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Research Article

Antibacterial activity of *Alchornea cordifolia* leaves found in Ebonyi state, Nigeria.

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ABSTRACT

This research work was carried out to investigate the antibacterial effect of aqueous and ethylacetate leaf extracts of *Alchornea cordifolia* plant using inhibitory zones of inhibition and killing rate studies. Antimicrobial activities of the extracts were performed against selected bacterial strains. Results show that different leaf extracts exhibited different levels of activities which were dependent on the nature of the test organisms. Gram-positive bacteria showed more susceptibility to the leaf extracts than the Gram-negative bacteria. The water extract and the ethylacetate extract exerted highest activity against *S. aureus* while good inhibitory activities were exerted by *Pseudomonas aeruginosa* and *Klebsiella* on the ethylacetate extract. This study reveals that aqueous and ethylacetate leaf extracts of *Alchornea cordifolia* plant exhibited strong inhibitory activities; the effect was bacteriostatic on some organisms and bacteriocidal on others. These differences in the susceptibilities of the isolates to the plant extracts can be related to the cell wall composition of the organisms. Aqueous and ethylacetate leaf extracts of *A. cordifolia* have potential to improve human health through its antimicrobial activities. Therefore, development of alternative antibacterial treatment drugs from medicinal plants are urgently needed for the treatment of infectious diseases from different sources.

Keywords: *Alchornea cordifolia,* antibacterial activities, aqueous and ethylacetate leaf extracts.

INRODUCTION

Medicinal plants are plants that have been used in human disease treatment for ages because they contain compounds that possess therapeutic values. Recently, due to pathogens' resistance against the available antibiotics and the recognition of traditional medicine as an alternative form of health care has reopened the research domain for the biological activities of medicinal plants[5]. The increased material worth of medical treatment and their strong physiological or chemical effects, contribute to the reason why individuals make use of herbal therapy[13]. The increasing demand of plant extracts used in the

cosmetic, food and pharmaceutical industries suggests that systematic studies of medicinal plants are very important in order to find active compounds and their use as a medicine for curing various diseases. In developing countries, it has been observed that the use of herbal remedy is a common practice to maintain good health[13]. In addition, the use of traditional medicine in developed societies have been recognized as the basics for the chemical analysis and development of different forms of drugs; and even those used for chemotherapy from medicinal plants that are traditionally used as herbal remedies[10].

Alchornea cordifolia belongs to the family of Euphorbiaceae. The common names are Christmas bush and Dovewood. In Nigeria, it is called "ububo" in Igbo; "ipaesinyin" in Yoruba and "banbani" in Hausa. The plant is a strangling shrub or small evergreen plant that can grow up to 32 feet tall in swampy locations. It is propagated through seed or stem cuttings and grows well in very moist soil. The leaves and stems are used traditionally as a therapeutic agent in many countries in Africa as remedies for venereal diseases, treatment of acute and chronic inflammatory disorders, cancer, ulcers, canker sores, to prevent miscarriage and cure various reproductive diseases[6]. The stem bark is tinctured with local gin for its aphrodisiac effect. It is also used as a local remedy for cold, in treatment of rheumatism, arthritis and muscle pains[5]. It is used as an antidote for poison, as a sedative and antispasmodic. The parts mostly used for medicine are the leaves and stem bark but the leaf is more potent.

Diseases caused by microorganisms are on the increase worldwide. A chemical substance derived from a mold or bacterium that can kill microorganisms and cure bacterial infections (antibiotic drug) are used for the treatment of bacterial infections. In recent years, many antibiotic drugs have lost the ability to generate strong chemical or physiological effects because of the development of bacterial strains that are resistant, mostly through the expressions of resistance genes[9]. In addition, antibiotic drugs are often linked with undesirable effects such as hypersensitivity, immune–suppression and allergic reactions[7]. Therefore, development of alternative anti-bacteria treatment drugs from medicinal plants are urgently needed for the treatment of infectious diseases from different sources. *Alchornea cordifolia* has been locally used for treatment of many ailments without detailed scientific bases. This study was therefore designed to investigate the antibacterial activities of *Alchornea cordifolia* leaves through MIC and killing rate studies.

