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Phytochemical and Antibacterial Studies of the Hexane Extract of *Alchornea cordifolia* Leaf

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1. Introduction

Alchornea cordifolia (Schum. & Thonn.) Muel. Arg. (Euphorbiaceae) is also known as Agyama in Ghana, Susu bolonta in Sierra Leone, Casamance bugong in Senegal, Tschiya in Togo, Bondji in Cameroon, Ewe ipa, Ubobo and Bambami in Nigeria. It is geographically distributed in secondary forest usually near water, moist or marshy places and it grows to a considerable height but is always of a shrubby or scrambling habit.

The plant leaf extracts have been reportedly used in various African countries such as Senegal in the treatment of venereal diseases, conjunctivitis, dermatoses, stomach ulcers, bronchitis, cough, toothache (Le Grand and Wondergem, 1987; Le Grand, 1989). In Zaire it was used in the treatment of urinary tract infections, infected wounds, diarrhoea, cough, dental caries, chest pain and anaemia (Kambu *et al.*, 1990; Muanza *et al.*, 1994). In Sierra Leone it was used for diarrhoea and piles (Dalziel, 1956; Macfoy and Sama, 1990) and in Nigeria for gonorrhoea, yaws, rheumatic pain and cough (Gbile and Adeshina, 1986; Ogungbamila and Samuelson, 1990).

A variety of plants or materials derived from plants are been used for the prevention and treatment of diseases virtually in all cultures. The potential of herbal medicines and medicinal plant research results in health care is no longer in doubt, having gained recognition in several nations of the world and the World Health Organisation (WHO).

Secondary metabolites which constitute important source of the pharmaceutical preparations have been reportedly isolated from different parts of plants. Some of these compounds have been reported to be present in *A. cordifolia* such as flavonoids (Ogungbamila and Samuelson, 1990), alkaloids and tannins (GHP, 1992), inulin and alchornine (Abdullahi *et al.*, 2003).

This work tends to investigate the phytochemical components and antibacterial activities of hexane extract.

2. Materials and methods

2.1 Collection, identification and preparation of plant leaf

Alchornea cordifolia leaves were collected in October from Abuja, Nigeria. They were authenticated in the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where a voucher with specimen number 4334 was kept for future reference. The leaves were air-dried at room temperature and then reduced to powder using mortar and pestle.

2.2 Preparation of the extract and its derived fractions

Using the Soxhlet extractor, 300 gm of the powdered leaves was extracted with 450 ml of hexane at room temperature until all the extractable components were exhausted. The extract was concentrated, dried, weighed and kept in a dessicator until needed.

Hexane extract was analysed for chemical composition using the bioassay-guided fractionation by employing the Accelerated Gradient Chromatography (AGC) technique. Silical gel G (E-Merck, Germany) was used as an absorbent. Gradient elution was effected using hexane and ethyl acetate sequentially with increasing polarity. A total of 77 fractions were collected. The thin layer chromatography (TLC) analyses of the fractions were carried out using Whatman TLC plates of size 10 × 20 cm precoated with K5 silical gel 150A (Whatman Limited Maidstone, England). The chromatograms were developed using solvent mixture specific for separating alkaloid compounds especially hexane and ethyl acetate, 3:1. After development, the chromatograms were dried and detection was made using ultra-violet light at both wavelength 254 nm and 365 nm. Similar fractions were pooled together giving 33 fractions.

2.3 Phytochemical screening of the hexane extract

The extract was subjected to phytochemical analysis to detect the presence of the chemical constituents using standard protocol (Trease and Evans 1996).

2.4 Extraction of the secondary metabolites

Extraction of the secondary metabolites present in the hexane extract was also carried out using standard methods of Marcek (1972).

3. Antimicrobial activity

3.1 Purification of organisms

The organisms: *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 12600 and *Escherichia coli* ATCC 11775 were collected from the Department of Pharmaceutical Microbiology, University of Benin, Benin City, Nigeria. While the clinical isolates *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus sp.* were from the

Staff Clinic, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The organisms were confirmed by sub-culturing into Nutrient broth and incubated at 37°C for 18 hours.

They were further streaked on the Nutrient agar and incubated at 37°C for 18 hours. Biochemical tests were used to confirm the organisms. The organisms were kept on agar slants at 4°C until needed.

