ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 7, Issue 2 , February 2020

Inhibitory activities of *Moringa oleifera* Lam leave and bark extracts against some clinically important bacterial isolates.

I. Titus , E. Joseph, M. Daji

Department of Biological Sciences, Faculty of Science, Abubakar Tafawa Balewa University, Bauchi, Nigeria
Department of Biological Sciences, Faculty of Science, Abubakar Tafawa Balewa University, Bauchi, Nigeria
Department of Microbiology, Faculty of Science, Federal University Wukari, Taraba, Nigeria

ABSTRACT: The Leave and bark of *Moringa oleifera* Lam. Were collected for this study. Bacteria of medical importance known to cause common diseases were used for the study, they include *Escherichia coli*, and *Salmonella typhi*. Ethanol and water extracts of leaves and bark of *Moringa oleifera* were tested against the isolates. The various extracts showed activity against the test organisms which are both Gram positive and Gram negative at varying concentrations and zones. Results of the antibacterial activity of the leave extract demonstrated higher activity against the test organisms compared to that of the bark of the plant. The results also showed that the organic extract (ethanol) had higher activity compared to the aqueous extract. The best and optimal interactions occurred with ethanol extract against *Escherichia coli*, followed by ethanol extract against *Salmonella typhi*. The use of *Moringaoleifera* plant in the treatment of diseases caused by these organisms is hereby recommended subject to further studies.

KEYWORDS: *Staphylococcus aureus*, *Salmonella typhi*, *Moringa oleifera*, *antimicrobialactivity*, *extracts*. Minimum Inhibitory Concentration, Bactericidal

I. INTRODUCTION

Before the advent of modern medicines, plants were the first line of disease treatment available to man. Humans have sought to control disease and pain with assistance, inspiration and guidance from natural products such as bark, stem, leaves, roots, flowers of plants. Plant parts have been known to have medicinal properties and are therefore used by man. These plant materials possess many active substances known to have significant antimicrobial activity against a wide variety of diseases. Sources from plants and its materials are relatively cheaper, locally available, and environmentally friendly and are potential sources for synthesis of new drugs (Ebena *etal*., 1991)

In recent past,emergence of ever-increasing number of antibiotic resistant microbial strainshas become a severe health threat(Alanis*etal*.,2005).This could be due to several factors which include the inappropriate and over-use of antibiotics, human and agricultural antibiotic consumption, microbial mutations among others are thought to be the major causative factors contributing to the appearance of strains with reduced susceptibility to antibiotics(WHO,2011) Thus, is has become critical to find new drugs with less resistance and side effects (Bhalodia *etal*., 2011). Consequently, medicinal plants are the best candidate for the development of such new drugs (Sofowora,1986).

Many studies showed that some plants contain many phyto chemical constituents such as saponins, tannins, alkaloids peptides, some essential oils, phenols and soluble compounds in water, ethanol, chloroform, methanol and butanol (Alma *etal*., 2003, Klausmeyer *etal*., 2004, Seyyednejad *etal*., 2008).

Moringa oleifera belongs to the monogenetic family, Moringaceae that is nature to many continents including Africa. The tree (also known as horseradish tree, drum stick tree, tree of life) is now widely cultivated and has become naturalized in many locations in the tropics, Nigeria inclusive. The plant extract of *Moringa oleifera* have been reported to contain the following phytochemical constituents; alkaloids, saponins, tannins, and phenols (Doughari *etal*; 2007). The presence of these phytochemical constituents has been reported to account for the exertion of antimicrobial activity by plants. This study was aimed at determining the antibacterial activity of Leave and barks extracts of *Moringa oleifera* Lam. on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typh*i and also determines the Minimum Inhibitory Concentration (MIC) ofLeave and bark extracts of the plant on some microorganisms

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II . METHODOLOGY

A) STUDY AREA

The research was carried out at Abubakar Tafawa Balewa University, Bauchi, Bauchi State Nigeria

B) COLLECTION OF ISOLATES

Isolates of *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli* were obtained from Government Health Facility in Bauchi and transported to the laboratory for further confirmation following standard microbiological identification procedures.

