ALCHORNEA CORDIFOLIA: A HERB WITH THE POTENTIAL FOR PHYTOTHERAPY AGAINST *CLOSTRIDIUM TETANI, PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT
The antibacterial activity of the extracts of the leaves and stem pith of *Alchornea cordifolia* were tested against three bacterial isolates. The bacteria isolates used were *Clostridiumtetani*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Juice from the stem pith of *A. cordifolia* showed the highest antibacterial activity against all the test organisms. *S. aureus* was most susceptible with a zone of inhibition (42.5±1.0 mm) higher than the control, gentamycin (41± 1.0 mm). All the isolates were susceptible to the leaf extract at a concentration of 575.0 mg/ml. However, the MICs were 143.7, 718.0 and 718.0 mg/ml for *C. tetani*, *P. aeruginosa* and *S. aureus*, respectively. Phytochemical analysis showed the presence of tannins, saponins and steroids in all the extracts tested. Phlobatannins were detected only in the fresh juice of the leaves. The results of this study show that extracts of this plant may be used for the treatment and management of wound infections caused by these test organisms.

Key words: *Alchornea cordifolia*, extracts, bacterial isolates, stem pith

INTRODUCTION

Plants have been used since time immemorial for prophylactic, management and treatment of many human and animal ailments. Most rural areas of developing countries continue to use herbs as a primary source of medicine. Globally, there is a renewed quest for the use of medicinal plants as an alternative for prevention, management and treatment of infections. This is prompted by a number of factors which include: an increase in disease incidence especially in developing countries; opportunistic infections in immuno-compromized individuals (Tichy and Novak, 1998; Badria and Zidan, 2004); herbal medicines being cheaper than conventional drugs, may not be associated with adverse side effects on the host (Saraf, 2010); and can replace conventional antibiotics to which human pathogens have developed resistance (Ali et al., 2011).

Among the multi-branched trees and shrubs found in tropical areas worldwide, is *A. cordifolia* of the family Euphorbiaceae commonly seen in secondary forests (Mavar-Manga et al., 2007). It is called 'agyama' in Ghana; 'cholei' in Guinea. In Nigeria, it is known as 'uwonmwe' in Edo; 'bambami' in Hausa; 'epa' in Yoruba; 'ubebe' in Igbo (Enpostng, 2010); 'ubobo' in Urhobo; and 'evwa' in Isoko languages of Delta State, Nigeria (Akpo and Owhe-Ureghe, 2013).

The decoction, crude and ethanolic extracts of *A. cordifolia* are used traditionally by rural people in most African countries for treating respiratory diseases such as sore throat, cough, and bronchitis, venereal diseases, mouth ulcers, toothache and dental caries, eye infections, wounds and intestinal infections. The leaf extracts have been reportedly used in Zaire for treating urinary tract infection, infected wound, dental caries, diarrhoea, cough, chest pain and anaemia (Kambu*et al.*, 1990; Muanza et al., 1994). In Senegal, the leaf extracts have been reported to be used in the treatment of venereal diseases, dermatoses, urinary tract infections, conjunctivitis, stomach ulcers, bronchitis, cough and toothache (Le Grand and Wondergem, 1987; Le Grand, 1989). Piles and diarrhoea were treated in Sierre Leone with the leaves (Macfoy and Sama, 1990). In Nigeria, they are used to treat gonorrhoea, yaws, rheumatic pain and cough (Gbile and Adeshina, 1986; Ogungbamila and Samuelson, 1990). The people of Wilberforce Island in Nigeria use the fresh leaves to stop bleeding from wounds. The leaves are also used

to treat athletes' foot, when they are squeezed and the juice placed in-between the toes (Amos-Tautua et al., 2011). Fresh leaves and stem pith extracts were earlier reported by us, Akpo and Owhe-Ureghe (2013) for their inhibitory effect against some oral bacteria.

