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In vitro Evaluation of Spectrum of Antimicrobial Activities of Leaves and Stem Bark Extracts of *Alchornea cordifolia* against Some Pathogenic Clinical Isolates from University of Uyo Medical Centre

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAA designed the study. Author MHE wrote the protocol and wrote the first draft of the manuscript authors CNN and IFU managed the analyses of the study. Author MAM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Antimicrobial activities of the leaves and stem bark in aqueouse and ethanol extracts of *Alchornea cordifolia* were evaluated using tube dilution assay to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the plant parts and to determine spectrum of activities considering the antimicrobial effect on Gram positive, Gram negative and yeast (*Candida albicans*), these

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organisms are known to be pathogenic in nature. Results showed leaves extracts, both aqueouse and ethanol exerted higher activities than the stem bark extracts with MIC values ranging from 1.95 mg/mL – 15.63 mg/mL, although the stem bark extract exhibited good inhibitory activities as well with MIC values ranging from 3.91 mg/mL – 62.50 mg/mL. The antimicrobial spectrum of activities against the organisms was determined by the ratio (R) of MBC/MIC. The leaf extract is bactericidal against the Gram-positive and Gram negative organism with the exception of *E. coli* that MBC was not determined and, fungicidal against *Candida albicans* with (R) MBC/MIC ≤ 2. The stem bark extracts were bactericidal against all the Gram positive organisms but bacteriostatic against *Salmonella spp* and fungistatic against *Candida albicans* with (R) MBC/MIC ≥ 2. This study revealed that the aqueous and ethanol extracts of leaf and stem bark of *A. cordifolia* plant exhibited strong antibacterial activities on *Staphylococcus aureus, Streptococcus pneumoniae, Bacillus Subtilis*, (Gram positive), *Escherichia coli, Pseudomonas aeruginosa*, *Salmonella spp* (Gram negative) and also exhibit anti-fungal activity against the *Candida albicans*. The leaf extract exhibited broad antimicrobial spectrum with bactericidal activities against Gram positive, Gram negative and yeast (*Candida albicans*).

Keywords: Alchornea cordifolia; aquoues; ehanol extracts; bacteriostatic; bactericidal; fungicidal activities.

1. INTRODUCTION

Infections due to pathogenic bacteria and fungi pose a critical problem to human health. Diseases caused by microorganisms are on the increase worldwide. Infectious diseases are among the most common causes of death [1]. The use of medicinal plants for traditional medicine in developed societies has been recognized as the basis for the chemical analysis and development of different forms of drugs [2].

Among many of such medicinal plants is *Alchornea cordifolia* belonging to the family of *Euphorbiaceae*. The common names of the plant are Christmas bush and Dovewood. In Nigeria, it is called *"ububo"* in Igbo, *"ipaesinyin"* in Yoruba, *"bambani"* in Hausa and *"mbom"* in Ibibio. This medium-sized, strangling shrub or small evergreen tree, either grows erect or half climbing, up to 8 m high and is widely distributed in Tropical Africa. It is found in secondary forests and riparian areas, mainly in moist or marshy areas and it is propagated through seeds or stem cuttings [3].

A. cordifolia is commonly used as a medicinal plant throughout its area of distribution. The plant has been locally used in ethnomedicine for the treatment of a variety of ailments, including inflammatory disease states and disorders associated with microbial infection, without detailed scientific basis. The effectiveness of *A. cordifolia* has also been highlighted through its traditional use in the treatment of convulsions, prostatitis, leprosy, jaundice, conjunctivitis, pain and nervous troubles, hormonal-related gynecological disorders, infertility, urinary, respiratory and intestinal problems as well as malaria like fevers [4,5].

The leaves stem bark, roots and fruits of *Alchornia cordifolia* contain terpenoids, steroid glycosides, flavonoids, phenolic acids, fatty acids, tannins, saponins, carbohydrates and the imidazo-pyrimidine alkaloids alchorneine, alchornidine, and several guanidine alkaloids. The plant parts also contain a range of hydroxybenzoic acids: gallic acid and its ethyl ester, gentisic acid, anthranilic acid, protocatechuic acid, and ellagic acid (alizarine yellow). A C20 homolog of vernolic acid named alchornoic acid can be found in the seed oil [3,6].