MATERIALS AND METHODS

Bacteriology Media

The following media were used in this study; MacConkey agar, Mueller-Hinton agar (MHA), Nutrient agar and Tryptone soy broth.

Test Organisms Used

Characterized bacterial isolates (*Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia and Klebsiella pneumoniae*) were collected from Medical Microbiology Laboratory, Ebonyi State University, Abakaliki, Ebonyi State. The isolates were preserved in slants for further use.

Plant Materials

The leaves of *Alchornea Cordifolia* were used for this study.

Collection of Plant Materials

Alchornea cordifolia leaves were collected from Ndiagu-Ogba in Ohaukwu Local Government Area of Ebonyi State. The leaves were identified and authenticated by a plant Taxonomist at Enugu state University of Science and Technology, Enugu (ESUT).

Extraction of Leaves of *Alchornea cordifolia*

Fresh leaves of *Alchornea cordifolia* were washed and dried under ambient temperature before they were ground into fine powder using manual grinder and stored in an air tight container.

Preparation of deionized water Extract

The homogenized sample (250 g) was soaked in 500 ml of deionised water for 48 hours. The solution was filtered using a muslin cloth. The aqueous filtrate was evaporated to dryness using a rotary evaporator.

Preparation of Ethylacetate Extract

The homogenized sample (250 g) was soaked in 500 ml of ethylacetate for 48 hours. The solution was filtered using a muslin cloth. The ethylacetate filtrate was evaporated to dryness using a rotary evaporator and stored in air-tight container.

Antimicrobial Screening

Inoculation of Mueller-Hinton (MH) agar plate

A sterile swab stick was dipped into standardized broth, rotated against the sides of the tube (above fluid level) to remove excess fluid. The surface of a MH agar plates were then inoculated by streaking

the swab three times over the entire surface; rotating the plate appropriately 60˚ each time to ensure an even distribution of the inoculums[12]. The inoculated plates were then allowed to sit for 3-5 minutes before perforating.

Antimicrobial Susceptibility Testing

Agar well diffusion method was used as described by Adeniyi *et al.*, 1996 to determine the antibacterial activity of the extracts. A sterile cork borer was used to bore equidistant cups into Mueller-Hinton agar plate. Dilution with 5 % DMSO gave the concentration of the extract. About 0.1ml of the extract was added to fill the bored holes, after which the plate were incubated a 37 ˚C for 18 hours.

Minimum Inhibitory Concentration (MIC)

The MIC is defined as the lowest concentration that completely incubated the growth of the microorganism for 24 hours. The MIC of the extract was done using the agar well diffusion technique. The different concentrations (100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml) was prepared by pipetting 1 ml of DMSO into the extract, after which 1 ml of the extract was poured into1 ml of water to prepare a serial dilution. The plates were then incubated at 37 ^{0}C for 24 hours and the lowest concentration of the extracts showing clear zone incubation was considered as the MIC.

Measurement of Zones of Inhibition

For all agents, the zone edge was read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye. The un-supplemented plates from were read from the back with reflected light while the plates were held above a dark background. The supplemented plates were read from the front with the lid removed and with reflected light. The diameters of zones of inhibition were measured to the nearest millimeter with a ruler and an automated zone reader[2].

Killing Rate Studies

The test organisms were grown in 5 ml of nutrient broth inside an incubator at 37 °C for 24 hours in order to achieve inoculum standard. Thereafter, the inocula were then sub-cultured in 3 ml of fresh nutrient broth and incubated at 37 °C for 1 hour to activate the organism. Sample (0.1 ml) were collected from all the test tubes and inoculated on dried nutrient agar plate for the estimation of the viable bacteria count. Samples were taken from test tubes at different time intervals of 0 mins, 10 mins, 20 mins, 40 mins, 60 mins, 90 mins, 24 hours and were incubated at 37 °C. The viable bacterial colonies were counted and results obtained were compared with the controls. The viable count on the control tube that contains only the test organism (bacteria) without extract were estimated and served as a control[2].