3.2 Preparation of inoculums

Eighteen-hour broth culture of the test organism was suspended into sterile nutrient broth. It was standardized according to National Committee for Clinical Laboratory Standards (NCCLS, 2002) by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/ml.

3.3 Susceptibility testing

The washed overnight broth cultures were diluted appropriately using sterile normal saline to 0.5 McFarland scales (0.5 McFarland is about 10^6 cfu/ml). The molten sterile nutrient agar (20 ml) was poured into sterile petri dish and allowed to set. The sterile nutrient agar plate was flooded with 1.0 ml of the standardized test organism and the excess was drained off and dried at 30°C for 1 hr. 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 extract will not sip beneath the agar. 0.1ml of the different concentrations (0.625 - 20.0 mg/ml) of the extract was added to fill the bored holes. Negative control was prepared by putting 0.1 ml of pure solvent in one of bored hole and aqueous solution of 2 µg of Gentamicin (for Gram positive bacteria) and 4 µg of Gentamicin (for Gram negative bacteria) (Sweetman, 2005) in another bored hole which served as positive control. One hour 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 triplicates and the mean of the triplicate results was taken.

3.4 Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal Concentration (M. B. C.)

The M.I.C. was determined by agar dilution method. Ten millilitre (10 ml) volume of double strength melted Mueller-Hinton agar at 45°C was diluted with equal volume of the test extract in graded concentrations of 0.625 - 20.0 mg/ml. These were poured aseptically into sterile Petri dishes and dried at 37°C for 1 h with the lid slightly raised. The solidified leaf extract-agar admixture plates were inoculated with 2.0 µl of standardized 18 h culture test organism. The inocula were allowed to diffuse into the test agar plates for 30 min. The test agar plates were then incubated at 37°C for 18 h. The M.I.C. value was taken as the least concentration of the extract showing no detectable growth.

The M. B. C. was carried out by inoculating the concentration of the extract in the test agar plates showing no visible growth into sterile nutrient broth test-tubes containing inactivating agents 3% v/v Tween 80. These test-tubes were then incubated at 37°C for 24 h after which they were examined for presence or absence of growth.

3.5 Preliminary antimicrobial activity test of the various fractions from the hexane extract

Exactly 5.0 ml of 20.0 mg/ml of the fraction was incorporated into 5.0 ml molten double strength sterile nutrient agar kept at 45°C and poured into sterile Petri dishes and allowed to set. The test organism was streaked on the poured plate and incubated at 37°C for 24 hours after which the activity/no activity was observed.

4. Results

The hexane extract (HE) of *Alchornea cordifolia* leaves was brown in colour. There was a yield of 40.22% of the extract.

The phytochemical screening of the hexane extract of *A. cordifolia* leaf revealed the presence of tannins, alkaloids, flavonoids and phenol with tannins having the highest percentage yield of 6.8 (Table 1).

Secondary Metabolites	Yield (%)
Tannins	6.8
Alkaloids	5.9
Flavonoids	4.2
Phenol	3.2

Table 1. Percentage yield of the secondary metabolites from the hexane extract of *A. cordifolia* leaf.

The susceptibility of the bacteria species to the secondary metabolites of the plant showed that test *Staphylococcus aureus* was more susceptible to the secondary metabolites than the other bacteria species (Table 2). The zones of inhibition observed from tannins and saponin were larger than those from the other metabolites (Table 2).

Test bacteria species	Zones of inhibition (mm)			
	Tannin	Saponin	Alkaloids	Phenols
<i>Ps.aeruginosa</i>	24± 0.1	20± 0.2	16± 0.0	14± 0.0
<i>Ps.aeruginosa</i> ATCC 10145	21± 0.1	19± 0.1	18± 0.1	15 ± 0.1
<i>Staph. aureus</i>	26± 0.3	21 ± 0.1	20± 0.0	17± 0.2
<i>Staph. aureus</i> ATCC 12600	22 ± 0.1	18± 0.2	21± 0.1	16± 0.0
<i>E.coli</i>	19± 0.2	16± 0.2	16± 0.0	13± 0.1
<i>E.coli</i> ATCC 11775	15± 0.0	14 ± 0.2	12± 0.1	11± 0.3
<i>Proteus sp.</i>	20± 0.0	22± 0.3	17 ± 0.2	12 ± 0.1

Table 2. Susceptibility of the bacterial species to the secondary metabolites of the hexane extract of *A. cordifolia* leaf.