C) BIOCHEMICAL TEST

The clinical isolates were Gram stained and further characterized using both biochemical test

D) CATALASE TEST

This is to demonstrate the presence of the enzyme catalase in an organism which aids to mediate the breakdown of hydrogen peroxide (H2O2) into O2 and H2O.Inoculums of the organisms were introduced into hydrogen peroxide (30% on a slide) and the rapid effervescence of oxygen bubbles if present was used to indicate the presence of catalase

E) OXIDASE TEST

This is used to determine the presence of bacterial cytochrome oxidase enzyme using colour change. Filter paper was soaked in Kovac's reagent and the test organism was inoculated into the filter paper, colour change was observed

F) UREASE TEST

The test organisms were inoculated heavily on the entire slope surface of the urea agar slants prepared in caped tubes. The tubes were placed in racks and incubated at 37ºC for up to 48 hours. Tubes were thereafter examined for change of colour from plain to pink (Cheesbrough, 2006).

G) HYDROGEN SULPHIDE PRODUCTION

Test organisms were inoculated into the triple sugar iron agar slants contained in test tubes. These were incubated at 35°- 37°C for up to 48 hours. After incubation the TSI agar media were checked for blackening and change in colour from amber to red at the bottom (butt) of the tube (Cheesbrough, 2006).

H. COLLECTION OF PLANT SAMPLE

Fresh leaves of *Moringa oleifera* were collected locally in Yelwa area of Bauchi Metropolis (Nigeria) and identified in the laboratory accordingly.

III PREPARATION OF PLANT EXTRACTS

Preparation of crude extracts was conducted using the method described by Predrag *etal*., 2005. Once sufficient amount sample was collected, the leave and bark materials were shade- dried at room temperature (32 – 35^{0}C) to constant weight over a period of days until completely dried. Exactly 50g of the plant materials each was coarsely grounded to powder using a mortar and pestle. The powdered shade-dried materials were extracted with water and ethanol. 25g of the powdered samples was suspended in 150ml of distilled water and another 25g of the powdered sample was suspended in 150ml ethanol. The mixed samples were shaken and allowed to sediment at room temperature for 72hrs and 24hrs with manual agitation after every 24hr, 72hr and 24hr. It was then filtered through Whatman filter paper No 1. Each of the resulting filtrate was then concentrated in a rotary evaporator.

A) STERILITY PROOFING OF THE EXTRACTS

Sterility proofing of the extracts was done by introducing 2ml of the extract into 10ml of Mueller Hinton broth, and incubated at 37oC for 24hr. The absence of turbidity or clearness of the broth after the period of incubation signifies the presence of a sterile extract.

B) PHYTOCHEMICAL SCREENING

The plant was screened for the presence of Flavonoids, Alkaloids, Saponins, and Tannins which are the active ingredients in the plants

C) TEST FOR TANNINS

About 0.5g of each of the extract was mixed thoroughly with 10ml distilled water and then filtered. 5ml of the filtrate was added to 1ml of 5% Ferric chloride solution. The appearance of blue black, greenish or blue green precipitate was observed which indicates the presence of tannins

D) TEST FOR FLAVONOID

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of each of the plant materials. Immediately, a red color was observed, which indicates the presence of flavonoids

E) TEST FOR SAPONINS

About 0.1g of the plant materials was boiled with 10ml of water for 5mins then filtered. After cooling, 5ml of filtrate was diluted with water and shaken vigorously. The formation of foam indicates presence of saponins

ISSN: 2350-0328

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F) TEST FOR ALKALOIDS

About 0.5g each of the extracts was stirred with 5ml of 1% hydrochloric acid on a steam bath and filtered. 1ml of the filtrate was then treated with Mayer's reagent. A white creamy precipitate indicates the presence of alkaloids

G) DETERMINATION OF ANTIBACTERIAL ACTIVITY : Antibacterial activity of the aqueous and ethanol extracts of the plant sample was evaluated using the agar well dilution method (Aida *et al*., 2001). The bacterial isolates were reconstituted and standardized then adjusted to 0.5 McFarland standard. Sterile cork borer was used to bore 3 wells of 6mm diameter on the nutrient agar plates. 0.5ml of each extract dilution (10^{-1}-10^{-5} dilutions) was introduced into the wells using a sterile pipette. The plates were then incubated at 37 ^{0}C for 18 to 24 hr. Antibacterial activity was determined by measuring the diameter of zones of inhibition formed around the wells. Each experiment was carried out in duplicate.

H) DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

Agar well dilution method was used to determine the Minimum Inhibitory Concentration (MIC). Extract dilutions with 80mg/ml, 70mg/ml, 60mg/ml, 40mg/ml, 20mg/ml and 10mg/ml of the samples were measured and analyzed. The lowest dilution of the extracts that produces inhibition was taken as the minimum inhibitory concentration and was assessed by measurement of the zones of inhibition formed around the wells. To determine the Minimum Bactericidal Concentration (MBC), the culture from the plates showing no growth was streaked onto sterile nutrient agar. Nutrient agar plates were re-streaked with the test organisms only to serve as control. The plates were then incubated at 37 ^{0}C for 24 hr. After incubation, the concentration showing no visible growth was noted as the minimum bactericidal concentration.

IV RESULTS

Table 1: Shows the effect of different extracts of *Moringa oleifera* Lam. on the test organisms used. All of the extracts were inhibitory to the three (3) test organisms used in this study.

Table 2: Shows the physicochemical constituents/active ingredients of *Moringa oleifera*

Table 3: Indicates the individual diameter (mm) zones of inhibition produced by the extracts of *Moringa oleifera* in replicates. The largest inhibition was produced by ethanol extract against *Escherichia coli* (with 13 and 14mm in diameter) followed by ethanol extract against *Salmonella typhi* (with 10 and 11mm in diameter). However, bark extracts showed the least activity against all the test organisms.

Fig. 1 shows the mean diameter (mm) of inhibitions produced by *Moringa oleifera* ethanol extract, water extract and bark ethanol extract on the test organisms. The largest mean diameter of inhibition was produced by ethanol extract against *Escherichia coli* (13.5mm) followed by ethanol extract on *Salmonella typhi* (10.5mm) while bark ethanol extract and bark water extracts showed the least mean diameter of inhibition against all the test organisms.

Table 4: Indicates the effect of the extracts on the test organisms at various concentrations (mg/ml). The concentrations ranged between 10mg/ml – 80mg/ml for each of the extracts on the three test organisms (*Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*).

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Table 1. Effects of *Moringa oleifera* extracts on the test organism

	Leaf ethanol extract (LEE)	Leaf water extract (LWE)	Bark ethanol extract (BEE)	Bark water extract (BWE)
Staphylococcus aureus	+	+	+	+
Salmonella typhi	+	+	+	+
Escherichia coli	+	+	+	+

Table 2. Phytochemical constituents of *Moringa oleifera*

Active ingredients	ethanol	Acqueous
Saponins	+ +	++
Flavoinoids	++	++
Alkaloids	++	- -
Tannins	--	++

Table 3: Diameter of zone of inhibition of the different extracts on the test organisms used at 100% concentration.

Test organism	Extract Replica							
	LEE		LWE		BEE		BWE	
	I	II	I	II	I	II	I	II
Staphylococcus aureus	10	10	9	9	7	8	6	5
Salmonella typhi	11	10	10	9	7	7	5	5
Escherichia coli	13	14	12	13	8	8	8	8

KEY: LEE = Leaf ethanol extract LWE = Leaf water extract BEE = Bark ethanol extract
BWE = Bark water extract

