



Evaluation of the Antibacterial Activity of Methanol and Chloroform Extracts of *Alchornea cordifolia* Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEL conceptualized and designed the work, author BOU. Wrote the first draft of the manuscript, authors PCU and MNI performed the experiment, did data acquisition and interpretation of result, author ANO revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Case Study

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ABSTRACT

Aims: To evaluate the antibacterial activity of the Methanol and Chloroform extracts of the leaves of *Alchornea cordifolia* and verify the claim that it is used to treat bacterial infections.

Study Design: This is an experimental study involving the extraction of the bioactive agents from *Alchornea cordifolia* leaf using methanol and chloroform, susceptibility tests (Zones of inhibition) and determination of the Minimum Inhibitory Concentration.

Place and Duration of Study: Study was carried out in Faculty of Pharmaceutical Sciences of Nnamdi Azikiwe University, Agulu Campus between March and June 2012.

Methodology: The leaves of the plant were harvested, dried at room temperature for two weeks and then pulverized. The leaves were divided into two parts of 200mg each. One part was soaked in 500 ml methanol and the other part in 500 ml chloroform (all for 72 hours) to extract the active principles. The extracts were exposed to air to allow the

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solvents to evaporate completely. The antibacterial activities of the residue against four pathogenic microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) were evaluated using agar diffusion and broth dilution techniques in accordance with Clinical Laboratory Standards Institute.

Results: The result showed that the methanol extract has antibacterial activity against the pathogens tested at concentrations 50 mg/ml and above while the chloroform extracts had activity against the micro organisms tested at 200 mg/ml concentration only.

Conclusion: The methanol extract of *Alchornea cordifolia* leaves has a more potent antibacterial activity than the chloroform extract. The leaves of *Alchornea cordifolia* can actually be used in the treatment of infection caused by these organisms aforementioned.

Keywords: Antibacterial activity; *Alchornea cordifolia*; medicinal plant; traditional medicine.

1. INTRODUCTION

Alchornea cordifolia (Schum & Thonn) Muell Arg. is a member of a plant family called the Euphorbiaceae. It is widely distributed in West and central African countries with the common names: Christmas bush, Christmas tree, dovewood (in English), Arbre de djeman (in French) and Bugi-bugi, bunce, pô d'arco in Portuguese languages [1]. The plant has also been found in other parts of the world including Antarctica, Asia, Australia, Europe, Oceania and North, Central and South Americas. *Alchornea cordifolia* has been extensively studied and shown to have some activity, *in vitro*, against seventy four bacterial strains [2,3,4 5]. The antibacterial activities of most medicinal plants depend on certain active principles such as alkaloids, saponnins, tannins, flavonoids and various oils [6]. In south-east Nigeria, the root epidermis is a good bitter while the leaf is used to treat eyes conditions. The leaves or leafy stems have sedative and antispasmodic activities and are used to treat a variety of respiratory problems such as sore throat, cough and bronchitis, to treat genital-urinary problems such as venereal diseases and female sterility, and intestinal problems such as gastric ulcers, diarrhoea, amoebic dysentery and worms [1]. The use of herbs in the treatment of man and animal disease has been practiced before the advent of modern antibiotics [7, 8]. For a plant to be described as a medicinal plant, the species of the plant from which the drugs are obtained, the chemical nature of the extracts from the plants and the purpose for which the plant species is used must be known [9]. In Nigeria, there appears to be an overwhelming increase in the public awareness and usage of herbal medical products in the treatments and/or prevention of diseases [10]. Some parts of plants contain substances that kill or inhibit the growth of some bacteria and so are said to have antibacterial activity [8]. The oil of *Melaleuca alternifolia* (tea tree) was shown to have good antibacterial activity against *Staphylococcus aureus* and *Moraxella catarrhalis* and has been used in the treatment of bronchitis and sinusitis [11]. In Nigeria, there is virtually no documentation of the isolation, selection, and preparation of medicinal plants in the earliest times. Information about them was passed from generation to generation through oral tradition [12]. Iwu [13,14], reported that over 207 herbal remedies from 188 plants genera and Edeoga et al. [15] about 10 medicinal plants from different families have been implicated in Ibo tribal ethno medicine. Suck [16], reported the use of more than 75 pure compounds derived from higher plants as herbal medicines. Aliyu et al. [17] reported the activity of some plant extracts against methicillin-resistance *S. aureus*.

The present study aims at evaluating the antibacterial activity of the leaves of *Alchornea cordifolia*.

2. EXPERIMENTAL DETAILS

2.1 Materials

2.1.1 Collection and identification of plant material

Alchornea cordifolia leaves was collected from a village farm at Agbor, Delta State of Nigeria and subsequently identified by Ozioko A. O., a taxonomist in the department of botany, University of Nigeria, Nsukka. The voucher (#: 035) specimen was deposited in the Herbarium of the department of botany for reference. The leaves were air-dried at room temperature ($26 \pm 2^\circ\text{C}$) for 2 weeks.