The clinical isolate of *Staphylococcus aureus* was more susceptible to the hexane extract of the plant leaf than the other bacterial species (Table 3).

The lowest Minimum Inhibitory Concentration of the hexane extract was found to be 2.5mg/ml against *Staph. aureus* and *Staph. aureus* ATCC 12600 (Table 4).

Test bacteria	Zones of Inhibition (mm)					
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	GTM
<i>Ps.aeruginosa</i>	13± 0.2	11± 0.1	NI	NI	NI	31 ± 0.0
<i>Ps.aeruginosa</i> ATCC 10145	11± 0.0	NI	NI	NI	NI	32 ± 0.2
<i>Staph. aureus</i>	23± 0.0	19± 0.1	18± 0.2	14± 0.2	NI	23± 0.0
<i>Staph. aureus</i> ATCC 12600	22± 0.2	17± 0.0	15± 0.1	12± 0.2	NI	25 ±0.1
<i>E.coli</i>	11± 0.2	NI	NI	NI	NI	20±0.2
<i>E.coli</i> ATCC 11775	16± 0.1	14± 0.3	11± 0.1	NI	NI	22±0.0
<i>Proteus sp.</i>	15± 0.2	13± 0.0	11± 0.1	NI	NI	21±0.0

The results are expressed as mean ± standard deviation, GTM = Gentamicin, NI = No Inhibition.

Table 3. Susceptibility of the bacterial species to different concentrations of the hexane extract of *A. cordifolia* leaf.

Test Bacteria	MIC (mg/ml)	MBC (mg/ml)
<i>Ps. aeruginosa</i>	20	NA
<i>Ps.aeruginosa</i> ATCC 10145	NA	NA
<i>Staph. aureus</i>	2.5	5
<i>Staph. aureus</i> ATCC 12600	2.5	5
<i>E .coli</i>	20	NA
<i>E. coli</i> ATCC 11775	10	20
<i>Proteus sp.</i>	10	20

Key: NA - No Activity

Table 4. M. I. C. and M. B. C. of the hexane extract against the test bacteria species.

Fractions	<i>Ps. aeruginosa</i>	<i>Ps. aeruginosa</i> ATCC 10145	<i>Staph. aureus</i>	<i>Staph. aureus</i> ATCC 12600	<i>E. coli</i>	<i>E. coli</i> ATCC 11775	<i>Proteus sp.</i>
HEF ₁₋₁₇	-	-	-	-	-	-	-
HEF ₁₈	-	-	IN	-	-	IN	IN
HEF ₁₉	-	-	IN	-	-	IN	IN
HEF ₂₀	-	-	IN	IN	-	IN	IN
HEF ₂₁	+	+	+	+	-	-	-
HEF ₂₂	-	-	IN	-	-	-	-
HEF ₂₃	-	-	IN	-	-	-	-
HEF ₂₄	-	-	IN	-	-	-	-
HEF ₂₅	-	-	IN	-	-	-	-
HEF ₂₆	IN	IN	IN	IN	-	-	-
HEF ₂₇	+	+	+	+	IN	IN	IN
HEF ₂₈	+	+	+	+	+	+	+
HEF ₂₉	IN	IN	IN	IN	IN	IN	IN
HEF ₃₀	+	+	+	+	+	+	+
HEF ₃₁	+	+	-	-	-	-	-
HEF ₃₂	-	-	IN	IN	-	-	-
HEF ₃₃	-	-	-	-	+	+	+

KEY: - = No Activity, IN = Inhibitory or bacteristatic, + = Activity or bactericidal

Table 5. Antibacterial activity of fractions of hexane extract of *Alchornea cordifolia*.

The hexane extract fractions (HEF) _{1 - 17} showed no antibacterial activity against any of the bacteria species while HEF₂₉ had bacteristatic effect and HEF _{28, 30} had bactericidal effect against all the bacteria species (Table 5).