RESULTS AND DISCUSSION

The antibacterial activity of properties of *A. cordifolia* has been extensively studied. The result of the effect of aqueous extract on bacteria killing rate showed that the extract is bacteriocidal to *Klebsiella, Staphylococcus, Pseudomonas* and *Streptococcus* but bacteriostatic to *E. coli* (Table 1, Table 2, and Figure 1). The effect of ethylacetate extract on bacteria killing rate showed that the extract is bacteriostatic to *E. coli* and *Klebsiella* but bacteriocidal to *Pseudomonas* and *Staphylococcus* (Table 1, Table 2, and Figure 2). The significant zone of inhibition caused by the extracts showed that they posses antimicrobial activity. As observed, all bacteria except *Klebsiella* exhibited high rates of inhibition at all concentrations. The antimicrobial activity might be as a result of phytochemicals present in the plant. All the extracts (aqueous and ethylacetate) were active against *E. coli* and other Gram-negative bacteria which causes travellers' diarrhoea, urinary tract infection and dysentery in men. Various extracts of medicinal plants have been reported to show antimicrobial activities[12]. According to Uwumarongie *et al.* (2007), this can be attributed to the presence of phenols and flavonoid compounds which have been employed as a disinfectant and remain the bench mark for comparing other bactericides[14]. The MIC study comparing aqueous and ethylacetate leaf extracts of *Alchornea cordifolia* showed that the extracts had activity against *Staphylococcus, Streptococcus, Klebsiella, E. coli* and *Pseudomonas* while the killing rate studies showed that the extracts had both bactericidal and bacteriostatic effects. Our study is in line with the work of Donatien *et al.* (2010) who reported that aqueous, methanol, ethanol and acetone extracts of *A. cordifolia* leaves were active against the microorganisms used, with inhibition diameters varying from 13 mm to 26 mm[8]. In their study, aqueous extract exhibited the highest antibacterial activity. Donatien *et al.* (2010) also reported that Ethyl acetate leaf extract of *A. cordifolia* was active against *E. coli* (13 mm), *P. aeruginosa* (13 mm) and *S. aureus* (14 mm) only[8]. Our work is also in concord with the work of Adeleye *et al.* (2008) who reported that both aqueous and ethanolic extract of *A. cordifolia* showed activity against *H. pylori* and the other bacteria screened[1]. They observed that the aqueous extract of *A. cordifolia* was both bacteriostatic and bacteriocidal to *H. pylori* at concentrations of 150 and 300 mg/ml

respectively whereas other extracts showed similar effects at higher concentration[1]. Similar trend was also observed for all other bacteria screened. Earlier, Okeke *et al.* (1999) had shown that the leaf extract of *A. cordifolia* was very active against 74 bacterial strains studied *in vitro*[11]. Ajali (2000) also made similar observations[4]. This current finding is in line with the earlier reports and our study. Our study is in agreement with the work of Adesina *et al*. (2012) who reported that the ethyl acetate and aqueous fractions of methanol extract of *Alchornea cordifolia* showed antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria; although, the ethyl acetate fraction was more active against Gram-positive bacteria[3]. The microorganisms used in this study were found to be susceptible to at least one extract of *A. cordifolia* leaves, suggesting that the antimicrobial principle contained in the leaves of this plant may be of broad spectrum since it was able to inhibit both Gram-positive and Gram-negative bacteria. These observations corroborate those of Okeke *et al*. (1999) confirming the use of this plant in the treatment of bacterial infections. *Alchornea cordifolia* has been widely reported to possess a broad spectrum of antimicrobial activities[12]. Therefore, the development of alternative antimicrobial drugs from medicinal plants is of urgent necessity for the treatment of infectious diseases.