2.1.2 Extraction of plant material

Approximately 400g of pulverized leaves were made and divided into two equal parts. One part was macerated in 500ml of methanol and the other part in chloroform using the cold maceration method as previously described by Esimone and Adikwu [18]. Briefly, the leaves were soaked separately in 500ml of chloroform and 70% methanol and macerated for 72 hours. The extracted materials were filtered and the filtrates exposed to air until the solvents evaporated to dryness. The residue recovered after drying was collected, weighed and kept in a container for further use.

2.1.3 Chemicals

The chemicals used are products of BDH, England. The chloroform and methanol used have 99% and 95% purity respectively.

2.1.4 Test organism

The test organisms used were clinical isolates identified and kept in the Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences of Nnamdi Azikiwe University Agulu Campus in Anambra State, Nigeria. The organisms include: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Stock cultures were sub-cultured in nutrient broth at 37°C for 24 hours before use.

2.2 Methods

2.2.1 Determination of antibacterial activity

The sensitivity of the isolates to the methanol and chloroform extracts was evaluated using a modified cup plate agar diffusion method as described by the Clinical and Laboratory Standard Institutes [19]. A 0.5 McFarland Standard of the organisms was swabbed on to sterile Mueller Hinton agar plates and incubated for 3 hours. Holes were then made with 6 mm diameter sterile cork borer in the agar plates to a depth of 3.5 mm and filled with the extracts dissolved in DMSO. Diameters of zones of inhibition were measured with a meter rule after incubating plates at 37°C for 24 hours. Inhibition Zone Diameter less than or equal to 12 mm were reported as resistant. The solvent dimethylsulphoxide was used as negative control as it was the solvent used to dissolve the extracts and has no standard or established antibacterial property. Gentamicin 3.125 $\mu\text{g/mL}$ (a preliminary work done - unpublished) was used as positive control.

2.2.2 Determination of minimum inhibitory concentration (M.I.C.)

The MIC of the methanol extract of *Alchornea cordifolia* leaves against the test organisms were evaluated using broth dilution method as described by Esimone and Adikwu [18]. Briefly, ten different sterile Mueller Hinton broth dilutions representing a concentration range of 0 – 200 mg/ml of the extract were made. Each dilution was made in triplicate (a – c). Then, a 0.1ml of 0.5 McFarland standards of the test organisms were diluted to 100 folds by suspending in a 99.9 ml of physiological saline. A 0.1 ml of the bacteria suspension was added to the tubes containing the different concentrations of the extract. The tubes were labeled appropriately and then incubated at 37°C for 18-24 hours and examined for activity. The lowest concentration of the extract in each replicate tube that shows no visible growth of the test organism after the 18 -24 hours of incubation was taken as the MIC of the extract in the tube for that particular organism. Then, the MIC of the extract for each organism is taken as the mean MIC of the replicates.

3. RESULTS AND DISCUSSION

Table 1 shows the antibacterial activities of methanol extract of *Alchornea cordifolia* leaves. The extract was most active against *E. coli* and least active against *P. aeruginosa*. The extract was active up to the concentration of 50 mg/ml except against *P. aeruginosa*, where the activity was reduced a little.

Table 2 shows the antibacterial activities of chloroform extract of *Alchornea cordifolia* leaves. The extract was weakly active. Only the highest concentration tested (200 mg/ml) showed activity against all the organisms tested. The 50 mg/ml concentration was active only against *E. coli*. The extract is therefore not considered effective and so further test on MIC determination was not done.

Tables 3a and 3b show the minimum inhibitory concentration (MIC) of methanol leave extract of *Alchornea cordifolia*. Methanol extract of *A. cordifolia* was found to be active against *E. coli*, *S. aureus*, *S. typhi* and *P. aeruginosa* at concentrations ranging from > 12mg/mL to >100mg/mL. The MIC of the methanol extract is 10.4 ± 3.6 for *S. aureus*, 12.5 ± 0 for *S. typhi*, 14.6 ± 9.5 for *E. coli* and 116.7 ± 28.9 (mg/mL) for *P. aeruginosa*. Generally, the methanol extract was more active than the chloroform extract on all the pathogens used. The susceptibility of *S. aureus* and *P. aeruginosa* to the extracts of *Alchornea cordifolia* leaves is of great importance because the infections caused by these bacteria are known to be difficult to control. The low effect of the chloroform extracts maybe due to the quantity and quality of the bioactive agents extracted by the solvent. The DMSO used did not show any activity against the bacteria isolates tested.

