

Preliminary Studies on the Phytochemical and Pharmacological Screening of *Alchornea Cordifolia*

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ABSTRACT

The study was to evaluate the classes of chemical compounds present, antifungal, antibacterial and effect on *Plasmodium* species by *Alchornea cordifolia* leaves extract used by local medicine practitioners for the treatment of malarial fever. The extract indicated the presence of alkaloids, saponins, unsaturated and carbonyl compounds. They exhibited activity against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Penicillium notatum* and *Plasmodium spp*. There was bulging of the plasmodial cells after 48h and total lyses after 72h incubation for the ethanol and water extracts while the ether extract showed no antiplasmodial activity. The study justifies the use of the plant for treatment of malarial fever.

INTRODUCTION

Alchornea cordifolia ((Schumach & Thonn). Mull Arg) commonly called Christmas bush (Yoruba: Akoyin; Benin: Ebe-uhosa) is an erect, sometimes scrambling, bushy perennial shrub or small tree up to 4m high, that reproduces from seeds and vegetatively from stem cuttings. The stem is woody, greyish, many branched and bushy when young. The leaves are simple and alternate, broadly-ovate 10-28cm long and 6.5 –16.5cm wide¹. It is a common plant in secondary forest re-growths and a weed of cultivated fields in the forest zone It is a widely distributed plant in Africa and is used in the traditional medicine in many African countries for the treatment of bacterial, fungal, parasitic and inflammatory disorders². In Ondo and Edo State of Nigeria, it is used in the preparation of remedies for urinary respiratory and gastro - intestinal disorders and in the treatment of malarial fever³.

Analyses of *Alchornea cordifolia* leaves have revealed the presence of tannins, phenolic acids,

gallic acid, ellagic acid, protocatechic acid, flavonoids, quercetin, hyperin and guaijaverin and an alkaloid tri-isopentenyl guanidine⁴.

Lamikanra *et al*⁵ attributed the antibacterial activity of *A. cordifolia* leaves extract on *Staphylococcus aureus* and *Escherichia coli* to isopentenyl guanidine found to be present in the plant. Phytochemical screening of the powdered leaf samples of another member of the genus *A. laxiflora* revealed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds and some terpenoid compounds⁶.

No report has been given on studies on the anti – plasmodial activity of *A. cordifolia*; thus in this study, the chemical constituents present were classified and the antimicrobial activity towards *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Penicillium notatum* and *Plasmodium spp*. activity were determined.

EXPERIMENTAL

Collection and treatment of plants material

The fresh leaves of *Alchornea cordifolia* were collected in June 2005 from a bush at Isiohor area of Benin City, Edo State, Nigeria. The plants were

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identified at the Botany Department in the University of Benin, Benin City, Nigeria. 10g each of the fresh leaves were crushed and extracted with water, ethanol and diethyl ether.

The rest of the leaves were air dried in the laboratory for about four weeks and then crushed mechanically with an all - steel grinding machine and stored in a polythene bag. 100g of the ground leaves were extracted using diethyl ether followed by 90% ethanol. The solutions were concentrated using a rotary evaporator. Phytochemical tests were then carried out on the ether and ethanol fractions of the dried leaf extract while anti - plasmodial test were carried out using water, ether and ethanol extracts of the wet and dry leaf.

Fractionation

A portion of the concentrated extract was fractionated based on the solubility of the constituents in 1M HCl, and 1M NaOH. The basic and acidic isolates were later subjected to TLC. Characterization of the isolates were done using Spectrophotometer 6405 for the UV analysis and Spectrophotometer MC500 for the IR analysis.

Test for unsaturation⁷

To 2ml of the extract, 2ml of ethanol was added to dissolve the mass; 2 drops of 0.2% potassium permanganate solution was added drop by drop to the solution while shaking till the colour persisted.

Test for carbonyl compound⁷

To 2ml of ethanol was added 2ml of the extract. 3ml of 2,4-dinitrophenylhydrazine was added to it and shaken vigorously. Formation of a red or orange yellow solid was an indication for the presence of a carbonyl.