Several pharmacological investigations have been carried out to validate some of the claimed ethnomedicinal uses of the plant. It has been reported to exhibit various pharmacological activities such as antibacterial, antifungal, antiplasmodial, anti-inflammatory, hepatoprotective, anticancer, antioxidant, wound healing, anti-diarrhoeal, antinociceptive, antidepressant, immunomodulatory, anxiolytic, antidiabetic and antispasmodic activities among others [7]. The anti-HIV potentials of the plant have been documented [8,9].

A number of constituents responsible for the observed activities have also been mentioned in the literature review [2] reported that the parts of the plant mostly used for medicine are the leaves and stem bark but the leaves exhibit more potency. However, no comprehensive study has been carried out to confirm this. This research

study is therefore geared towards validating or repudiating this claim. The antimicrobial activity of the leaves and stem bark extracts of *A. cordifolia* was evaluated and compared.

2. MATERIALS AND METHODOLOGY

2.1 Plant Collection and Aunthentication

Fresh leaves and stem barks of *Alchornea cordifolia* (Schum. & Thonn) Müll. Arg. used for the study were collected from a farmlandwithin Itak community inIkono Local Government Area of AkwaIbom State, Nigeria in the month of June 2018. The plant was identified and authenticated by Mr. O. U. Etefia, a naturalist of the Pharmacognosy Department, Faculty of Pharmacy, University of Uyo.

2.2 Samples Preparation and Plant Extarction

Four samples of the plant extracts were prepared. Aqueous leaf and stem bark extarcts and ethanol leaf and stem bark extracts of *Alchornea cordifolia* were used for the study.

2.3 Extraction

The650g of the powdered leaves and 650 g of the powdered stem barks of *Alchorne acordifolia* were weighed and macerated separately in 1000 mL of distilled water and 70 % ethanol in a 1000ml conical flask at room temperature for 24 hours for aqueouse extract and 72 hours for ethanol extract with intermittent stirring. The samples were filtered separately for three times eachon sterile cotton wool and filter paper and the filtrates obtained were concentrated to dryness using a water bath at 40°C for about three days [10].The extracts obtained were 59.90 g and 15.50 g for the aqueouse leaves and stem barks, while 74.10 g and 25.50 g for the ethanol leaves and stem barks respectively. The concentrated extracts were transferred to beakers sealed with aluminium foils, labelled appropriately and stored in the refrigerator pending analysis.

2.4 Isolation, Identification and Inoculum Standardization

All the test organisms were clinical isolates of human pathogens obtained from the Medical Centre Laboratory of University of Uyo, Uyo. The culture media used in this study was Mueller Hinton agar. The streak plate technique was used for isolating into pure cultures of the organisms. Loopfuls of the samples were inoculated by streaking on sterile Mueller Hinton agar plates for the bacteria and Sabourauddestrose agar containing 0.4% chloramphenicol was used for the *Candida albicans* isolate, maintained by weekly sub culturing on SDA slant, before each experiment and stored at 37°C for 24 hours. The resulting visible colonies of pure cultures of bacteria isolates were subjected to simple but specific tests for identification and confirmation which included colony and cell morphology, biochemical tests and Gram staining.

The microbial isolates selected for this investigation were all pathogenic clinical isolates; three Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pneumonia, and Bacillus subtilis*), three, Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*) and onefungal isolate(*Candida albicans*). This is to evaluate the spectrum of activities across pathogenic bacteria and fungal isolates. The suspensions were adjusted to a turbidity of 10^6 colony forming unit CFU/ml which is equal to 0.5 Mcfarlandstandard using visual comparison.

2.5 Susceptibility Test

The agar well diffusion method modified for its suitability [11] was used for the bacteria susceptibility test. The media used was prepared according to manufacturer's instructions and asceptically poured into sterile Petri dishes and allowed to solidify. An overnight culture of each of the test organisms adjusted to a turbidity of 10^6 using the the 0.5 Mcfarland standard was introduced into each dish. A 4 mm sterile cork borer was used to bore holes equidistant from each other on the plates. Using a sterile pipette, different concentrations of the extract were introduced into the wells. 1 mg/mL of ampicloxand miconazol nitrate was introduced into the wells as control measures. The plates were allowed to stand for one hour before incubation to allow for the diffusion of the agent into the media. They were then incubated for 24 hours at 37^oC. The diameter of the zones of inhibition was then measured to the nearest millimeter confirming the susceptibility of the extracts.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the plant extracts was determined using the broth dilution method, as described in [12] with some modifications. Stock solutions of the plant extracts were prepared at a concentration of 250 mg/mL by reconstituting 2.50 g of each plant extract in 10 ml of sterile water. The mixture was filtered through a filter paper to obtain a stock solution free of insoluble particles from the extracts. Two-fold serial dilution of each stock solution was carried out aseptically using five sets of ten tubes each, containing 2 ml of sterile nutrient broth labelled appropriately for the respective test organisms. 2 ml of the respective stock solutions was transferred using a sterile pipette into Tube 1 for each set of tubes and the tube was agitated thoroughly to ensure proper mixing. This step was repeated for Tube 2 by transferring 2ml from Tube 1 into Tube 2. This was repeated consecutively for the remaining tubes until Tube 10 from which 2ml was discarded. The resulting concentrations from the dilution procedure for Tube 1 to Tube 10 were: 125.00 mg/mL, 62.50 mg/mL, 31.25 mg/mL, 15.63 mg/mL, 7.81 mg/mL, 3.91 mg/mL, 1.95 mg/mL, 0.98 mg/mL, 0.49 mg/mL and 0.24 mg/mL respectively.