Table 1: Antibacterial activities of methanol extract of *Alchornea cordifolia*

Concentration (mg/mL)	Inhibition zone diameter (IZD) (mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>
200	24.0	21.5	23.0	18.0
100	22.0	19.0	22.5	13.0
50	20.5	14.5	16.0	11.5
25	17.5	11.5	7.5	8.0
Gentamicin	20.0	17.5	16.0	21.0
DMSO	0	0	0	0

Note ≤ 12.0 = Resistant.

Table 2: Antibacterial activities of chloroform extract of *Alchornea cordifolia*

Concentration (mg/mL)	Inhibition zone diameter (IZD) (mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>
200	19.0	15.5	14.5	12.0
100	16.5	12.5	11.5	8.5
50	13.5	10.0	9.5	6.5
25	10.0	2.0	5.0	5.5
Gentamicin	20.0	17.5	16.0	21.0
DMSO	0	0	0	0

Note ≤ 12.0 = Resistant.**Table 3a: Minimum inhibitory concentration (MIC) of methanol extract of *Alchornea cordifolia***

S/N	Concentration (mg/mL)	Organisms' growth											
		<i>E. coli</i>			<i>S. aureus</i>			<i>S. typhi</i>			<i>P. aeruginosa</i>		
		a	b	c	a	b	c	a	b	c	a	b	c
1	200.00	+	+	+	+	+	+	+	+	+	+	+	+
2	150.00	+	+	+	+	+	+	+	+	+	+	+	-
3	100.00	+	+	+	+	+	+	+	+	+	-	-	-
4	50.00	+	+	+	+	+	+	+	+	+	-	-	-
5	25.00	+	-	+	+	+	+	+	+	+	-	-	-
6	12.50	-	-	+	+	-	-	-	-	-	-	-	-
7	6.25	-	-	-	-	-	-	-	-	-	-	-	-
8	3.125	-	-	-	-	-	-	-	-	-	-	-	-
9	1.565	-	-	-	-	-	-	-	-	-	-	-	-
10	0	-	-	-	-	-	-	-	-	-	-	-	-

*Note: + means no microbial growth seen while – means there was microbial growth.

Table 3b: Minimum inhibitory concentration (MIC) of methanol extract of *Alchornea cordifolia*

Organisms	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>
MIC (mg/ml)	> 12	< 12.5	12.5	> 100
MIC $\pm$ SD	14.6 $\pm$ 9.5	10.4 $\pm$ 3.6	12.5 $\pm$ 0	116.7 $\pm$ 28.9

The microorganisms used in this study were found to be susceptible at least to one extract of *Alchornea cordifolia* leaves, suggesting that the antibacterial principle contained in the leaves of this plant may be of broad spectrum as it was able to inhibit both Gram-positive and Gram-negative bacteria. This report agrees with the findings of Okeke et al (8) and Amos-Tautua et al (20).

Earlier work carried out on the phytochemical screening of the methanol and chloroform extracts of *Alchornea cordifolia* leaves showed the presence of the secondary metabolite: tannins, alkaloids, anthraquinones, saponins, flavonoids, phenols, sterols, cardiac glycosides and phlobatannins [20,21], tannins, phenolic acids, gallic acid, ellagic acid, protocatechic acid [22], flavonoids: quercetin, hyperin and guaijaverin [23,11] and an alkaloid: triisopentenylguanidine [24]. The antibacterial properties of plants have been linked

to the presence of these secondary metabolites [25, 26, 27, 28 and 29]. This can explain the broad spectrum of bacteria activity shown by the methanol extract against Gram positive and Gram negative bacteria.

Staphylococcus aureus has been reported by many workers to have developed resistance to most antibiotics and *P. aeruginosa* is an opportunistic organism which has been reported to really receive resistance carrying plasmid from other bacteria species [30]. The antibacterial activity against *E. coli* is also noteworthy. *E. coli* is known as a major cause of infantile diarrhoea in developing countries and of travelers' diarrhoea in visitors to these countries [31].

The MICs of the extract against these pathogenic organisms *E. coli*, *S. aureus*, *S. typhi* and *P. aeruginosa* were found to be 5.1 – 24.1 mg/ml, 3.6 – 14.0 mg/ml, 12.5 and 28.9 – 145.6mg/ml respectively in our study. This give the MIC range of the extract for the organisms tested [32]. This figure correlates with previous investigation in Nigeria [21].

4. CONCLUSION

Alchornea cordifolia methanol leaves extract possess broad spectrum of antibacterial activity against the pathogenic test organisms. The result obtained from this work gives high hopes for the development of new antibacterial agents from the methanol extract of *Alchornea cordifolia* leaves.

CONSENT

It was not sought for because it is not needed.

ETHICAL APPROVAL

It was not sought for because it is not needed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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