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Phytochemical and Antibacterial Study of Ethanol and Ethyl acetate Extracts from Leaves of *Alchornea Cordifolia* Against Isolates from Infected Wounds

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Abstract

Alchornea cordifolia leaves have been reported to be used traditionally to treat wound infections. The leaves of *Alchornea cordifolia* were extracted with ethanol which was partitioned with ethyl acetate to obtain the ethanol extract and ethyl acetate fraction respectively. The extracts were screened for basic phytocompounds and subjected to antibacterial assay against bacterial isolates from infected wounds which include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella spp*, *Enterobacter spp*, *Citrobacter spp*, *Escherichia coli*, *Proteus mirabilis* and *Proteus vulgaris*. Agar well diffusion method was used to determine the antibacterial activity of the extract and fraction. Phytochemical investigation revealed the presence of carbohydrates, cardiac glycosides, saponins, flavonoids, tannins and alkaloids. The zone of inhibition ranged between 1.0 - 32.2mm for the entire microorganisms. This variation in level of the activity among the extracts could be due to the difference in solubility of the active ingredient in each solvent on one hand and to the constitutional or structural variability of the tested organisms on the other hand. The results of the study showed that the ethyl acetate fraction of *Alchornea cordifolia* leaves possess high level of antibacterial activity against bacteria associated with wound infection. This gives credence to traditional medicine application of the plant for treating wound infections.

Keywords: *Alchornea cordifolia*, Ethyl acetate fraction, wound infection, antibacterial

1. Introduction

Despite advances that have been made scientifically in wound management, wound infection has been considered as the most common nosocomial infection [1]. The significance of wound infections, in both economic and human terms, should not be under-rated [2]. Organisms frequently isolated from infected wounds include Gram positive cocci such as *Staphylococcus aureus, Streptococcus spp,* Gram negative bacilli mostly Acinetobacter, Enterobacter, *Escherichia coli, Proteus spp, Pseudomonas aeruginosa* and anaerobic bacteria such as *Propionibacterium spp* [3] and *Klebsiella spp* [4].

Alchornea cordifolia (Schumach. & Thonn) Müll. Arg belongs to the Family Euphorbiaceae. The plant is geographically distributed in secondary forests usually near water, moist or marshy places. It is frequently used as a medicinal plant around its area of distribution. The leaves are mostly used, but also the stem bark, stem pith, leafy stems, root bark, roots and fruits are used in local medicine. The plant is used for treating infected wounds, diarrhorea, gonorrhea, urinary tract infections, conjunctivitis, fever and malaria [5-8].

Alchornea cordifolia leaf extracts have been reported to inhibit the growth of bacteria such as *S. aureus, Klebsiella pneumoniae, E. coli, P. aeruginosa* and *S. albus* [9-12]. The aim of the study was to carry out phytochemical screening and evaluate the antibacterial activities of the crude ethanol extract and Ethyl acetate fraction of the leaves of *Alchornea cordifolia*

2. Materials and Methods

2.1 Sample Collection

Alchornea cordifolia leaves were collected from Idu village, Abuja and identified at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The sample of the plant has been deposited in the herbarium for reference purpose with Voucher No. 401.

2.2 Extraction of plant material

The leaves of *Alchornea cordifolia* were air dried under shade and then reduced to coarse powder using wooden mortar and pestle. About 500 g of the coarsely powdered leaves was extracted with 1500 ml of 70 % ethanol by cold maceration and left for 24 h. The extract was filtered and evaporated to dryness on a hot water bath at 100 ºC. The extract was kept in suitable amber-coloured containers until ready for use.

One hundred and eighty (180) grams of Ethanolic extract was suspended in 1000 ml of distilled water. The filtrate was partitioned in separating funnel with 750 ml of Ethyl Acetate. The Ethyl Acetate fraction was concentrated on a water bath.

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2.3 Phytochemical Screening

The extracts were screened for the presence of phytocompounds using standard qualitative methods [13].

