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Activities of Stem Barks of *Alchornea Cordifolia* (Schumach. & Thonn.) Müll. Arg. and *Moringa Oleifera* Lam. on some Meningitis - causing Bacteria

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ABSTRACT

This work was aimed at studying the antibacterial activity of *Alchornea cordifolia* and *Moringa oleifera* against four meningitis-causing bacteria namely: *Staphylococcus aureus, Bacillus subtilis*, *Micrococus* spp and *Escherichia coli.* The technique employed was agar well diffusion and spread plate inoculation. The aqueous extract of *A. cordifolia* inhibited all the bacteria tested with a range of zones of inhibition of 7.0mm to 17.5mm. On the other hand, the aqueous extract of the stem bark of *M. oleifera* did not inhibit any of the test bacteria. However, the ethanolic extract inhibited *S. aureus, B. subtilis and Micrococcus* at a range of zone of inhibition of 3.0-9.0mm but *E. coli* was resistant to all concentrations of the extract, while all the test bacteria were resistant to the ethanolic extract of *A. cordifolia*. The minimum inhibitory concentrations of the aqueous extract of *A. cordifolia* ranged from 50 mg/ml to 200 mg/ml for the susceptible bacteria. On the other hand, the minimum inhibitory concentration of *M. oleifera* for the susceptible bacteria was at a range of 100 mg/ml to 200 mg/ml. The gram negative bacteria, *E. coli*, displayed the most resistance for both extracts. In conclusion, the aqueous stem bark extract of *A. cordifolia* and the ethanolic extract of the stem bark of *M. oleifera* can respectively be exploited for use as antibacterial agent against the susceptible bacteria.

Keywords: *Alchornea cordifolia, Moringa oleifera,* stem bark, extract, susceptibility

INTRODUCTION

Meningitis is a swelling of the meninges, the protective membranes which cover the brain and spinal cord. Bacterial meningitis is a serious condition which can be fatal, with death occurring within a few hours. It may also result in permanent disabilities which include hearing loss, persistent difficulties in learning, impaired short term memory (Grimwood *et al.*, 2000) and brain damage. However, the recovery rate from this condition is high. The mode of transmission of bacterial meningitis is from person to person, through contaminated food, coughing, sneezing and kissing. It is worthy of note that most people who spread the infection to susceptible persons are carriers, who never become sick. Those susceptible to infection are babies, the aged, persons with weak immune systems, those who undergo certain surgeries, medical laboratory scientists and travelers (CDC, 2019). Bacterial meningitis is caused by a number of bacteria which include *Streptococcus pneumonia*, Group B *Streptococcus*, *Neisseria meningitides*, *Haemophilus influenza* and *Listeria monocytogenes* (CDC, 2019). Plants have been used as a source of medicine since the time of creation. God made all the plants as food for man and exploitation for medicine. Scarce resources, antibiotic resistance and complications of diseases have led more people, especially those with low income and rural dwellers to the use of herbal remedy. Medicinal plants such as *Alchornea cordifolia, Moringa oleifera,* among others are considered home remedies to cure several illnesses such as diarrhea, constipation, menstrual disorders, fever, asthma and cold etc. However, more scientific research to verify the effectiveness of plants and elucidate their safety profile must be carried out. The plant *Alchornea* c*ordifolia*, Order – *Malpighales* and Family – *Euphorbiaceae*, is commonly called dovewood or Christmas Bush. It is very abundant in African countries like Guinea, Ghana and mostly Nigeria. In the Niger Delta, the Urhobos call it '*Ubobo'* while the Isokos call it '*evba'* (Akpo and Owhe-Ureghe, 2016). *Moringa oleifera Lam.* is a multi-purpose tropical tree of the order *Capparales* and it is the most widely cultivated species of the monogenic family, the *Moringaceae.* The name is derived from the Tamil word ''Murunggai'' (Mangale *et al.*, 2012). The common name is drumstick plant a name that was originated from India (Abalaka, *et al.*, 2012). The antibacterial activities of these plants have been investigated by different workers.

