aqueous extract showed the least activity at l00 mg/mL.
The leaves are the most commonly used part of the plant. The leaves are used in Traditional medicine in several countries. Nutrition content of 100 g offers *Moringa oleifera* leaves is shown in the table below. The leaves are the most nutritious part of the plant being a significant source of vitamin B and C, provitamin A as carotene, vitamin K, manganese and protein among other essential nutrients [12]. According to Olson [13], some of the calcium in Moringa leaves is bound as crystals of calcium oxalate though at levels 1/25 th to 1/45 th of that found in spinach, which is negligible amount. The leaves are cooked and used like spinach and are commonly dried and crushed into a powder used in soups and sources. As with most foods heating Moringa above 14˚C of 28˚C destroys some of the nutritional value. Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. One author state "the nutritional properties of Moringa are now well known that there seems to be little doubt of the substantial health benefit to be realized by consumption of Moringa leaf powder in situations where starvation is imminent [14].
Moringa has been used in folk medicine [14] including Sidtha medicine and Ayurveda traditional medicines and in the Philippines [8]. It is a natural anthelmintic and possible adjuvant [8]. Its leaf powder was as effective as soap for hand washing when wetted in advance to enable antiseptic and detergent properties from phytochemical in the leaves [15]. Moringa leaves are given to nursing mother in the belief that they increase lactation [16]. This study was aimed at investigating the antibacterial properties and preliminary phytochemical analysis of different extracts of *Moringa oleifera*.

METHODS

Sample Collection

Fresh leaves of *Moringa oleifera* were collected from Attakwu in Akegbe Ugwu, Nkanu West Local Government, Enugu State, Nigeria. They were collected very early in the morning between 6:00am to 6:30am. This was to avoid denaturing of the nutrient by sunlight. The leaves were identified at the Department of Botany, University of Nigeria Nsukka. The leaves were then transported in aerated polythene bag to Project Development Institute Enugu (PRODA) for phytochemical screening.

Preparation of Leaves for Extraction

This was carried out using modified method of Nwobu et al. [17]. The leaves of plants were plucked off, properly washed and dried under shade at room temperature for 14 days. The dried leaves were ground using electric grinder into powdered form and kept ready for extraction of active ingredients and antibacterial activities.

Extraction for Antibacterial Activity

Extraction was done using cold maceration method. Solvents used include water, ethanol, diethyl ether and ethyl acetate. An amount of (40 g) of each of the four pulverized leaf samples was placed in a conical flask and solvent added. The flask was covered and left for 24 hours. The resulting extracts were subsequently filtered using what man No 1 filter paper. Sterility proof was conducted on the leaves extracts. The liquid extracted was placed into a conical flask and was evaporated at 50˚C in a water bath to obtain the semi solid crude extracts except for aqueous extract which was condensed to half its volume. The weight of the residue was obtained by subtracting the weight of the empty dish from the weight of the dish and residue. All extracts obtained were stored in the refrigerator until required for use.

Sterility Proofing of the Extracts

The extracts were tested for sterility by inoculating 1ml of the sterile extract in 10ml of sterile nutrient broth. Incubation was done at 37˚C for 24 hours. A sterile extract was indicated by the absence of turbidity or clearness of both after incubation.

Collection and Maintenance of the Test Organisms

Pure culture of six organisms was used for the tests. They were collected from the Department of Microbiology, University of Nigeria, Nsukka, Enugu State. The organisms are *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes* and *Salmonella typhi*. All the bacterial species were maintained on nutrient agar slants except *Streptococcus pyogenes* which was maintained in Todd-Hewitt broth supplemented with 0.2% yeast extract and stored in the refrigerator at a temperature at 4˚C from where they were subcultured into fresh media at regular intervals.

Standardization of the Bacterial Cell Suspension

A volume (10 mL) of nutrient broth was pipetted using 10 mL syringe for each organism into McCartney bottles and incubated for 24 hours at 37˚C. The turbidity produced by these organisms was adjusted and used to match the turbidity standard prepared as described by Monica [18].