2.4 Collection of Bacteria

Samples were collected from Aminu Kano Teaching Hospital, Kano in North West Nigeria. It is the largest Tertiary Health Institution in Kano State. It has a bed space of four hundred and twenty-two. Ethical approval was obtained from the Medical Advisory Committee of the Teaching Hospital. A total of 150 wound swabs collected from different wards of the hospital and submitted at the general culture bench of the Microbiology department were used.

2.5 Identification of Bacterial Pathogens and Culturing

Gram staining was done according to standard techniques [14]. The specimens were inoculated on Blood and MacConkey Agar plates. The plates were incubated aerobically at 37 ^{o}C for 24 – 48 h. Pure colonies were isolated on to nutrient agar slants. The nutrient agar slants were incubated at 37°C for another 18-24 h before storage in the refrigerator for biochemical analysis. Biochemical tests such as catalase, coagulase, oxidase, Voges Proskauer, Hydrogen sulphide production, urease, Methyl red, Indole, Citrate and sugar utilization tests were carried out according to standard techniques [14].

2.6 Antibacterial Assay of Crude Extracts and Fractions of Alchornea cordifolia Leaf

Agar well diffusion method was used to determine the antibacterial activity of the extract and fraction. Molten sterile nutrient agar (20 ml) was poured into sterile petri dish and allowed to set. The sterile nutrient agar plates were flooded with 1.0 ml of the standardized inoculums (equivalent to 10^5 - 10^6 CFU/ml) and the excess was drained off. A sterile cork borer (No. 4) was used to bore equidistant cups into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the test agent will not sip beneath the agar. Exactly 0.1 ml of the different concentrations (1.0 – 6.25 mg/ml) of the extract/fraction was added to fill the bored holes. Negative control was prepared by putting 0.2 ml of pure solvent in one bored hole. One h pre-diffusion time was allowed, after which the plates were incubated at 37 °C for 18 h. The zones of inhibition were then measured in millimeter. The above method was carried out in duplicates and the mean of the duplicate results was taken [15].

2.7 Data Analysis:

The diameter of zones of inhibition for antibacterial assays were expressed as mean ± SEM. Simple percentage was used to analyze the distribution of bacterial isolates.

3. Results and Discussion

Figure 3.1 shows the percentage distribution of bacteria isolated from wound infection sites. The most frequently isolated organism was *S. aureus* (22 %), followed by *P. aeruginosa* (19.9 %).

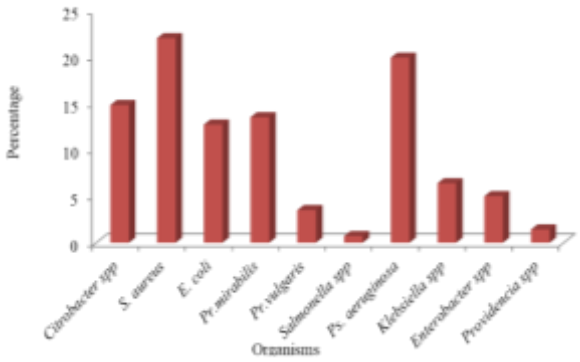

Figure 3.1: Percentage Distribution of Bacteria Isolates from Wound Infection Sites

The phytochemical screening revealed the presence of several chemical constituents in the extract/fraction (Figure 3.1).

Furthermore, the diameters of zones of inhibition exerted by 50 mg/ml concentration of the various extracts against 90 % of the isolates were presented in Table 3.2. The Ethyl acetate fraction exerted the highest inhibition against the various organisms with values ranging from 17.8 ± 0.8 mm for *Enterobacter spp* to 32.2 ± 2.8 mm for *S. aureus*. Also, the extract had good inhibitory activity against *S. aureus* and *P. aeruginosa*; little inhibitory activity against *Citrobacter spp* and *Proteus spp*.

S. aureus and *P. aeruginosa* have been reported to be the most frequently isolated organisms in wound infections [2,4]. *S. aureus* is commonly associated with skin bacteria and easily contaminates wounds. *P. aeruginosa* tends to be endemic in hospital environment by being easily transferred from object to object and it also tend to be resistant to common antiseptics, making it difficult to eradicate in the long term.