Each tube was aseptically inoculated with a loopful of the respective microbial suspensions and the tubes were incubated at 37°C for 24 hours after which they were observed visually for the presence or absence of turbidity as an indication of the presence or absence of growth respectively. The lowest concentration of each of the plant extracts obtained from the first tube which inhibited the growth of the microorganisms after 24 hours of incubation was reported as the MIC of the plant extracts against the various test organisms.

2.7 Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of the plant was derived from the MIC tubes which showed no growth. A loopful aliquot from each tube was aseptically streaked on sterile antibacterial free Muller Hilton agar plates and SDA for *Candida albicans* which were labelled appropriately with corresponding concentrations. The plates were further incubated at 37°C for 24 hours after which they were examined for the presence or absence of growth against the respective concentrations. The plate with the least concentration which killed the organisms (or allowed less than 0.1% of the original inoculums to survive) after 24 hours of incubation was taken as the MBC of the plant extracts for the various test organisms.

3. RESULTS

3.1 Antimicrobial Activities of *Alchornea cordifolia* Extracts

The antimicrobial activities of respective concentrations of each extract against the selected test organisms are recorded in Tables 1 –6. *A. cordifolia* extracts showed broad spectrum antibacterial activities against *Staphylococcus aureus, Streptococcus pneumonia, B. Subtilis Escherichia coli, Pseudomonas aeruginosa and Salmonelatyphi*. Sterling antifungal activities of the extracts were also observed against *Candida albicans*.

Table 1. Summary of minimum inhibitory concentrations of the leaves and stem barks extracts of *A. cordifolia* against the selected test organisms

Test organism	Plant extract/MIC (mg/mL)			
	ALE	ELE	ASE	ESE
Staphylococcus aureus	3.91	1.95	7.81	3.91
Streptococcus pneumoniae	1.95	3.91	7.81	3.91
Bacillus subtilis	7.81	3.91	15.63	7.81
Escherichia coli	7.81	3.91	15.63	15.63
Pseudomonas aeruginosa	15.63	15.63	62.50	15.63
Salmonela spp.	15.63	7.81	7.81	15.63
Candida albicans	3.91	1.95	7.81	3.91

Keys: ALE = Aqueouse leaves extract of Alchornea cordifolia; ELE = Ethanol leaves extract of Alchornea cordifolia; ASE = Aqueouse stem barks extract of Alchornea cordifolia; ESE = Ethanol stem barks extract of Alchornea cordifolia

Table 2. Summary of minimum bactericidal concentrations of the leaves and stem barks extracts of *A. cordifolia* against test organisms

Test Organism	Plant extract/MBC (mg/mL)			
	ALE	ELE	ASE	ESE
Staphylococcus aureus	7.81	3.91	31.25	7.81
Streptococcus pneumoniae	3.91	7.81	15.63	7.81
Bacillus subtilis	15.63	7.81	15.63	15.63
Escherichia coli	-	-	-	-
Pseudomonas aeruginosa	62.50	31.25	125.00	62.50
Salmonela spp.	31.25	15.63	62.50	125.00
Candida albicans	15.63	31.25	62.50	-

Keys: ALE = Aqueous leaves extract of Alchornea cordifolia; ELE = Ethanol leaves extract of Alchornea cordifolia; ASE = Aqueous stem barks extract of Alchornea cordifolia; ESE = Ethanol stem barks extract of Alchornea cordifolia

Table 3. Antimicrobial activity of aqueouse leaves extract of *A. cordifolia* against selected test organisms