Stem pith is chewed to treat tachycardia and rubbed on the chest to treat respiratory problems in Senegal (Mavar-Manga et al., 2007). It is also chewed to treat enteric infections, notably, diarrhoea in Nigeria. Guineans use the pith and leaves of this plant as an antiseptic and anti-cough agent (Sugiyama and Koman, 1992). This study was aimed at determining the antibacterial activity of the juices from the leaves and pith of *A. cordifolia* against *C. tetani, P. aeruginosa* and *S. aureus*; carry out *in vitro* test to validate the traditional use of the plant for treating wound infections; determine the susceptibility of the selected organisms to the extracts of the plant under investigation and to investigate the phytochemical constituents present in the plant.

In this study, the antibacterial activities of the fresh as well as concentrated extracts of leaves and stem pith of *A. cordifolia* were determined *in vitro.*

MATERIALS AND METHODS

Plant selection and preparation

A. cordifolia whole plants were collected from the banks of River Ethiope, behind Ethiope Hall,Delta State University, Abraka. They were were identified and authenticated at the herbarium of the University of Nigeria, Nsukka. The old, damaged leaves were removed while the fresh ones were cleaned by washing with distilled water. Tender stems of *A. cordifolia* were broken and the pith neatly removed.

The plant materials were homogenized using a porcelain pestle and mortar. Juice was extracted from the pith of *A. cordifolia* by squeezing through a sterile handkerchief to obtain sterile filtrates.

Phytochemical screening

Phytochemical analysis of the plant extracts were done in the laboratory of the Departments of Chemistry and Pharmaceutical Chemistry, Delta State University, Abraka. The standard methods described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993) were employed.

Test isolates

The palms of some pupils of Avwaeke Primary School were swabbed, using sterile swab sticks soaked in sterile normal saline. The swab sticks were immediately taken in ice packs to the laboratory and streaked on the surface of sterile blood agar plates. Bacterial species were isolated, characterized and identified according to standard methods described in the Manual of Clinical Microbiology (Murray et al., 2007 and Holt, 1994).

Antimicrobial susceptibility testing

Standardization of test organisms

A standard stock of the bacteria isolates were prepared by suspending a loopful of each microbial growth in about 10 ml of nutrient broth. After incubation at 37°C for 12 h, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about $1 - 2 \times 10^8$cfu m/l (Murray et al., 2007).

Preparation of dilutions

Doubling dilutions of each crude extract was made with sterile distilled water as described by Harrigan and McCance (1976). For each extract, 2 ml of sterile distilled water was placed in five test tubes with a sterile pipette. Using a fresh sterile pipette, 2 ml of the extract was added to the first tube and mixed thoroughly, resulting in a 1:2 dilution. Then 2 ml of this first dilution was taken and transferred to a second tube of sterile diluent, to make a 1:4 dilution. This process was carried out until a dilution of 1:32 was obtained with the last tube.

Preparation of concentrated extract

The plant parts were each weighed before the juice was extracted and concentrated. Exactly 459.1 g of *A. cordifolia* leaves yielded 230 g of juice and 261 g of *A. cordifolia* pith yielded 80.2 g of fluid. The method of Ellof (2000) was employed with modifications. The juice from each plant material was centrifuged at 3000 rpm for 5 min, the supernatant was then spinned at the same speed until a clear supernatant was obtained for each plant juice. Clear supernatant was discarded and the resulting fluid weighed. The

concentrated extracts weighed 17.7 and 1.61 g, for *A. cordifolia* leaves and *A. cordifolia* pith, respectively. The volume obtained for each was 30.8 and 3.0 ml, respectively. The method of Harrigan and McCance (1976) with modifications was used for the doubling dilution. *A. cordifolia* leaf concentrations were 575.0, 287.5 and 143.7 mg/ml; and 535.0, 267.5 and 133.7 mg/ml of *A. cordifolia* pith were used.

