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Inhibitory activities of leave and bark extracts of *moringaoleifera* lam against some medically important bacterial isolates.

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ABSTRACT :*Based on its traditional usage in the treatment of some bacterial infections, the Leave and bark of Moringaoleiferalam. were collected for this study. The causative agents of Common bacterial infections that are of immense medical importance, such as Escherichia coli, Staphylococcus aureus and Salmonella typhi were therefore chosen for the study. Ethanol and water extracts of leaves and bark of Moringaoleifera were tested against these three selected bacteria and they all showed activity against the test organisms. 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. It has been reported that different solvents have different extraction capacities and different spectrum of solubility for the phytoconstiuents. The best and optimal interactions occurred with ethanol extract against Escherichia coli, followed by ethanol extract against Salmonella typhi.*

Keywords:*Staphylococcus aureus, Salmonella typhi, E. coli. Moringaoleifera, antimicrobial activity*

I. INTRODUCTION

Traditionally, man has sought to fight and control disease and pain with assistance, inspiration and guidance from nature. Before the advent of modern medicines, herbal remedies were the first art of treatment available to man. The use of plants with medicinal properties by man to treat ailment dates back to a very long time in the history of man, as he has been able to find one use or the other from plants as from them he gets food (directly or indirectly), materials for clothing, building, furniture, cosmetics and medicine [1].Many substances known to have antimicrobial activity are basically from natural sources (plants, bacteria, fungi). Sources from plants and its materials are relatively cheaper, locally available, and environmentally friendly and are potential sources for synthesis of drugs [2].Studies have shown that 300 plants from Southern Africa has antimicrobial activity, 48% had a medium activity, 31% high activity, and 21% no activity when tested against a range of pharmacological parameters. And a wide range of plants in West Africa were also shown to have antimicrobial activity against a wide range of organisms [3].

Plants are the source of many important scientific drugs of modern medicine, for example, the roots, leaves and flowers of *Calotropisprocera* shows activity against *Escherichia Coli* and *Shigelladysentearae.* Hot water extraction of Acalyphatorta has significant inhibiting effects in vitro on the growth of *Staphylococcus aureus*, *Pseudomonas aero*genosa, *Escherichia Coli*, *SalmonellaparatyphiandKlebsiellasp* [3]. The use of the roots of *Rauwolfiasepertina* in traditional treatment of illnesses, insanity has led to the isolation of modern sedative, tranquilizing and hypertensive drugs [4].

The indiscriminate use of antibiotics and several other factors has led to the emergence of multidrug resistance to human pathogenic infections by bacterial strains [5].Besides, plants of medicinal value are said to have minor side effects compared to the chemical agents [6].

Based on the traditional importance and continued use of *Moringaoleifera* for food and medicinal purposes as well as several other uses by cultures in separate and distant parts of the world, it is therefore important to ascertain and evaluate its antimicrobial activity. The plant extract of M*oringaoleifera* have been recently found to contain the following phytochemical constituents; alkaloids, saponins, tannins, and phenols [7]. The presence of these phytochemical constituents has been reported to account for the exertion of antimicrobial activity by plants [8]. This study was aimed at determining the antibacterial activity of Leave and bark extracts of *Moringaoleifera* Lam. on some selected clinical isolates of medical importancesuch as *Escherichia coli, Staphylococcusaureus* and *Salmonella typhi*and also determinethe minimum inhibitory concentration (MIC) of *Moringaoleifera* Lam. Leave and bark extracts on the selected clinical isolates that are evaluated.

II. MATERIALS AND METHODS

2.1 *Collection of Plant Sample*

Fresh leaves of *Moringaoleifera* were collected in Bauchi Metropolis (Nigeria) and identified in the laboratory according to [4].