Test Organism	ALE MIC (mg/mL)	ALE MBC (mg/mL)	R = MBC/MIC	Inference
Staphylococcus aureus	3.91	7.81	2.00	Bactericidal
Streptococcus pneumoniae	1.95	3.91	2.00	Bactericidal
Bacillus subtilis	7.81	15.63	1.99	Bactericidal
Escherichia coli	7.81	-	-	ND
Pseudomonas aeruginosa	15.63	62.50	4.00	Bactericidal
Salmonelaspp	15.63	31.25	2.00	Bactericidal
Candida albicans	3.91	15.63	3.99	Fungicidal

Key: R = Ratio; ND = Not Determined

Table 4. Antimicrobial activity of ethanol leaves extract of *A. cordifolia* against selected test organisms

Test Organism	ELE MIC (mg/mL)	ELE MBC (mg/mL)	R = MBC/MIC	Inference
Staphylococcus aureus	1.95	3.91	2.00	Bactericidal
Streptococcus pneumoniae	3.91	7.81	2.00	Bactericidal
Bacillus subtilis	3.91	7.81	2.00	Bactericidal
Escherichia coli	3.91	-	-	ND
Pseudomonas aeruginosa	15.63	31.25	2.00	Bactericidal
Salmonela spp.	7.81	15.63	1.99	Bactericidal
Candida albicans	1.95	31.25	16.00	Fungistatic

Table 5. Antimicrobial activity of aqueous stem bark extract of *A. cordifolia* against selected test organisms

Test Organism	ASE MIC (mg/mL)	ASE MBC (mg/mL)	R = MBC/MIC	Inference
Staphylococcus aureus	7.81	31.25	3.99	Bactericidal
Streptococcus pneumoniae	7.81	15.63	1.99	Bactericidal
Bacillus subtilis	15.63	15.63	1.00	Bactericidal
Escherichia coli	15.63	-	-	ND
Pseudomonas aeruginosa	62.50	125.00	2.00	Bactericidal
Salmonela spp.	7.81	62.50	7.99	Bacteriostatic
Candida albicans	7.81	62.50	7.99	Fungistatic

Key: R = Ratio; ND = Not Determined

Table 6. Antimicrobial activity of ethanol stem barks extract of *A. cordifolia* against selected test organisms

Test organism	ESE MIC (mg/mL)	ESE MBC (mg/mL)	R = MBC/MIC	Inference
Staphylococcus aureus	3.91	7.81	2.00	Bacteriocidal
Streptococcus pneumoniae	3.91	7.81	2.00	Bacteriocidal
Bacillus subtilis	7.81	15.63	1.99	Bacteriocidal
Escherichia coli	15.63	-	-	ND
Pseudomonas aeruginosa	15.63	62.50	4.00	Bacteriocidal
Salmonela spp.	15.63	125.00	8.00	Bacteriostatic
Candida albicans	3.91	-	-	ND

Key: R = Ratio; ND = Not Determined

3.2 Spectrum of Antimicrobial Activities of *A. cordifolia* Extracts

Antimicrobial substances are considered as bactericidal agent when the ratio MBC/MIC ≤4 and bacteriostatic when the ratio MBC/MIC is> 4 [1].

4. DISCUSSION

The objective of this study is to determine the antimicrobial activities of *A. cordifolia* extracts against different microbiological strains including yeast. The values of the MICs and MBCs obtained from this study showed that the degree of activity varied with the organisms and the extracts. The antimicrobial activities of aqueous leaf extract (ALE) on the Gram positive organisms (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus subtilis*) were bacteriocidal with the ratio(R) MBC/MIC≤ 2, also bactericidal for two of the Gram negative organisms (*Pseudomonas aeruginosa*, *Salmonela spp*), but the antimicrobial property for *Escherichia coli* was not determined since there was no MBC for the clinical strain isolated for this study and the yeast (*Candida albicans*), the activity was fungicidal withratio (R) MBC/MIC≤ 2 as shown in (Table 3). The effect of ethanol leaf extract (ELE) was similar to that of ALE on Gram positive and Gram negative organisms but fungistatic on *Candida albicans* as the (R) MBC/MIC ≥ 2 as observed in (Table 4). The antimicrobial activities of the plant stem bark seems to reduce as demonstrated in this study, the aqueous stem bark extract (ASE) on the microbial strains showed that the extract was also bacteriocidal for all Gram positive organisms and one Gram negative (*Pseudomonas aeruginosa*) and bacteriostatic for *Salmonela spp* and fungi static to *C. albicans*, with the activity of *E. coli* undetermined (Table 5). The effect of ethanol stem bark extract (ESE) showed the least activity on the microbial strains, though remained same on Gram positive organisms, and Gram negative organisms as in ASE but the antimicrobial properties for *E. coli* and *C. albicans*were not determined (Table 6) because the MBC for the two organisms isolated for this study were not determined. The result obtained from the aqueous leaf extract is in line with the work of Ebenyi, et al. [2] who assessed the antibiotic activities of the aqueous and ethylacetate extracts of the leaves of *A. cordifolia* against *S. aureus*, *S. pneumoniae*, *E. coli*, *P. aeruginosa* and *Klebsiella pneumoniae* through antimicrobial susceptibility testing, MIC and killing rate studies. In their study, the result ofthe effect of aqueous extract on bacteria killing rateshowed that the extract is bactericidal to *S. aureus*, *S. pneumoniae*, *P. aeruginosa* and *K. pneumoniae* but bacteriostatic to *E. coli*. These findings suggest that all extracts used in this survey may be classified as bactericidal agents against Gram positive organisms and some Gram negative organisms, while only the aqueous leaf extract may be classified as a fungicidal agent with the rest of the extracts being fungistatic.