Reconstitution of Extract

A quantity (2 g) of the semi solid crude extract of was dissolved in 5ml of 20% dimethyl sulphoxide (DMSO) to get 200 mg/mL of solution of extract for ethanol and diethyl ether acetate extract of *Moringa oleifera* and used as the stock. 1ml of 100% tween 80 with 5 mL of 20% of DMSO was dissolved to give perfect dissolution acetate. Weaker concentration 160 mg/mL of the extracts were prepared by serial dilution of the stock

Antibacterial Tests

Antibacterial activity of each plant extract was determined by using modified kirby bauer or agar well diffusion method [7]. Broth culture of test bacteria was spread on the Muller Hinton agar Media in duplicates and microbes broth culture was applied on media by swabbing using swab stick under aseptic conditions. Three holes were bored with 8 mm cork borer for 200 mg/mL of each plant and mixture of 200 mg/mL of both plants. The same was applied for 160mg/ml for each plant extract used (aqueous, ethanol, diethyl ether and ethyl acetate). The extracts was inoculated and allowed to diffuse for 1hour. It was incubated at 37˚C for 24 hours. The diameter of the inhibition zones were measured in millimeters. Standard an-

tibiotics, tetracycline and ciprofloxacin of 250 mg/mL were used as control. All assays were performed in duplicates to consider the mean values as a standard one.

RESULTS

The qualitative phytochemical components of *Moringa oleifera* in aqueous extract showed the presence of alkaloids, saponin, flavonoids, phenol, glycosides and tannin. In ethanol extract it showed the presence of alkaloids, saponin, phenol, glycoside and tannin. In ethyl acetate, it showed the presence of alkaloids, phenol and tannin while in diethyl ether, alkaloids, phenols and tannins were present (Tables 1, 2, 3, 4, and 5 and Figs 1 and 2).

Table 1: Qualitative Analysis of *Moringa oleifera* in Aqueous, Ethanol, Diethyl Ether and Ethyl Acetate

Phytochemicals	Aqueous Extract	Ethanol Extract	Ethyl Acetate Extract	Diethyl Ether Extract
Alkaloids	+	+	+	+
Saponins	_	+	+	_
Flavonoids	+	_	_	_
Phenols	+	+	+	+
Glycosides	+	+	_	_
Tannins	+	+	+	+

Key: +, Present; -, Absent.

Table 2: Physical Appearance of *Moringa oleifera* and Weight Obtained After Evaporation

Physical Properties							
Colour				Weight, g			
Diethyl Ether	**Ethanol**	**Aqueous**	**Ethyl Acetate**	**Diethyl Ether**	**Ethanol**	**Aqueous**	**Ethyl Acetate**
Oily and dark	Oily and dark green	Brown	Oily and dark green	6.44	5.45	15.95	5.95

Table 3: Quantitative Phytochemical Analysis

Sample	Alkaloid, % w/w	Flavonoid, % w/w	Phenol, % w/w	Glycoside, mg/g	Tannin, n mg/g
Moringa oleifera	2.94	10.54	1.72	0.21	0.01

Table 4: Antibacterial Activities of Aqueous, Ethanol, Diethyl Ether and Ether Acetate of

Antibiotics (Controls)	Zone of Inhibition on Test Organisms in Millimeter, mm					
	Escherichia coli	**Pseudomonas aeruginosa**	**Staphylococcus aureus**	**Salmonella typhi**	**Streptococcus pyogenes**	**Klebisella aerogenes**
Ciprofloxacin 250 mg/mL	20	26	27	21	25	20
Tetracycline 250 mg/mL	10	5	12	21	27	9

Table 5: Antibacterial Activities of Aqueous, Ethanol, Diethyl Ether and Ether Acetate of *Moringa oleifera* at Various Concentration on Test Organisms

Concentration of extracts, % and mg/mL	Zone of Inhibition on Test Organisms in Millimeter, mm					
	Escherichia coli	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Salmonella typhi***	***Streptococcus pyogenes***	***Klebisella aerogenes***
Aqueous 100%	-	-	-	-	-	-
Aqueous 80%	-	-	-	-	-	-
Ethanol 200 mg/mL	7	-	3	7	1	6
Ethanol 160 mg/mL	4	-	-	3	-	-
Diethyl ether 200 mg/mL	8	-	4	1	7	1
Diethyl ether 160 mg/mL	2	-	-	-	-	-
Ethyl acetate 200 mg/mL	8	-	9	10	12	4
Ethyl acetate 160 mg/mL	-	-	7	7	7	2 (-) means No zone of inhibition

