Preliminary Antimicrobial Activities of Extracts of *Alchornea cordifolia, Nauclea latifolia* and *Spondias mombin* against Antibiotic-Resistant *Pseudomonas aeruginosa* and some other Pathogenic Bacteria

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Abstract

Ethanolic and aqueous extracts of three local medicinal plants viz Alchornea cordifolia, Nauclea latifolia and Spondias mombin were screened against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Salmonella typhi. The results reveal that ethanolic extract of A. cordifolia showed activity against P. aeruginosa, E coli and S .typhi with mean minimum inhibitory concentration (MIC) values of 2.733, 2.887 and 0.602 mg/ml respectively, while its aqueous extract was active against S .typhi only, (MIC 0.912 mg/ml). Ethanolic extract of S. mombin was only active against S. typhi (MIC 1.445 mg/ml) while its aqueous extract exhibited activity against S. aureus and S. typhi with MIC values of 0.622 and 1.514 mg/ml respectively. Results of Independent Samples t-test infer that there was no significant difference (P > 0.05) in the susceptibilities to the plant extracts between antibiotic–resistant and sensitive P. aeruginosa. These findings underscore the possibility of using A. cordifolia and S. mombin extracts in developing alternative drugs for cases of drug resistance in infections caused by S. aureus and S. typhi

Keywords: *Pseudomonas aeruginosa, Alchornea cordifolia, Spondias mombin, Nauclea latifolia,* antibacterial activity

Introduction

Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Salmonella species are often involved in a number of infectious diseases (Horan *et al.,* 1988; Cheesbrough, 1994; Van Asperon *et al.,* 1995). Strains of these pathogens have often been found to be resistant to the mainstream antibiotics used in hospitals (Oduyebo *et al.,* 1997; O' Brien, 1992).

P. aeruginosa is particularly dreaded for its resistance especially to the older and more commonly used antibiotics. Many strains of *P. aeruginosa* are resistant to gentamicin and cephalosporin (Udo *et al.,* 1998; Kesah *et al.,* 1998). Some strains are even resistant to the fluoroquinolones (Olayinka *et al.,* 2005; Rastegar Lari *et al.,* 2005).

P. aeruginosa, S .aureus and E. coli are nosocomial pathogens and the circulation of resistant pathogens in a hospital setting poses a danger which cannot be over-looked and calls for constant effort in the development of new treatments (Nester *et al.,* 2001; Fauci, 1998).

With regard to the development of new treatments, attention has increasingly been turned to plants. Historically, plants have been used to cure diseases in all parts of the world. In developing countries, about 90% of people still depend on traditional medicine based on different species of plants, for their primary health care (Taylor, 2005). In Ibo ethnomedicine, local herbs including *Alchornea cordifolia, Spondias mombin* and *Nauclea latifolia* are used in the treatment of diseases such as bronchitis, tonsillitis, toothache and septic mouth, vaginal infections, wounds and malaria (Iwu, 1993; Oyewole and Shonukan, 1999; Okeke *et al.,* 1999).

This study was undertaken to evaluate the in-vitro activities of these plants against the above-named pathogens and the possibility of using them as alternative drugs in cases of drug resistance.

Materials and Methods

Test organisms

1. Fifteen strains of *P. aeruginosa:* P_1 to P_8 are sensitive to antibiotics. (P_8 is a typed strain *P. aeruginosa* ATCC 10145). P_9, P_{10} and P_{12} are sensitive to ciprofloxacin only. P_{11}, P_{12}, P_{13} and P_{14} are resistant to all antibiotics.
2. Two *E. coli* strains
3. Two *S. aureus* strains(vancomycin-resistant)
4. One strain of *Salmonella typhi(resistant to 6 antibiotics including perfloxacin and ofloxacin)*

These were collected from Departments of Microbiology and Veterinary Microbiology, University of Nigeria, Nsukka, National Orthopaedic Hospital, Enugu and Federal Medical Centre, Abakaliki, Ebonyi State.

Plant materials: The plants collected were *Alchornea cordifolia* leaves, *Nauclea latifolia* root and leaves and *Spondias mombin* leaves. They were collected from Nsukka in Enugu State, and Iboko in Izzi Local Government Area of Ebonyi State. The plants were identified by Prof. J.C. Okafor and Mr. A.O. Ozioko of Department of Botany, Faculty of Biological Sciences, University of Nigeria, Nsukka. Voucher specimens were deposited at Department of Botany herbarium, University of Nigeria, Nsukka with numbers 97G, 303i, 241D and 578 for the *Alchornea cordifolia* leaves, *Nauclea latifolia* root and leaves and *Spondias mombin* respectively.

Extraction: The plants were dried at room temperature and pulverized. A 50g portion of each pulverized material was macerated separately in 250ml of ethanol (Analar) and distilled water, for 48hr. The mixtures were then filtered with Whatman No.1 filter papers.