Alchornea cordifolia has been widely reported to possess abroad spectrum of antimicrobial activities [13]. This was confirmed as the microorganisms used in this study were found to be susceptible to the extracts of *A. cordifolia* leaves and stem bark, suggesting that the antimicrobial principle contained in these plant parts may be of broad spectrum since they were able to inhibit both Gram-positive and Gram-negative bacteria as well as fungi, [14] also made similar observations. This validates its use as an antimicrobial agent in the treatment of numerous diseases and conditions caused by microbial infections. It was earlier mentioned in the literature review that the antimicrobial activity can be attributed to the presence of phytochemicals

present in the plant such as phenols and flavonoid compounds [15,16] which have been employed as a disinfectant and remain the benchmark for comparing other bactericides. Alkaloids, steroids and terpenes also play a major role in antimicrobial activity [17].

The results from this study validate the claim by the aforementioned research group. The MIC and MBC study comparing leaf and stem bark extracts of *A. cordifolia* showed that the extracts had activity against *S. aureus*, *S. pneumoniae*, *E. coli*, *P. aeruginosa* and *Candida albicans* but they exhibited varying range of activities with one being more active than others. The MIC and MBC of the leaf extracts recorded were significantly lower than that of the stem bark extracts, indicating greater activities of the leaf extracts compared to the stem bark extracts. However, this study is inconclusive of which leaves extract is more promising in terms of antimicrobial activity although a study by Gasting et al. [12] reported that the aqueous leaf extract exhibited the highest antibacterial activity in comparison to methanol, ethanol, ethyl acetate and acetone extracts of *A. cordifolia* leaves stating that the active principle could be very polar in nature. The ethanol stem bark extract exhibited relatively good antimicrobial activities comparable to the aqueous leaf extract. The aqueous stem bark extract had the weakest antimicrobial activity of all the extracts although it exhibited good activity against the test organisms. This variation in the activity of the extracts could be due to the difference in solubility of the active ingredient in each solvent.

In addition to the difference in solubility of the active ingredient in each solvent and the constitutional or structural variability of the tested organisms, the variation in the degree of activity of the germs and the extracts could also be due to the capacity of the microorganisms to modify the structure of the active principle [18]. Also, it could be that the abundance of the phytochemicals present in the leaves which are implicated in antimicrobial activity work synergistically to produce increased antimicrobial activity in the leaves than in the stem bark.

5. CONCLUSION

The antimicrobial activity of the leaves and stem barks extracts of *Alchornea cordifolia* have been evaluated against some Gram-positive bacteria, Gram-negative bacteria and yeast (*Candida albicans*), all pathogenic clinical isolates from vaginal swab, faecal excrete and abscess from patients of University of Uyo medical centre.

These results confirmed the leaves and stem barks extracts of *A. cordifolia* to have potential to improve human health through its antimicrobial activities. This was confirmed as the microorganisms used in this study were found to be susceptible to the extracts of *A. cordifolia* leaves and stem barks, suggesting that the antimicrobial principle contained in the leaves of the plant part may be of broad spectrum, since they were shown to have bacteriostatic and bactericidal effects against both Gram-positive and Gram-negative bacteria as well as fungi. This validates its use as an antimicrobial agent in the treatment of numerous diseases and conditions caused by microbial infections.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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