5. Discussion

The results from the phytochemical screening of the hexane extract revealed the presence of tannins, saponins, alkaloids and phenol. Several plants which are rich in tannins have been shown to possess antibacterial activities against a number of microorganisms (Doss *et al.*, 2009). Saponins though are haemolytic on red blood cells, are harmless when taken orally and they have beneficial properties of lowering cholesterol levels in the body (Amos-Tautua *et al.*, 2011). Alkaloids have been shown to possess both antibacterial (Erdemoglu *et al.*, 2009) and antidiabetic (Costantino *et al.*, 2003) activities. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (Uwumarongie *et al.*, 2007).

The antibacterial activities exhibited by the secondary metabolites: tannins, saponins, alkaloids, and phenols extracted from the hexane extract of *A. cordifolia* leaf can be responsible for the antibacterial activity of the extract. The presence of secondary metabolites in plants have been reported to be responsible for their antibacterial properties (Rojas *et al.*, 2006; Nikitina *et al.*, 2007; Udobi *et al.*, 2008; Rafael *et al.*, 2009; Adeshina *et al.*, 2010). The broad spectrum of antibacterial activity showed by the hexane extract against Gram positive and Gram negative bacteria can be attributed to the presence of the secondary metabolites. All the secondary metabolites showed more antibacterial activity against the gram-positive bacteria than the gram-negative bacteria. This is similar to the results of Adeshina (2005) who discovered that tannins, saponin, alkaloid and phenol from the leaf methanol, water and ethyl acetate extracts of *Alchornea cordifolia* had antibacterial activities against gram-positive bacterial strains more than gram-negative bacteria. Banso and Adeyemo (2007) also detected that tannins and alkaloids from *Dichrostachys cinerea* possessed antibacterial activities against gram-positive bacterial strains more than gram-negative bacteria.

The hexane extract appeared to be more active against the gram positive bacteria, *Staph. aureus*, than the Gram negative bacteria species. Gram negative bacteria are known to be resistant to the action of most antibacterial agents including plant based extracts and these have been reported by many workers (Kambezi and Afolayan, 2008; El-Mahmood, 2009). Gram negative bacteria have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impermeable to antimicrobial agents.

The bactericidal action of HEF₂₈ and HEF₃₀ against all the tested bacteria species can be an indication that these fractions possess the active ingredients responsible for antibacterial activity of the hexane extract of *Alchornea cordifolia* leaf. HEF₂₁ and HEF₂₇ displayed notable antibacterial activities against *Staph. aureus* and *Pseudomonas aeruginosa* that is of great importance because the infections cause by these bacteria are known to be difficult to control. *Staphylococcus aureus* has been reported by many workers to have developed resistance to most antibiotics and *Pseudomonas aeruginosa* is an opportunistic organism which has been reported to readily receive resistance carrying plasmid from other bacteria species (Wiley *et al.*, 2008). HEF₃₃ also showed noteworthy bactericidal action against the

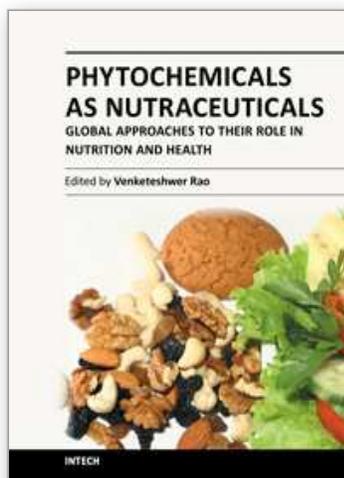
tested enteric bacteria-*E. coli* and *Proteus sp.* Enteric bacteria are known to transmit resistance plasmid among themselves (Brooks *et al.*, 2008) therefore developing resistance to many antibiotics. In view of all these observations, these fractions: HEF₂₈, HEF₃₀, HEF₂₁, HEF₂₇ and HEF₃₃ can further be worked on to get their structures and other necessary properties needed for formulation into newer antibiotics.

In conclusion, the hexane extract and fractions of *Alchornea cordifolia* leaf possess broad spectrum of antibacterial activity against the test bacteria species. Five out of the thirty-three fractions displayed potential antibacterial activity that can be explored as remedy for human bacterial infections. The results obtained from this work gives high hope for the development of new antibacterial agents.

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