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Mambe and Kuete (2015) studied the antibacterial activities of the methanol extracts of the stem bark of *Alchornea cordifolia* and four other Cameroonian plants against multidrug-resistant (MDR) bacterial infections especially those caused by sensitive and resistant strains of *Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter aerogenes, Escherichia coli* and *Providencia stuartii.* Another study on the phytochemical and antimicrobial activity of the methanol and chloroform extracts of the leaves, stem bark and root of *Alchornea cordifolia* against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Candida albicans* has been reported (Amos –Tautua *et al.,* 2011). *Moringa oleifera* aqueous stem bark extracts have shown varying degrees of antibacterial activity against four bacteria species, *Staphylococcus aureus, Citrobacter freundii, Bacillus megaterium* and *Pseudomonas fluorescens* (Zaffer *et al*., 2014). In another report, the methanolic, n-hexane and aqueous extracts of the seeds and bark of *Moringa ovalifolia* and *Moringa oleifera* were used against *Escherichia coli, Enterococcus faecalis* and *Bacilus cereus. Moringa oleifera* extracts showed more inhibitory activity than that of *M. ovalifolia* (Patel *et al.,* 2010). This study is aimed to determine the effect of aqueous and ethanolic bark extracts of both *A. cordifolia* and *M. oleifera* against three gram positive (*Staphylococcus aureus, Bacillus subtilis* and *Micrococcus)* and one gram negative (*Escherichia coli*) meningitis-causing bacteria.

MATERIALS AND METHODS

Materials

Nutrient agar (Titan Biotech, India), Sabouraud Dextrose Broth, (Guangdong Huankai HKM, China), Mueller Hinton's agar (Titan Biotech, India). Nutrient Broth (L;S BIOTECH). Petri dishes, swab stick, aluminum foil, 5ml and 2ml syringes pack, absolute ethanol (Poole England), bottles, disinfectant (Dettol, Reckitt Benckiser Ltd, Nigeria), spirit lamp, jar cotton wool (Vagen cotton wool, Nabos Global Services, Edo State Nigeria), distilled water obtained from Pharmaceutical Technology Laboratory of the Department of Pharmaceutics, Faculty of Pharmacy, Delta State University, Abraka.

Plant Collection and Preparation

The plant *Moringa oleifera* was obtained from the Staff Quarters in Site II, Delta State University Abraka. *Alchornea cordifolia* on the other hand, was obtained from Otorho, Abraka. Both plants were identified and authenticated at the herbarium of the University. Stems of each plant were harvested in the month of March. The barks were peeled off the stems, washed to remove debris and air-dried. They were ground and stored at room temperature. Thereafter, a total of 100g of each plant material (*Moringa oleifera* and *Alchornea cordifolia* respectively) were weighed, soaked in 500 ml of distilled water and labeled A and B respectively. They were extracted by cold maceration for three days with constant agitation at various intervals. Another 100g of each plant was weighed and soaked in 500ml of 70% ethanol (99%) and labeled respectively A2 and B2. The four decoctions were extracted to obtain 250ml each for aqueous *A. cordifolia* and *Moringa Oliefera* and 200ml each for ethanolic *A. cordifolia* and *Moringa Oliefera* respectively. The extracts obtained were concentrated by heating in a water bath. The aqueous extracts of *A. cordifolia* and *M. oleifera* were diluted serially with sterile water to obtain the following concentrations 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. For each of the ethanolic extracts, 70% ethanol was used for the serial dilution to obtain 200mg/ml, 100mg/ml, 50 mg/ml and 25 mg/ml concentrations.

Bacterial Isolates

The stock culture of the test bacteria *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Micrococcus spp,* were obtained from two laboratories (Pharmaceutical Microbiology laboratory and Department of Microbiology laboratory) in Delta State University, Abraka. The isolates were sub cultured into nutrient agar (Titan Biotech India) prepared according to the manufacturer's instruction and incubated for 24 hours at 37^{o}C. Confirmation was done by performing some simple, specific tests such as Gram staining, fermentation of sugars, catalase and coagulase tests.