2.2 *Preparation of Crude and Organic Extracts*

This was carried out as described by Predrag et al; [9]. After collection, the leave materials were shade-dried at room temperature (32 – 35^{o}C) to constant weight over a period of five (5) days. 50g of the plant tissue was coarsely grounded to powder using a mortar and pestle. The powdered shade-dried material was extracted with water and ethanol. 25g of the powdered sample was resuspended in 150ml of distilled water and another 25g of the powdered sample was resuspended 150ml ethanol. The mixed samples were shaken and allowed to sediment at room temperature for 72hrs and 24hrs with manual agitation after every 24hr After 72hr and 24hr, each extract was filtered rapidly through Whatman filter paper No 1. Each of the resulting filtrate was then concentrated in a rotary evaporator. 50g of bark extract (powdered) was mixed with 150ml of ethanol and extracted using some procedure as that of the leave.

2.3 Collection and Cultivation of Test Organisms

Isolates of *Salmonella typhi*, *staphylococcus aureus* and *Escherichia coli* were obtained from state specialist hospital, Bauchi and these clinical isolates were further characterized using both morphological (Gram staining) and biochemical test (urease, catalase, coagulase test, etc) to confirm the exact species of isolates. The clinical isolates were inoculated into nutrient agar slants and then stored in the refrigerator at 4^{o}C until required.

2.4 Determination of Antibacterial Activity

Antibacterial activity of the aqueous and ethanol extracts of the plant sample was evaluated using the agar well dilution method [10]. The bacterial isolates were reconstituted and inoculated on nutrient agar plate. 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^{o}C for 18 to 24hr. Antibacterial activity was determined by measuring the diameter of zones of inhibition formed around the wells. Each experiment was carried out in duplicate.

2.5 Determination of Minimum Inhibitory Concentration MIC

Agar well dilution method as described by [10] 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 analysed.

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^{o}C for 24hr. After incubation, the concentration showing no visible growth was observed and noted as the minimum bactericidal concentration.

III. RESULTS

Table 1 shows the effect of the different extracts of *Moringaoleifera lam*. (ethanol extract EE, water extract WE and bark ethanol extract BE) 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 individual diameter (mm) zones of inhibition produced by the extracts of Moringaoleifera in the duplicated plates. 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 ethanol extract showed the least activity against all the test organisms.

Fig. 1 shows the mean diameter (mm) of inhibitions produced by *Moringaoleifera* 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 showed the least mean diameter of inhibition against all the test organisms.

Fig. 2 shows 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 (*Staphylococcusaureus, Salmonella typhi* and *Escherichia coli*).

Table 1Effects of *Moringaoleifera* extracts on the test organisms.

Organism	**Leaf**		**Bark Ethanol Extracts (BEE)**
	Ethanol Extract (LEE)	**Leaf Water Extract (LWE)**	
Staphylococcus aureus	+	+	+
Salmonella typhi	+	+	+
Escherichia coli	+	+	+

Key: + = Inhibition

Table 2Diameter of zones of inhibition of the different extracts on the test organisms used at 100% concentration (mm).

Test Organisms	***Extract Replica***					
	EE		*WE*		*BEE*	
	I	*II*	*I*	*II*	*I*	*II*
Staphylococcus aureus	*10*	*10*	*9*	*9*	*7*	*8*
Salmonella typhi	*11*	*10*	*10*	*9*	*7*	*7*
Escherichia coli	*13*	*14*	*12*	*13*	*8*	*8*