Table 1: Inhibition zone diameters (IZDs) of *A. cordifolia* measured in millimeters (mm)

Test Organism	Concentration of extracts in mg/ml									
	Ethanolic extracts					Aqueous extracts				
	40	20	10	5	2.5	20	10	5	2.5	1.25
***P.aerug.*1**	12.5±0.50	11.5±0.23	10.5±0.23	9.80±0.17	0	0	0	0	0	0
P.aerug 2	12.5±0.50	11.5±0.23	10.5±0.00	0	0	0	0	0	0	0
P.aerug.3	15.8±0.44	14.5±0.23	11.5±0.23	0	0	0	0	0	0	0
P.aerug.4	12.0±0.58	10.0±0.00	0	0	0	0	0	0	0	0
***P.aerug.*5**	12.5±050	11.0±0.00	0	0	0	0	0	0	0	0
***P.aerug.*6**	16.0±023	14.2±0.17	11.8±0.17	0	0	0	0	0	0	0
***P.aerug.*7**	14.0±0.50	12.0±0.23	11.0±0.00	10.0±0.00	0	0	0	0	0	0
***P.aerug.*8 ATCC 10145**	14.0±050	12.5±0.23	12.0±0.00	11.0±0.00	0	0	0	0	0	0
***P.aerug.*9**	15.0±0.50	12.7±0.34	10.5±0.23	0	0	0	0	0	0	0
***P.aerug.*10**	13.0±050	12.5±0.23	11.2±0.17	10.2±00	0	0	0	0	0	0
***P.aerug.*11**	13.0±0.58	10.5±0.50	0	0	0	0	0	0	0	0
***P.aerug.*12**	13.0±0.44	11.7±0.34	10.0±0.00	0	0	0	0	0	0	0
***P.aerug.*13**	12.0±0.00	10.0±0.00	0	0	0	0	0	0	0	0
***P.aerug.*14**	17.0±0.58	15.5±050	14.0±0.50	12.0±00	0	0	0	0	0	0
***P.aerug.*15**	14.0±0.50	12.3±0.34	10.0±0.00	0	0	0	0	0	0	0
***E. coli.* 1**	12.0±0.00	11.0±0.00	10.5±0.50	0	0	0	0	0	0	0
***E. coli.* 2**	12.0±0.23	10.8±0.17	10.0±0.00	0	0	0	0	0	0	0
***S.aureus* 1**	0	0	0	0	0	0	0	0	0	0
***S.aureus* 2**	0	0	0	0	0	0	0	0	0	0
S. typhi	17.3±0.34	16.0±0.23	14.0±0.23	12.0±0.00	15.0±0.23	14.3±0.34	12.8±0.18	11.0±0.00	0	0

n = 3, Values represent means ± standard error of the mean

Table 2: Inhibition zone diameters (IZDs) of *S. mombin* (mm)

Test Organism	Concentration of extracts in mg/ml									
	Ethanolic extracts					Aqueous extracts				
	20	10	5	2.5	1.25	20	10	5	2.5	1.25
***P.aerug.* isolates**	0	0	0	0	0	0	0	0	0	0
***E. coli* 1**	0	0	0	0	0	0	0	0	0	0
***E. coli* 2**	0	0	0	0	0	0	0	0	0	0
***S. aureus* 1**	0	0	0	0	0	17.5±0.50	15.3±0.34	14.0±0.23	12.0±0.00	10.0±0.00
***S. aureus* 2**	0	0	0	0	0	16.5±0.23	15.0±0.00	13.0±0.00	11.5±0.50	0
S. typhi	12.0±0.50	12.0±0.00	10.5±0.23	0	0	14.0±0.58	13.0±0.50	11.2±0.17	0	0

Ethanolic extracts were concentrated using a Rotary evaporator under low pressure. Aqueous extracts were air-dried in stainless steel trays under an electric fan.

Stock solutions of each extract were prepared in five dilutions as follows: - 0.02g was introduced into a sterile bijou bottle containing 1 ml of solvent and thoroughly mixed to give a 20 mg/ml solution. A sterile pipette was used to transfer 1ml of this dilution into another bijou bottle containing an equal amount of solvent to obtain a 10mg/ml dilution. This procedure was used to prepare dilutions of 5mg/ml, 2.5mg/ml, and 1.25 mg/ml.

Antimicrobial screening: The agar-well diffusion method was used (Baron and Finegold, 1990). Wells (8mm) were bored into Mueller-Hinton agar seeded with the test organisms. The plates were seeded from cell suspensions with turbidity matching the 0.5 Mcfarland standard. A sterile pipette was used to add 0.1 ml of the dilutions of extracts into each labeled well. The same quantity of diluent was used as control. Triplicate plates were prepared. The plates were allowed to stand on the bench for 1hr for proper diffusion and subsequently incubated at 37^0C for 24hr.

After incubation, inhibition zone diameters (IZDs) around each well were measured with a ruler. The IZD (mm) was recorded by calculating the mean of IZDs for each set of triplicate plates.