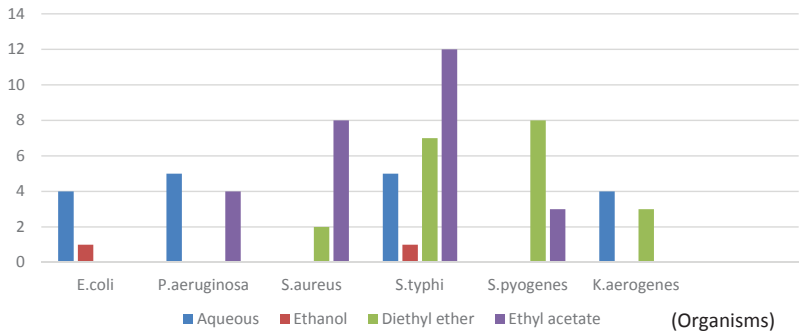

Figure 1: Bar chart Representation of Total Zones of Inhibition at 100% Aqueous and 200 mg/mL of Ethanol, Diethyl Ether and Ethyl Acetate Extracts of *Moringa oleifera* on Test Organisms

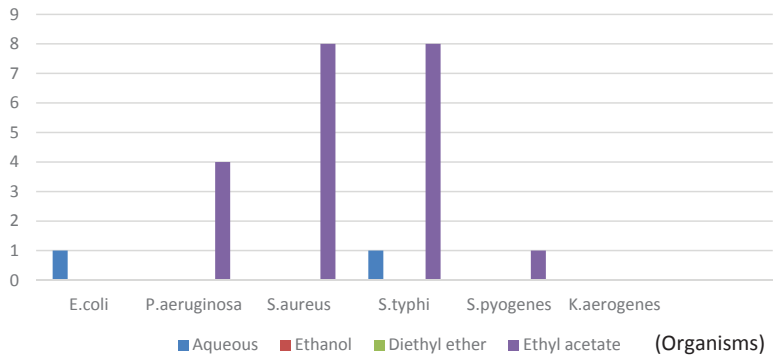

Figure 2: Bar Chart Representation of Total Zones of Inhibition at 80% Aqueous and 160 mg/mL of Ethanol, Diethyl Ether and Ethyl Acetate Extracts of *Moringa oleifera* on Test Organisms

DISCUSSION

The findings of the preliminary phytochemical investigations and the results of antibacterial activity were depicted in the respective tables and figures. The plant extracts was tested on two Gram positive bacteria (*Staphylococcus aureus*, and *Streptococcus pyogenes*) and four Gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Klebsiella aerogenes* and *Pseudomonas aeruginosa*). Different parts of plant extracted with different types of solvents have been used by different researchers for investigating their medicinal purposes. In this present study, water, ethanol, ethyl acetate and diethyl ether were used as extractive solvents. The essence of using different solvents was to utilize their differential extractive properties.

Aqueous extract contains most of the phytochemical constituents tested in this study. Alkaloids, phenol, and tannin were present in all the solvent extracts. The presences of these phytochemical constituents in leaves of tested plants were being responsible for the antibacterial activities exhibited by the plants which confirms with the works of Animashaun et al. [6]. In the leaf extracts, flavonoids were the highest phytochemical component while the least was tannin. This confirms with the work of Udochukwu et al. [19] and Animashaun et al. [6].

The overall result of antibacterial tests using the extract at 100% aqueous and 200 mg/L of diethyl ether, ethyl acetate and ethanol of *Moringa oleifera* on test organisms were 5mm on *Echerichia coli*, 9 mm on *Pseudomonas aeroginosa*, 16 mm on *Staphylococcus aureus*, 25 mm on *Salmonella typhi*, 11 mm on *Streptococcus pyogenes* and 7 mm on *Klebsiella aerogenes* showing that *Moringa oleifera* has the highest antibacterial activity on *Salmonella typhi* and least on *Klebsiella aerogenes*. 80% and 160mg/ml of aqueous diethyl ether, ethyl acetate and ethanol extracts on/ against test organisms showed least zone of inhibition. These agree with the work of Swee et al. [20].

Ciproflaxacin had more antibacterial properties than Tetracycline on the test organisms in the range of 20 mm-27 mm and 5 mm-27 mm respectively (Table 4). Findings from present work showed zone of inhibition equal to some of the selected antibiotics. This contradicts the work of Udochukwu et al. [19].

The result obtained from this research work indicates that the leaves of *Moringa oleifera* contain antimicrobial substances which is both bacteriostatic and bacteriocidal on several species of bacteria. However the bacteria varied widely in their degree of susceptibility to the plants extracts. The plant extracts should therefore be seen as a potential source of useful drugs. The phytochemical constituents can be investigated further to achieve lead molecules in the search of novel herbal drugs.

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None declared.

CONFLICTS OF INTEREST

There is no conflict of interests.

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