Test for alkaloids⁸

Dragendoff's reagent, Wagner's reagent and picric acid were used to test for alkaloids in both the petroleum ether and ethanol extract. 1ml of the

extract was transferred into three different test tubes labelled A, B and C.

- a. To portion A; 2ml of Dragendoff's reagent (made by a mixture of potassium bismuth iodide salt) was added. Reddish brown precipitate indicated a positive test.
- b. To portion B; 2ml of Wagner's reagent was added. Reddish brown precipitate indicates a positive test.
- c. To portion C, 2ml of picric acid was added. A yellowish precipitate showed a positive test.

Test for eugenol

2ml of the extract was mixed with 5ml of 5% potassium hydroxide solution. The aqueous layer was separated and filtered with filter paper and drops of dilute HCl were added to the filtrate. A pale yellow precipitate indicated a positive test.

Test for tannins^{9,10}

To 2ml of the extract, 10ml of distilled water was added. The mixture was boiled for 5 minutes and then filtered.

- i. To 2 drops of the filtrate, ferric chloride solution was added; formation of a bluish precipitate indicated hydrolysable tannin.
- ii. To 5 drops of the filtrate, dilute HCl was added and boiled for 5 minutes. Red precipitate indicated condensed tannin.

Test for saponins

To a 2ml portion of the sample extract, 3ml water was added. The mixture was boiled for three minutes and later filtered. To the filtrate, 5ml of distilled water was added and then shaken vigorously. Persistent frothing indicated the presence of saponins.

Test for flavonoids^{9,11}

2ml of the extract was boiled in 10ml of distilled water and filtered. The filtrate was divided into two different portions A and B of 5ml each:

- i. To portion A, 10% lead acetate solution was added in few drops. Yellowish precipitate indicated positive result.
- ii. To portion B, 5ml of 20% NaOH was added and drops of dilute HCl were also added to the solution. Formation of a colourless solution indicated the presence of flavonoid.

Test for phenolic compound⁸

1ml of the extract was added to 5ml of 90% ethanol, a drop of 10% FeCl₃ was added, pale yellow colouration indicated a positive result.

Determination of antimicrobial activities

Sourcing and preparation of micro organisms

Four clinical isolates consisting of *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Penicillium notatum* were obtained from the Microbiology Department of the University of Benin, Benin City. The organisms were then inoculated into 10ml of nutrient broth. Overnight broth cultures of *S. aureus*, *E. coli*, *A. niger* and *P. notatum* were prepared in sterile 20ml universal bottles.

Preparation of nutrient agar medium

The media used were nutrient agar and nutrient broth. 28mg of powdered nutrient agar was dissolved in 1 litre of sterile distilled water. It was allowed to dissolve and then sterilized for 15 minutes using the autoclave at 121⁰C and then cooled to 47⁰C for 15-20 minutes. 20ml of this was poured into sterile petri dishes aseptically.

Determination of inhibitory action using agar dilution method

The sensitive disks used were the Whatman's No 4 filter paper. They were cut by mechanical perforator into circular forms measuring 0.6cm in diameter and sterilized in hot air oven at 46⁰C for 1hr and allowed to cool. The diluted organisms were used to flood the dried nutrient agar. The sterilized papers were immersed in the various dilutions of the extracts and

impregnated on the surface of the nutrient Agar already flooded with the organism using a sterile pasture pipette. These were incubated for about 16-24 hours at 37⁰C and zones of inhibition were measured in millimetres. The procedures above were used for anti fungal test with the substitution of PDA (Potato dextrose agar) broth for nutrient broth¹².