Key:
EE = Ethanol Extract
WE = Water Extract
BEE = Bark ethanol Extract

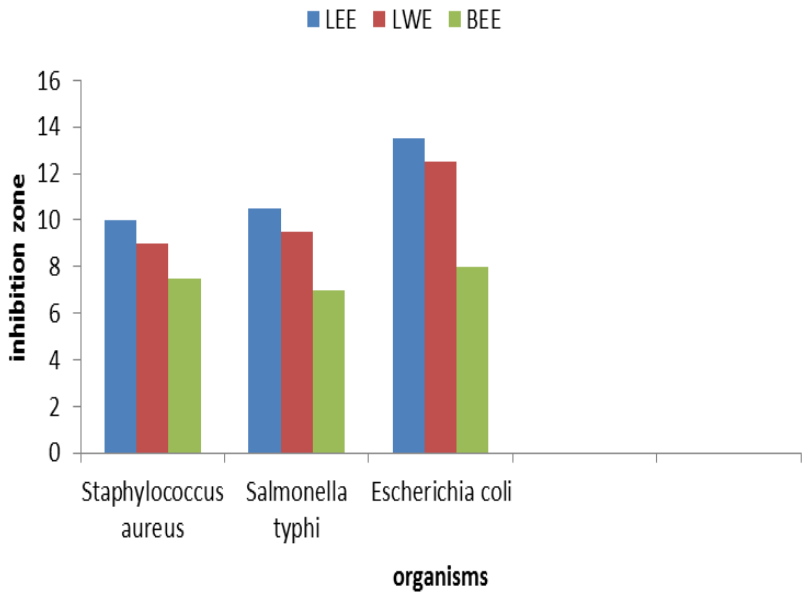

Fig. 1 Mean diameters of zones of inhibition for the different extracts at 100% concentration (mm) (undiluted).

Key:
LEE = LEAF Ethanol Extract
LWE = LEAF Water Extract
BEE = Bark ethanol Extract

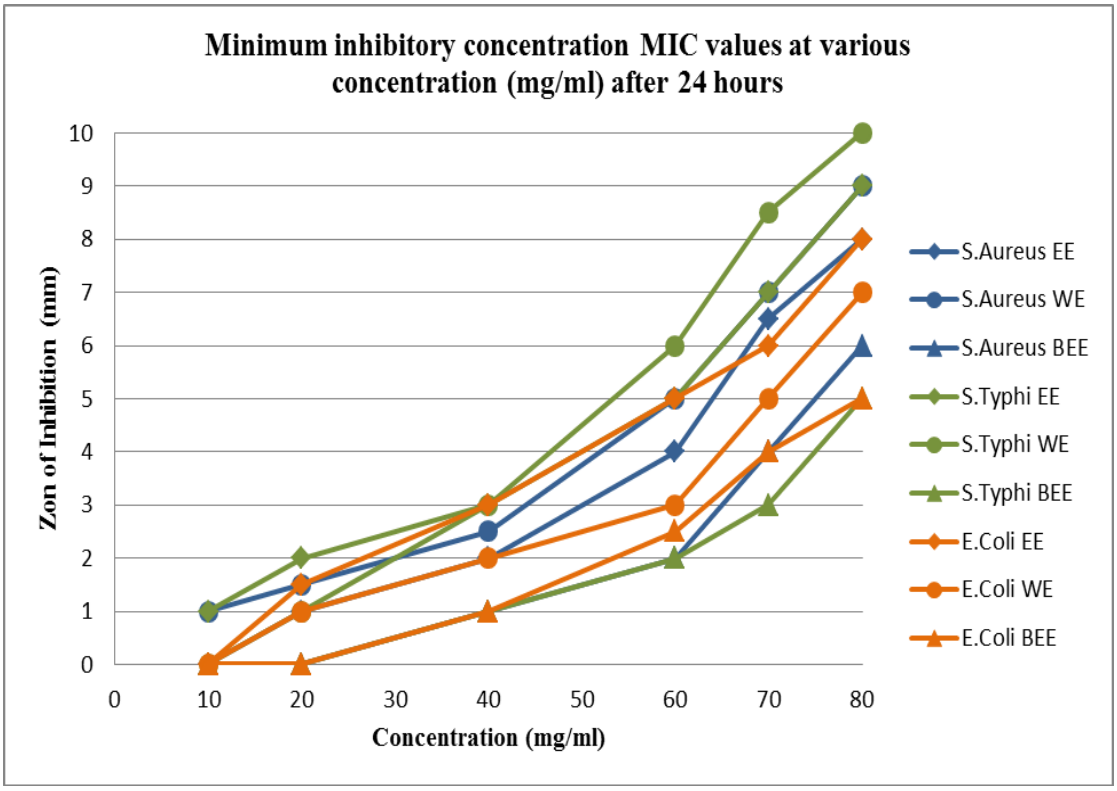

Figure 2. Minimum inhibitory concentration MIC values at various concentration after 24 hours With different organism and different extraction method.