Susceptibility test

This was done in triplicates. The NCCLS (2000) method of susceptibility testing was adopted in this investigation. A sterile swab dipped in each standardized suspension was spread uniformly on freshly prepared Mueller Hinton agar(MHA). Wells of 5 mm diameter were punched on the MHA using a sterile cork borer. These wells were labeled accordingly and aliquots (100 µl) of the plant extracts were dropped into each, allowed to stabilize and incubated at 37°C for 24 h. Thereafter, the resulting zones of inhibition were measured in millimetres and recorded. Gentamycin (35 mg/ml) was used as positive control.

Determination of minimum inhibitory concentration (MIC)

The tube dilution test for determining minimum inhibitory concentration (MIC) was done. A serial dilution of each of the extracts was prepared aseptically. Fresh colonies of each bacterial isolate were suspended to an appropriate turbidity in 5.0 ml of Mueller-Hinton Broth (MHB) to give a slightly turbid suspension. This suspension was aseptically diluted by drawing 0.2 ml and placing in 40 ml MHB. Thereafter, 1.0 ml was added to each tube containing the extract and incubation of all tubes was at 37°C overnight. Tubes were examined for visible bacterial growth. The highest dilution without growth is the MIC.

Statistical analysis

The data obtained were analyzed by descriptive statistics. The mean and standard deviation of the triplicate susceptibility tests were calculated. Results were expressed as Mean ± SD.

RESULTS

Phytochemical analysis

The result of the phytochemical analysis presented in Table 1 shows that the extracts of the pith of *A. cordifolia* contain tannins, terpenoids, saponins and steroids. Similarly, the leaf extract contain tannins, terpenoids, saponins, steroids as well as phlobatannins. Phlobatannins were detected only in *A. cordifolia*leaves.

Susceptibility testing

The result obtained with the crude extracts of the leaves and pith of *A. cordifolia*is is summarized in Table 2. Gentamycin at a concentration of 35 mg/ml was used as positive control and the inhibition zones were between 39.5±1.0 (*P. aeruginosa*) and 42.0±1.0 mm (*C. tetani*).The pith juice of *A. cordifolia* significantly inhibited the three isolates with mean zones of inhibition ranging from 27.0 ± 1.5 to 42.5± 1.0 mm. *S. aureus* was most susceptible with a zone of inhibition higher than the control, gentamycin (41.0±1.0 mm), and *P. aeruginosa* was least susceptible. The leaf juice of *A. cordifolia* inhibited *C. tetani* and *S. aureus* with 9.5±1.6 and 9.0±1.6 as mean zones of inhibition, respectively. *P. aeruginosa* was resistant to the leaf juice of the plant.

Dilutions of crude extracts

Dilutions of the crude extracts of the plant parts used for this work did not inhibit any of the bacterial isolates tested.

Concentrated extracts

The result obtained with the concentrated extracts of the leaves and pith of *A. cordifolia* is summarized in Table 3. The control, gentamycin, was used at a concentration of 35mg/ml and the inhibition zones between 37.0±1.0 (*P. aeruginosa*) and 42.6±0.6 mm (*C. tetani*). All the isolates were susceptible to the leaf extract at a concentration of 575.0 mg/ml and the zones of inhibition ranged from 12.0±0.5 mm (*C. tetani*) to 253.0 ±0.5 mm (*S. aureus*). At a concentration of 287.5mg/ml, zones of inhibition ranging from 9.5±0.5 mm (*S. aureus*) to 15.5±2.5 mm (*P. aeruginosa*) were obtained. All isolates were susceptible to the leaf extracts of *A. cordifolia*at a concentration of143.7 mg/ml and the range of the inhibition zones is 5.3±0.6 mm (*C. tetani*) to 13.0±1.0 mm (*P. aeruginosa*). On the other hand,

Table 1. Phytochemical analysis of crude leaf and stem extract, and *A. cordifolia*pith and leaf extracts.

Constituent	*A. cordifolia*pith extract	*A. cordifolia* leaf extract
Alkaloids	ND	ND
Anthraquinones	ND	ND
Flavonoids	ND	ND
Phenols	ND	ND
Phlobatannins	ND	+
Tannins	+	+
Terpenoids	+	+
Saponins	+	+
Steroids	+	+

+ = Present; ND= Not detected.