Test For antiplasmodial activity

Blood samples containing malaria parasites were collected in EDTA bottles from patients at the Medical Microbiology Department, University of Benin Teaching Hospital (UBTH). The blood was authenticated by staining with Giemsa stain diluted 1/10. Using an applicator stick, 1 to 2 drops of blood was smeared on a clean grease free slide. It was allowed to air dry and then flooded with 1/10 diluted Giemsa stain. This was left for 10-35 minutes and then rinsed in water (not running water), air dried and stained with immersion oil, and viewed under a microscope to show the presence of the parasites. 2ml of the plant extract (water, diethyl ether and ethanol) were separately added to about 10ml of the blood. Each mixture was vigorously shaken to enhance homogeneity; the control contained just the solvent used for the extraction. It was then incubated for 0hr, 24 hours, 48 hrs, and 72hrs respectively and the Giemsa stain applied (as described above). The effect of the extract on the plasmodium parasites were observed under a microscope.

RESULT AND DISCUSSION

The results of the chemical and microbial tests are presented in Tables 1 to 4.

This work confirms the presence of tannins, flavonoids and phenolic compounds as earlier reported by Maver - Manga *et al*⁴ with the new report of the presence of alkaloids, saponins and eugenol which have been variously implicated in antiplasmodial activities in plant¹³. The IR spectra of the various isolates showed the presence of phenolic compound, organic acid salt, an unsaturated amine or

Table 3 Anti-microbial Test on *Alchornea cordifolia* extract

Vol. of Extract in 10ml	Zone of inhibition (mm)							
	<i>E. coli</i>		<i>S. aureus</i>		<i>P. notatum</i>		<i>A. niger</i>	
	Diethyl ether	Ethanol	Diethyl ether	Ethanol	Diethyl ether	Ethanol	Diethyl ether	Ethanol
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4ml	16.00	12.00	25.00	14.00	10.00	16.00	10.20	11.70
3ml	15.50	10.00	18.00	13.00	9.50	11.00	9.50	9.50
2ml	15.00	9.50	13.00	11.50	8.00	9.00	8.70	9.00
1ml	14.00	8.00	9.00	9.00	7.70	8.50	8.00	8.30

Table 4a Antiplasmodial Test on *Alchornea cordifolia* fresh leaf extract

MP Blood Sample plus Plant Extract	0hrs	24hrs	48hrs	72hrs
W ₁	++	++	SCD	NP
W ₂	++	++	SCD	NP
E ₁	++	++	SCD	NP
E ₂	++	++	SCD	NP
D ₁	++	++	++	++
D ₂	++	++	++	++

TABLE 4b Antiplasmodial Test on *Alchornea cordifolia* dry leaf extract

MP BLOOD SAMPLE PLUS PLANT EXTRACT	0hrs	24hrs	48hrs	72hrs
Ethanol Extract	++	++	SCD	NP
Diethyl ether Extract	++	++	SCD	NP

KEYS

MP	=	Malaria parasite	NP	=	No parasite
++	=	Parasite number (11-100 per 100high power fields)			
W ₁	=	Dry leaves water extract			
W ₂	=	Wet leaves water extract			
E ₁	=	Dry leaves ethanol extract			
E ₂	=	Wet leaves ethanol extract			
D ₁	=	Dry leaves diethyl ether extract			
D ₂	=	Wet leaves diethyl ether extract			
SCD	=	Swollen chromatin dot.			

The antimicrobial test of the plant extracts using four microbes, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Penicillium notatum* showed sensitivity to both extracts. While the non-polar petroleum ether fraction was more effective towards *E. coli*, *S. aureus*, the polar ethanol fraction was more effective toward the fungi *A. niger* and *P. notatum*. This is because most of the values fall between 12 to 15mm¹⁴. As dilution of the plant extract increased, the zone of inhibition decreased, this means that the high concentration of the plant extract will speed up inhibition.

This in-vitro studies of the action of *Alchornea cordifolia* extract on *Plasmodium* species have shown a possible mechanism for its action. Some plants are known to exert antiplasmodial action by either causing elevation of red blood cell oxidation¹⁵ or inhibiting protein synthesis¹⁶; the possible route for this plant may be the alteration of the cell membrane of the parasite leading to swollen chromatin dot and subsequently lysis.

CONCLUSION

This study supports the use of the plant for malarial treatment; however, studies need to be carried out on its toxicity. The work is also being extended to identify the isolated compounds and to determine the dosage induced blood schizontocidal activity against malarial infection in mice..

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