Susceptibility testing

Mueller Hinton agar (Titan Biotech, India) was prepared according to manufacturer's instruction, sterilized and aseptically poured into petri dishes to solidify. Wells were made on the agar by means of a sterile cork borer and labeled with the extract concentrations: 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml respectively and two wells in the middle for the positive and negative controls. A suspension of each bacterial isolate was standardized to 3.0×10^5 CFU/ml (compare with McFarland standard) using the dilution methods prescribed by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Thereafter, Mueller Hinton plates were seeded by spread plate method. The various concentrations of the extracts and the drugs were aseptically filled into their respective labelled wells with the aid of a pasture pipette; while the positive

control well was filled with the standard drug (gentamicin 40 mg/ml), the negative control well was filled with distilled water. This preparation was done in triplicates. Culture plates were incubated for 24 hours and at the end of the interval, zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined by serial dilution in solid medium. Mueller Hinton agar was prepared according to the manufacturer's instruction and 19 ml of molten agar was mixed with 1ml of each concentration of plant extract and poured into each petri dish and allowed to solidify. Each petri dish was divided into six parts and with the aid of a sterile glass rod, each organism was streaked on the surface of the agar plate on the part labeled for it. The plates were then incubated for 24 hours at 37°C. The smallest concentration that inhibited the growth of the test organism was taken as MIC.

RESULTS

Table 1: Mean zones of inhibition (mm) of aqueous bark extract of *A. cordifolia* and *M. oleifera*

Concentration / Bacterial Isolates	200mg/ml		100mg/ml		50mg/ml		25mg/ml		Control (20mg/ml) Gentamycin
	AC	*MO*	*AC*	*MO*	*AC*	*MO*	*AC*	*MO*	
S. aureus	16.0	NI	14.0	NI	11.0	NI	NI	NI	24.0
B. subtilis	17.5	NI	12.5	NI	11.0	NI	NI	NI	26.0
Micrococcus	13.5	NI	9.5	NI	7.0	NI	NI	NI	18.5
E. coli	NI	NI	NI	NI	13.0	NI	NI	NI	22.0

No inhibition, *AC: Alchornea cordifolia and MO: Moringa oleifera*

Table 2: Mean zones of inhibition (mm) of ethanolic bark extract of *A. cordifolia* and *M. oleifera*

Concentration / Bacterial Isolates	200mg/ml		100mg/ml		50mg/ml		25mg/ml		Control (20mg/ml)
	AC	*MO*	*AC*	*MO*	*AC*	*MO*	*AC*	*MO*	
S. aureus	NI	7.0	NI	7.0	NI	5.0	NI	4.0	32.0
B. subtilis	NI	5.0	NI	5.0	NI	4.0	NI	3.0	18.0
Micrococcus	NI	9.0	NI	5.0	NI	4.0	NI	NI	21.5
E. coli	NI	NI	NI	NI	NI	NI	NI	NI	24.0

NI: No inhibition, *AC: Alchornea cordifolia and MO: Moringa oleifera*

Table 3: Minimum Inhibitory Concentration (mg/ml)) of the aqueous bark extract of *A. cordifolia* on bacterial isolates

Bacterial isolates	MIC
S. aureus	100mg/ml
B. subtilis	50mg/ml
Micrococcus	200mg/ml

Table 4: Minimum Inhibitory concentration of *M. oleifera* ethanolic bark extract on bacteria isolates

Bacterial isolates	MIC
S. aureus	200mg/ml
B. subtilis	100mg/ml
Micrococcus	200mg/ml

DISCUSSION

The result of the mean zones of inhibition (in milliliters) of the aqueous bark extracts of *A. cordifolia* and *M. oleifera* on *S. aureus, B. subtilis, Micrococcus* and *E. coli* is represented in Table 1. The aqueous stem bark extract of *A. cordifolia* gave clear zones of inhibition (7mm -17.5mm) against all bacterial isolates at concentrations of 50 mg/ml to 200 mg/ml. *B. subtilis* was most susceptible while *Micrococcus* sp was least susceptible. However, *E. coli* was inhibited only at a concentration of 50 mg/ml. None of the bacterial isolates was inhibited by the aqueous bark extract of *M. oleifera.* Table 2 expresses the Mean zones of inhibition of the ethanolic bark extract of *M. oleifera* and *A. cordifolia* on bacterial isolates. The result shows *E. coli* resistance to the ethanolic extracts of both plants. On the other hand, clear zones of inhibition were