Table 2. Zones of inhibition by the fresh juices of *A. cordifolia* pith and leaves on bacterial isolates.

Bacteria	Zones of inhibition (mm)		
	A. cordifolia pith	*A. cordifolia*leaves	Gentamycin control (35 mg/ml)
Clostridium tetani	31.5±3.1	9.5±1.6	42.0±1.0
Pseudomonas aeruginosa	27.0±1.5	NI	39.5±1.0
Staphylococcus aureus	*42.5±1.0	9.0±1.6	41.0±1.0

Values are written as mean ± SD for n=3 plates per isolate. *Compares well with the control. NI= No inhibition.

Table 3. Zones of inhibition of concentrated *Alchorneacordifolia*leaf and pith extracts on isolates.

Bacterial isolates	Zones of inhibition (mm)							
	Leaf extracts			Pith extracts			Sterile distilled water	Gentamycin
	Concentration (mg/ml)							
	575.0	287.5	143.7	535.0	267.5	133.7		35
C. tetani	12.0±0.5	10.3±0.6	5.3±0.6	NI	NI	NI	_	42.6±0.6
P. aeruginosa	21.5±1.8	15.5±2.5	13.0±1.0	NI	NI	NI	_	37.0±1.0
S. aureus	25.3±0.5	9.5±0.5	9.30±0.5	8.5±1.6	NI	NI	_	41.5±1.1

Values are written as mean ± SD for n=3 plates per isolate. *Compares well with the control. NI= No inhibition.

the pith extract at a concentration of 535.0mg/ml inhibited only *S. aureus* with a zone of inhibition of 8.5±1.6 mm.

Minimum inhibitory concentrations

The minimum inhibitory concentrations of the leaf and pith extracts of *A. cordifolia* are shown in Table 4. The highest dilution of the leaf extract (714.0 mg/ml) was observed for *P. aeruginosa* and the least dilution of 575.0 mg/ml was recorded for *C. tetani*. It is interesting to note that *S. aureus* which had the largest zone of inhibition had a minimum inhibitory concentration of 143.7mg/mL.

DISCUSSION

The presence of phlobatannins, tannins, terpenoids, saponins and steroids were detected in the leaf juice of *A. cordifolia*. This result is similar to that of Adeshina et al. (2010) who had earlier reported the presence of tannins and flavonoids in the ethyl acetate extract of *A. cordifolia* leaves. Tannic acid has been found to be inhibitory to the growth of intestinal bacteria such as *Bacteroides fragilis*, *Clostridium perfringens*, *Escherichia coli* and *Enterobacter cloacae* amongst others (Akiyama *et al.*, 2001). The methanolic and chloroform extracts of these leaves have been reported to contain among others, saponins, tannins and steroids (Amos-Tautua et al., 2011). Saponins, tannins, alkaloids and flavonoids were reported to be present in the ethanolic leaf extracts of *A. cordifolia* (Olaleye et al., 2006).

The stem pith of *A. cordifolia* had tannins, terpenoids, saponins and steroids. Amos-Tautua et al. (2011), reported that the methanolic extract

Table 4. Minimum inhibitory concentrations of extracts on isolates.

Isolates	*A. cordifolia* leaf (mg/ml)	*A. cordifolia* pith
Cl. tetani	575.0	—
S. aureus	143.7	535.0
P. aeruginosa	714.0	—