Table 1
Zones of inhibition of bacterial isolates on the extracts

Microorganism	Aqueous extract Average zone of inhibition (mm)	Ethylacetate extract Average zone of inhibition (mm)
Staphylococcus	18	17
Streptococcus	19	18
Klebsiella	0	0
E. coli	19	17
Pseudomonas	18	18

Table 2
Minimum inhibitory concentration (MIC) of the bacterial isolates on the extracts

Microorganism	Ethylacetate extract MIC (mm)	Aqueous extract (mm)
Staphylococcus	50	50
Streptococcus	50	25
Klebsiella	50	50
E. coli	50	50
Pseudomonas	50	25

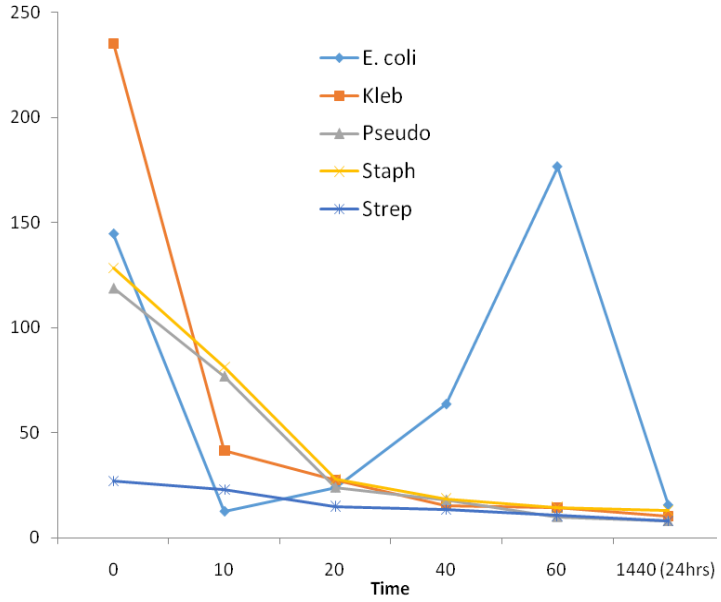

Fig. 1
Killing rate kinetics of aqueous extract of A.cordifolia on selected bacterial species

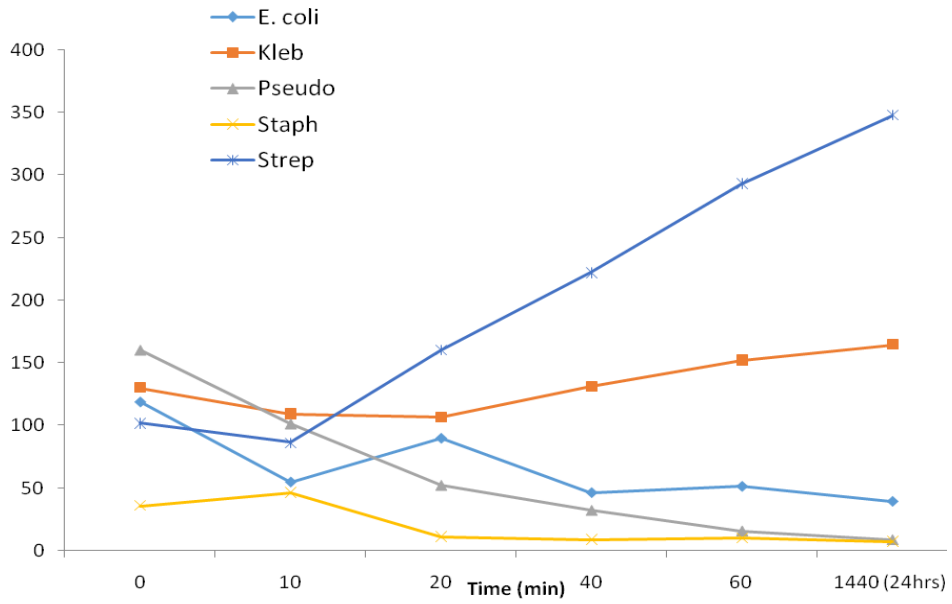

Fig. 2
Killing rate kinetics of ethylacetate extract of A.cordifolia on selected bacterial species

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