The minimum inhibitory concentrations (MICs) of the active extracts were determined by plotting a graph of mean IZD^2 against log drug concentration, using Microsoft Excel.

Independent Samples t-test was used to compare susceptibilities of antibiotic-sensitive and resistant test organisms to plant extracts.

Phytochemical analysis: Phytochemcial analysis of the active extracts were carried out based on procedures outlined by Harbourne (1973) and Trease and Evans (1989).

Results and Discussion

Results of the screening show that ethanolic extract of *A. cordifolia* exhibited activity against *P .aeruginosa (*mean MIC 2.733 mg/ml) *E. coli* (mean MIC 2.887 mg/ml) and *S. typhi* (MIC 0.602mg/ml), while it's aqueous extract was active against *S. typhi* only, (MIC 0.912mg/ml). Ethanolic extract of *S. mombin* did not show activity against *P. aeruginosa, S. aureus* and *E. coli* but was active against *S. typhi* (MIC 1.445mg/ml). Its aqueous extract exhibited activity against *S. aureus* and *S. typhi* with MICs 0.621 and 1.514 mg/ml respectively. Extracts of *Nauclea latifolia* root and leaves did not show activity against the test organisms. The results are shown in Tables 1-4.

Table 3: Minimum inhibitory concentrations (MICs) of extracts (mg/ml)

Test Organism	Acet	Acaq	Smet	Smaq
***P. aerug.* 1**	1.318	0	0	0
***P. aerug.* 2**	3.020	0	0	0
***P. aerug.* 3**	3.311	0	0	0
***P. aerug.* 4**	1.445	0	0	0
***P. aerug.* 5**	4.365	0	0	0
***P. aerug.* 6**	3.055	0	0	0
***P. aerug.* 7**	3.020	0	0	0
***P. aerug.* 8**	4.074	0	0	0
***P. aerug.* 9**	3.034	0	0	0
***P. aerug.* 10**	4.365	0	0	0
***P. aerug.* 11**	0.603	0	0	0
***P. aerug.* 12**	3.062	0	0	0
***P. aerug.* 13**	3.055	0	0	0
***P. aerug.* 14**	1.950	0	0	0
***P. aerug.* ATCC 15 10144**	1.318	0	0	0
***E. coli* 1**	2.754	0	0	0
***E. coli* 2**	3.020	0	0	0
S. aureus1	0	0	0	0.331
S. aureus 2	0	0	0	0.912
S. typhi	0.602	0.912	1.445	1.514

Key: *Acet: A. cordifolia leaf ethanolic extract, Acaq: A. cordifolia leaf aqueous extract, Smet: S. mombin leaf ethanolic extract, Smaq: S. mombin leaf aqueous extract*

Table 4: Mean MICs of extracts (mg/ml) for the test organisms

Extract	*P. aeruginosa*	*E. coli*	*S. aureus*	*S. typhi*
Acet	2.733 ± 0.30	2.887 ± 0.13	0.000	0.602
Acaq	NI	NI	0.000	0.912
Smet	NI	NI	0.000	1.445
Smaq	NI	NI	0.622±0.290	1.514

NI = No Inhibition

Table 5: Comparing mean MICs of *A. cordifolia* for antibiotic–sensitive and resistant *P. aeruginosa* using Independent samples t-test

P. aeruginosa strains	Mean MIC (mg/ml)	t-value	Sign. (2-tailed) P-value
Antibiotic-Sensitive	2.601 ± 0.344	-0.457	0.655
Antibiotic-Resistant	2.883 ± 0.532	-0.445	0.665

Inference from Independent Samples t-test analysis showed that there were no significant differences (P>0.05) in the mean MICs of *A. cordifolia* for antibiotic- sensitive and resistant *P. aeruginosa.* This signifies that the antibiotic–resistant and sensitive isolates were equally susceptible to *A .cordifolia* leaf extract.

The findings of this study corroborate the reports of Oyewole and Shonukan, (1999) that *A. cordifolia* ethanolic extract was active against *P. aeruginosa* and *E. coli and that S. mombin* exhibits high activity against *S. aureus.* In addition, *S. mombin* in this study exhibited a high activity against *S. typhi*. However, the lack of activity of *A. cordifolia* aqueous extract against *P. aeruginosa, E. coli* and *S. aureus* in this study do not seem to corroborate an earlier report of its broad spectrum activity (Okeke et al, 1999, Oyewole and Shonukan 1999). *Nauclea latifolia* root and leave extracts did not show appreciable activity against any of the test organisms. This result is at variance with earlier reports of its activity against *P. aeruginosa* and *Salmonella* species *(*Deeni and Hussain, 1991)

The preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, steroidal aglycones, glycosides, proteins and an absence of cyanogenic glycosides.

The findings of this study correlate with the local use of these plants in the treatment of bacterial infections. It is also significant to note that the extracts were equally effective against antibiotic – sensitive and resistant organisms. Further studies on the plants need to be carried out.

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