of the stem bark of the plant obtained from Wilberforce Island in Bayelsa State, Nigeria, contain saponins, alkaloids, flavonoids, tannins, sterols and cardiac glycosides, while the chloroform extract contain saponins, sterols and cardiac glycosides. Hafiza et al. (2002) reported that crude saponins also inhibited the growth of microbes. The antibacterial activities of saponins against *S. aureus* have been demonstrated (Soetan et al., 2006; Kaur and Arora, 2009). Saponins, when taken orally play the role of lowering cholesterol levels in the body (Price et al., 1987). Steroids have been shown to have antibacterial activity against *S. aureus* (Aslam, 2009). Steroidal derivatives have been reported to inhibit the growth of Gram-positive and Gram-negative bacteria (Khan et al., 2008). Tannins have been reported to hasten the healing of wounds, inflamed mucous membranes and to arrest bleeding (Shivananda et al., 2007; Manjunatha et al., 2007). Tannins are antidiarrheal and antihaemorrhagic (Asquith and Butler, 1986). They have shown inhibitory activity against *P. aeruginosa* and *S. flexneri* (Kaur and Arora, 2009).

Phlobatannins were detected only in the leaves of *A. cordifolia.* This is contrary to the reports of Adeshina et al. (2010), Gatsing et al. (2010) and Amos-tautua et al. (2011), who respectively, did not detect phlobatanins in the dried-leafalcoholic extracts of *A. cordifolia*. The absence of phlobatannins in the alcoholic extracts may be due to the effect of alcohol on the phytochemical compound or they may have been lost during the extraction process.

S. aureus was most susceptible to the fresh crude extracts of the stem pith of *A. cordifolia* with a zone of inhibition higher than the control, gentamycin. This organism was also inhibited the most with the concentrated leaf extracts of the plant. Similarly, *S. aureus* have been reported to be most susceptible to the ethyl acetate extract of the leaves of *A. cordifolia* (Adeshina et al., 2010). Okwu and Ukanwa (2010) reported the inhibition of *S. aureus*by 5–methyl 4*'* - propenoxyanthocyanidines 7-*O-β-D* – diglucopyranoside isolated from the ethanolic extract of the leaves of *A. cordifolia*. The susceptibility shown by *S. aureus* over other bacterial isolates is of great significance since reports abound that this organism has developed resistance to many antibiotics, sometimes making its clinical management difficult (Willey et al., 2008).

This study demonstrated that the leaves of *A. cordifolia* are more potent when concentrated. Similarly, Adeshina et al. (2010) reported zones of inhibition of a range of 10-35 mm for *P. aeruginosa* and *S. aureus* from the ethyl acetate extract of these leaves. *S. aureus* and *P. aeruginosa* have been reported to be susceptible to the methanolic extracts with zones of inhibition which ranged between 16-20 mm (Amos-Tautua et al., 2011). *A. cordifolia* leaf extracts have been reported to have shown the widest spectrum of antibacterial activity against *S. pyogenes, S. aureus* and *P. aeruginosa*, among a number of plants tested (Pesewu et al., 2008).

The results of this study show that the fresh juice from the stem pith of *A. cordifolia* inhibited the three isolates. The zone of inhibition observed with *S. aureus* was comparable to that of the standard (gentamycin). This validates its traditional use for the treatment of respiratory infections caused by *S. aureus* and *P. aeruginosa* and revealed that it can also be used to treat wound infections including the deadly tetanus.In contrast, the concentrated pith juice at 535.0 mg/mL inhibited only *S. aureus.*

From the results of this study, it can be concluded that saponins, steroids and tannins, which were present in all the extracts (Table 1) have antibacterial properties. The leaf juice of *A. cordifolia* contained phlobatannins, which may be responsible for its antibacterial activity against all the isolates at a concentration of 575.0 mg/mL. It was also observed in this study that the diluted fresh juices from these plant parts did not inhibit

any of the bacterial isolates tested; they are more useful as crude extracts and this justifies why traditional medicine practitioners make use of the crude extracts for the management of the infections.The *in vitro* potency of these plant parts against these aetiologic agents of wound, respiratory and eye infections have been demonstrated in this work. The fresh pith and leaves of this plant can serve as a broad-spectrum antimicrobial agent.

It is recommended that the phytochemical compounds present in these plant parts are tested for safety and further work done on the pith juice of *A. cordifolia*.

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