All over the world, pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents especially for microbial effects [16]. As clearly demonstrated in this study, the extracts possess antibacterial properties but to varying degrees. Several reports have shown the secondary metabolites to possess inhibitory properties against different organisms. Saponins, flavonoids and tannins are well known to possess antimicrobial activities [17]. The higher efficacy of the ethyl acetate fraction might be attributed to the higher proportion of some of these secondary metabolites in this extract. Similar activity was also reported [11].

Table 3.1: The Phytochemical Screening of the *Alchornea Cordifolia* Leaf Extracts/Fractions.

Test	Ethanol Extract	Water Extract	Ethyl acetate Fraction	n-Butanol Fraction
1.Carbohydrate				
a. Molisch's test	+	+	+	+
b. Fehling's test	+	+	+	+
2.Glycosides				
a. Fehling's solution	+	+	+	+
b. Ferric Chloride	+	+	+	+
3.Anthraquinones				
a. Bontrager's test	+	-	-	-
4. Cardiac glycosides				
a. Kella-killiani	+	+	+	+
b. Salkowski	+	+	+	+
5. Saponins				
a. Frothing test	+	+	+	+
b. Lieberman-Burchard test	Triterpenes Present	Triterpenes Present	Triterpenes Present	Triterpenes Present
6. Flavonoids				
a. Shinoda	+	+	+	+
b. Sodium hydroxide	+	+	+	+
7. Tannins				
a. Lead sub-acetate	+	+	+	+
b. Dragendoff's test	+	+	+	+
c. Wagner's test	+	+	+	+
d. Picric acid test	+	+	+	+

Table 3.2: Mean Diameter of Zones of Inhibition of Extracts/Fractions at 50 mg/ml against 90% of the isolates (IZD_{90}).

Organism	Ethanol Extract	Ethyl Acetate Fraction
S. aureus (n=12)	24.4±4.5	32.2±2.8
Ps. aeruginosa (n=10)	19.0±2.2	27.7±1.7
Klebsiella spp (n=5)	0	21.5±2.0
Enterobacter spp (n=5)	0	17.8±0.8
Citrobacter spp(n=8)	0	18.6±2.4
Pr. mirabilis (n=7)	6.5±6.5	24±2.0
Pr. vulgaris (n=3)	6.5±6.5	23.0±3.0
E. coli (n=16)	1.0±1.9	24.6±1.9

The study demonstrated that different extracts exhibited varying levels of activities which were also dependent on the nature of the test organisms. Generally, Gram positive bacteria showed more susceptibility to the extracts and fractions than the Gram-negative bacteria. The ethyl acetate fraction exerted highest activity against *S. aureus* isolates at a concentration of 50 mg/ml. similar level of activity was reported [12]. While good inhibitory activities were exerted against *P. aeruginosa* especially by the ethyl acetate fraction, the Enterobacteriaceae isolates were more resistant to the activity of the ethanol extract. The differences in the sensitivity of the isolates to the plant extracts can be attributed to the cell wall composition of the organisms. Gram positive bacteria have a cell wall of peptidoglycan with teichoic acid in between. Therefore, they are more sensitive than Gram negative bacteria which have their cell wall surrounded by lipopolysaccharides and lipoproteins, which prevent penetration of antibiotics through their cell wall.

This variation in level of the activity among the extracts could be due to the varying levels of solubility of the active ingredient in each solvent on one hand and to the constitutional or structural variability of the tested organisms on the other hand.

4. Conclusions

Bacteria isolated from wound infections in this study which were mostly *S. aureus*, enteric bacteria and *P. aeruginosa* are consistent with reports of similar studies conducted globally and in various parts of the country. Extracts of *A. cordifolia*, particularly ethyl acetate fraction possess useful antibacterial activity that can be used in the therapy and management of infections of various wound sites. This study, therefore, justifies and

authenticates the use of extract of *A. cordifolia* by herbalists in the treatment of wound infections. It is recommended that long term toxicity studies be carried out on the leaf extracts of *A. cordifolia* and the Ethyl acetate fraction formulated into appropriate dosage form (cream and lotion) for use in treating wound infections.

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