Key:
EE = Ethanol Extract
WE = Water Extract
BEE = Bark Ethanol Extract

IV. DISCUSSION AND CONCLUSION

Plant tissues from leave and bark of *Moringaoleifera Lam*. from Bauchi region, Nigeria were collected and ethanol and water extract were tested against some common infectious bacterial isolates; Escherichia coli, *Staphylococcusaureus* and *Salmonella typhi*. Ethanol and water extracts of leaves and bark of *Moringaoleifera* showed varying level of activities against the test organisms.

Our results indicated 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 [11]; [12]. 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 *Escherichia coli* followed by ethanol extract against *Salmonella typhi*.

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*, *Salmonella typhi* 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 [13].

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. More research is needed since it is known that the active compound of Moringa crude extracts is a cationic protein with a molecular weight between 17-26kDa, composed of 3 subunits of different molecular weight (around 10 kDa) not observed before [14].

REFERENCES

[1]. E. Kafaru, *Immense help from natures Workshop*,(Elikaf Health services Limited, 1994). 1-5 and 59 – 104.

[2]. R.V. Ebena, R.E. Madunagu, E.D. Ekpe, and I. Itugu,Microbial exploitation of Cardiac Glycoside and alkaloid from Garcinia kola, Bonreniaoccurnoides, kola nitida and citrus aurantifolia.*Journal of Applied Bacteriology, 71*, 1991, 398 – 401.

[3]. SofoworaA,*Medicinal Plants and Traditional Medicine in Africa*. (Spectrum Books Ltd., Ibadan, Nigeria 1984). 191-289.

[4]. E.A Sofowora, *Screening of plants for bioactive agents "in" Medicinal plants and traditional medicines in Africa*. (Spectrum books limited, 1991). (1st edition) 128 – 160.

[5]. F.E Berkowitz. Antibiotic resistance in bacteria, *South,Med. J, 88* 1995, 797-804.

[6]. M. Maghrani,N.Zeggwagh, J. Michel, M. Eddouks,Antihypertensive effects of LepidiumSativum L. in spontaneously hypertensive rats *J. Ethnophamacol,100(200)*, 2005, 193 – 197.

[7]. Doughari, J.H; Pukuma, M.S and De.N. (2007). Antibacterial activity of BalanitesaegyptiacaL. Drel and Moringaoleifera Lam. On Salmonella typhi. *African Journal of biotechnology, 6 (19)* Pp. 2212 – 2215.

[8]. C.J. Pretorius, E. Watt,Purification and identification of active components of Carpobrotusedulis L. *J. Ethnopharmacol, 76*, 2001, 87 – 91.

[9]. L. Predrag,S.Hui, C. Uri,A.Hasswan,B.Arieh, The effects of aqueous extracts prepared from leaves of Pistacialentiscus in experimental liver disease, *J. Ethnopharmacol, 100 (1 – 2)*2005, , 198 – 204.

[10]. P. Aida, F. RosaBlamea; A.Tomas,C.Salvador,Paraguyan plants used in traditional medicine.Shortcommunication, *J. Ethnopharmacol,16*, 2001, 93 – 98.

[11]. M.C. Majorie, Plant products as antimicrobial agents.Clin.Microbiology. Rev. 12(4), 1999, 564-582.

[12]. D. Scrinivasan,L.P.Perumalsamy, S. Nathan,T.Sures, Antimicrobial activity of certain Indian medicinal plants used in Folkloric medicine Z, *J. Ethnopharmacol 94*, 2001, 217 – 222.

[13]. K.A. Hammer, (Arson, C.F and riley, T.V. Antimicrobial activity of essential oils and other plants extracts. *Journal of Applied microbiology 86*, 1999, 985 – 990.

[14]. B. García-Fayos, J.M.Arnal,G.Verdú, I. Rodrigo, purification of a Natural Coagulant Extracted from Moringaolifeira Seeds: Isolation and Characterization of Active Compounds. *International Conference on Food Innovation, 3*. 2010.