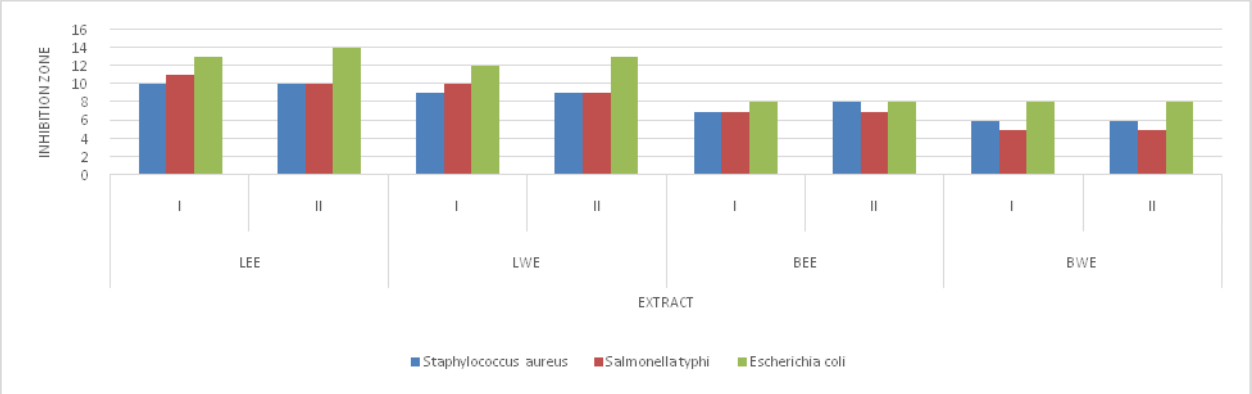

Fig1:Zone of Inhibition of the Different Extracts Organisms Used at 100% Concentration (mm)

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Table 4. Minimum Inhibitory Concentration value at various concentration (mg/ml) after 24 hrs.

Organisms	Zones of inhibition (mm)						
	Extract Methods	80mg/ml	70mg/ml	60mg/ml	40mg/ml	20mg/ml	10mg/ml
S. aureus	LEE	9	8	5	3	2	-
	LWE	8	7	5	4	-	-
	BEE	6	5	5	4	1	-
	BWE	4	4	3	2	-	-
S. typhi	LEE	9	7	5	3	2	1
	LWE	10	9	6	3	1	-
	BEE	6	5	3	2	1	1
	BWE	5	4	4	3	2	-
E. coli	LEE	12	12	10	8	5	4
	LWE	11	10	10	6	3	1
	BEE	8	7	5	4	-	-
	BWE	6	5	5	2	-	-

V: DISCUSSION AND CONCLUSION

Many of the existing synthetic drugs cause various side effects. Hence, development of plant-based compounds is required to meet this demand for production of newer drugs with minimal side effects. Plant tissues from leave and bark of *Moringaoleifera* Lam. from Bauchi region, Nigeria were collected and ethanol and water extract were tested against Escherichia coli, *Staphylococcusaureus* and *Salmonellatyphi*. Ethanol and water extracts of leaves and bark of *Moringaoleifiera* showed varying level of activities against the test organisms. Active ingredients such as saponins, tannins, alkaloids and flavonoids were found to be present in the plant which is responsible for antibacterial activity.
Findings in this study indicates that the antibacterial activity of the leave extract demonstrated higher activity against the test organisms compared to that of the bark of the plant. The results also showed that the organic extract (ethanol) had higher activity compared to the aqueous extract. It has been reported that different solvents have different extraction capacities and different spectrum of solubility for the phytoconstiuents, Majorie 1999, Srinivasan *etal*., 2001. However, it was unclear if the different level of activities was due to the nature of solvent or the type of active ingredient being extracted. The best and optimal interactions occurred between ethanol extract against *Escherichiacoli* followed by ethanol extract against. Statistical analysis was carried out using the Analysis of variance (ANOVA) and the antibacterial study of *Moringaoleifera* leave and bark shows significant inhibitory effect on test organisms. Significant differences in response were observed between the Gram negative bacteria E. coli, *Salmonellatyphi* and the Gram positive bacteria S. *aureus*. This shows that the Gram negative bacteria are more sensitive than the Gram-positive bacterium. This is probably due to the thickness in cell wall of S. *aureus* as compared with those of the Gram- negative bacteria (Hammer, 1999). In conclusion, this study demonstrated the antibacterial activity of leaves of *Moringaoleifera* which could potentially serve to treat those infections that otherwise has become highly resistant to most of the conventional antibiotics used for its treatment. Moreover, that the plant is available, cheaper and affordable makes it an alternative for conventional antibiotics provided toxicological investigations is further carried out

ISSN: 2350-0328

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