observed for the ethanolic bark extract of *M. oleifera* for the three gram positive bacterial isolates (*Micrococcus, B. subtilis, S. aureus*) with *S. aureus* having the highest zone of inhibition of (7.0mm at 200mg/ml) and *B. subtilis* having the lowest zone of inhibition of 3.00mm at 25mg/ml. The mean zones of inhibition of the control antibiotic is very high when compared with that obtained with the extracts. Minimum Inhibitory Concentration (MIC) in mg/ml of the aqueous bark extract of *A. cordifolia* and *M. oleifera* ethanolic bark extract on bacteria isolates are presented in tables 3 and 4 respectively. The MIC of the aqueous bark extract of *A.* cordifolia on the bacterial isolates are 100mg/ml, 50mg/ml and 200mg/ml for *S. aureus, B. subtilis* and *Micrococcus* respectively. The MIC of the ethanolic bark extract of *M. oleifera* on *S. aureus, B. subtilis* and *Micrococcus* are 200 mg/ml, 100 mg/ml and 200 mg/ml respectively. The susceptibility of all the gram positive bacteria to the aqueous stem bark extract of *A. cordifolia* at all concentrations was observed in this study (Table 1). This is similar to the work done by Akpo and Owhe-Ureghe (2013) on the antibacterial activity of the stem pith extract of *A. cordifolia* against four oral, gram positive bacteria, where all the isolates were susceptible. This gives credence to the traditional use of this plant part for the treatment of ailments such as cough, respiratory infections, sore throat, among other diseases. As shown in Table 1, all the bacterial isolates were resistant to the aqueous bark extract of *M. oleifera*. This is similar to the report of Upadhye *et al.* (2012) where all the test bacteria were resistant to the aqueous extract of the plant but susceptible to other solvents. In contrast to these, Juvatkar *et al.* (2002) reported that the aqueous bark extracts of the plant *Moringa oleifera*, screened against ten bacterial strains including *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* for *in vitro* qualitative evaluation, showed pronounced antibacterial activity at concentration range between 50 µg and 300 µg/0.1ml. This variation in test reports for the same solvent may be due to varying environments as well as the season the test was performed. The resistance of *E. coli* to the ethanolic extracts of *M. oleifera* and *A. cordifolia* is observed in this research (Table 2). On the contrary, Ngoupayo *et al*. (2015) reported the best inhibitory activities for *Escherichia coli* among other bacteria, at 3.125 mg/mL MIC with methanolic stem extracts of *A. cordifolia*. On the other hand, clear zones of inhibition were observed for the ethanolic bark extract of *M. oleifera* for the three gram positive bacterial isolates (*Micrococcus, B. subtilis, S. aureus*). A result of the methanolic extracts of *A. cordifolia* against *S. aureus, E. coli, B. subtilis* among others, with zones of inhibition from 16.0 – 20.0mm has been reported (Amos-Tautua *et al.*, 2011). Similarly, susceptibility has been previously reported by Yao and Moellering (1995) on the *in vitro* analysis of *M. oleifera* bark extract using ethanol against the pathogenic gram positive bacteria, *B. subtilis* and *S. aureus*. In this study, the positive control gentamicin gave larger zones of inhibition against all the bacteria tested, than the plant extracts. It shows that the plant extracts were not very potent *in vitro* against the bacteria tested. This do not rule out their potency *in vivo* since other factors may be present in man and animals that may promote their potency. The minimum inhibitory concentration (MIC) of the aqueous bark extract of *A. cordifolia* on the gram positive bacterial isolates are 100mg/ml, 50mg/ml and 200mg/ml for *S. aureus, B. subtilis* and *Micrococcus* respectively (Table 3). The MIC of the ethanolic bark extract of *M. oleifera* on *S. aureus, B. subtilis* and *Micrococcus* are 200 mg/ml, 100 mg/ml and 200 mg/ml respectively (Table 4). Ngoupayo *et al*. (2015) have reported a 3.125mg/ml MIC of the methanolic stem extract of *A. cordifolia* for *E. coli*.

CONCLUSION

This study has shown that the aqueous extract of the barks of *A. cordifolia* is effective against all the bacterial isolates tested. The study also show that the ethanolic bark extract of *M. oleifera* is active against the gram positive bacteria while the gram negative E. coli, was resistant. Though the extracts were not as effective against the tested isolates as the control antibiotic gentamicin, they constitute potential antibacterial activity that can be explored as remedy for infections caused by *S. aureus, B. subtilis and Micrococcus.*

Recommendation

It is recommended that further work on these plants are done to enhance their antibacterial activity with focus on the effect of the barks of these plants against *E. coli* and other gram negative bacteria implicated in meningitis.

The *in vivo* activity of these extracts may be carried out in animals and humans to ascertain their potency against infections such as meningitis.

The active compounds of the stem barks of these plants may be studied in detail for the development of new drugs in the future.

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