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Full Length Research Paper

In vitro antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri*

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The comparative studies of extracts of leaves, barks and roots of *Nauclea latifolia* and *Daniella oliveri* with respect to their phytochemical and antibacterial properties was carried out. Preliminary screening for phytochemical components showed that both plants had similar constituents, namely tannins, alkaloids and glycosides, but variable proportions of saponins. The antibacterial screening of the water and ethanolic extracts of the various plant materials were carried out against pathogenic bacteria including *Pseudomonas aeruginosa, Klebsiealla pneumonia, Escherichia coli, Staphylococcus aureus* and S*higella dysenteriae.* Ethanolic extracts were more potent than aqueous extracts and activity were concentration dependent. The Gram positive bacteria were more sensitive to the ethanolic extracts of both plants.

Key words: *Nauclea latifolia, Daniella oliveri,* phytochemical, antibacterial properties, Gram positive bacteria.

INTRODUCTION

Infectious diseases are the number one causes of death, accounting for approximately one half of all deaths in tropical countries (Iwu et al., 1999). Infact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to available agents. The use of plant parts as a source of medicine to treat infectious diseases predates history. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines (Erdemeier et al. 1996; Lino and Deogracious, 2006) to cure infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control. Even the World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine boards to license and regulate the practice of herbal practitioners under the supervision of ministries of health.

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In this study, we report the finding of a research designed to asses the antimicrobial properties of crude extracts from two selected African medicinal plants that are widely used in Northern Nigeria, both as sources of food (Aiyela`agba et al., 1996), medicine and timber.

MATERIALS AND METHODS

Plant collection and pre-extraction preparation

The plants were chosen based on reports from the local population in Girei town, a superb of Yola, Adamawa state of Nigeria. Voucher specimens were prepared and taken to Federal University of Technology Yola herbarium for identification. The leaves, barks and roots of *Nauclea latifolia* and *Daniella oliveri* were collected, sun dried for 7 days, pounded using pestle and wooden mortar.

Extraction procedure

100 g of the ground powder of each of the *D. oliveri* plant parts were soaked separately in 500 ml of 95% ethanol contained in a 1 L capacity flask for five days. The samples were then strained to remove solids. The solutions were again filtered using Whatman's filter paper No.1 to obtain a solution free of solids. The solution was then concentrated in a rotary evaporator to remove the ethanol and stored at 2 - 4ºC. *N. latifolia* was extracted in a similar way as *D. oliveri.* The same procedure was followed for water extracts that 100 g of each sample was dissolved in a 1 l of distilled water, the

Table 1. Phytochemical components of *N.* s*latifolia* and *D. oliveri*.

Phytochemical component	Plant	
	N. latifolia	*D. oleivera*
Saponin	-+	++
Tannin	++	++
Alkaloid	++	++
Glycoside	++	++

-+ = Trace; ++ = appreciable amount.

solution filtered, solids removed, and then concentrated by drying at 37ºC and stored at 2 - 4ºC until required.

Preparation of bacteria

The bacteria used were *K. pneumonieae, P. aeruginosa, E. coli and S. aureus* isolated from clinical specimens obtained from the pathology department of the Federal Medical Centre, Yola. Isolation and identification of the organisms were done following standard procedures in handling clinical specimens (Potashmercher et al., 1979; Cheessbrough et al., 2002). The organisms were maintained on nutrient agar slants at 2 - 8°C. Purity of the organisms was checked at regular intervals by plating and staining (Acheampong et al., 1988). The bacterial cultures were standardized using the method of Baker et al. (1983). The test organisms were suspended into sterile universal bottles containing nutrient broth and normal saline added gradually to it so as to compare the culture turbidity to that of Mc Farland standard, which corresponded to approximately 1.0 x 10^7 cell.ml^{-1}.

Screening for phytochemical components

The method described by Odebiyi and Sofowora (1978) was used to screen for the presence of the bioactive constituents of the plant materials.

Determination of antibacterial activity

The antibacterial screening was done as described by Lino and Deogracious (2006) and Akoma et al. (2002). Briefly, 1 ml of the test culture (1.0x10^7cell.ml^{-1}) was placed into a sterile plate and 19 ml molten agar at 45ºC poured and the plate shaken for even spread and proper mixing of the organisms and agar. This agar was allowed to solidify. Four wells of approximately 6 mm in diameter and 2.5 mm deep were made on the surface of the agar plates using a sterile cork borer. The plates were turned upside down and the wells labeled with a marker. The extract was reconstituted by dissolving 1 gm of each aqueous extract in 1 ml of distilled water and each three of the four well was filled with 0.5 ml of extract. The fourth well was filled with 0.5 ml of the pure solvents and served as control and of 12.5 µg equivalent gentamicin was used as a positive control. The plates were incubated at 37°C for 24 h and zones of inhibition measured with a pair of calipers and a millimeter ruler and results tabulated. The same test procedure was repeated for the ethanolic extract. The MIC of the extracts against the test organisms was determined using the broth dilution method (Sahm and Washington, 1990). Briefly 1 ml of the extract solution at concentration of 100 $mgml^{-1}$ was added to 1 ml of nutrient broth and subsequently transferred. 1 ml from the first test tube to the next, for up to the seventh test tube. Then 1 ml of 24 h culture of test organism (1.0x10^7cell.ml^{-1}) was inoculated into each test tube and mixed thoroughly on a vortex mixer. The test tubes were then incubated at 37°C for 24 h. The tube with the lowest dilution with no detectable growth was considered as the MIC. The same procedure was repeated using 5 – 50 $µgml^{-1}$.

RESULTS AND DISCUSSION

The results of phytochemical screening of the leaves, barks and roots of *Nauclea lotifolia* and *D. oliveri* for the bioactive components are presented in Table 1. Both plant parts contain glycosides, alkaloids and tannins with variable amounts of saponins. The result for antibacterial screening to determine diameters of zone inhibition is given in Table 2. The extracts were used at a concentration of 100 $mgml^{-1}$ in the respective solvents. The ethanolic extracts showed larger zones of inhibition and therefore higher activity than the aqueous extract. The root extracts showed more potency in terms of zones of inhibition sizes than the leaves and barks for both plants in all the test microorganisms used in this study. The MIC of the extracts against the organisms is shown in Table 3 and is similar for both plants. The MIC values ranged from 6.25 to 25 $mgml^{-1}$ for *K. pneumoniea*; 50 – 100 $mgml^{-1}$for *E. coli*; 12.5 -100 $mgml^{-1}$ for S*. aureus*; 6.25 - 50 $mgml^{-1}$ for *S. dysentriea* and 50 - 100 $mgml^{-1}$ *P. aeruginosa*. The MIC for *D. oliveri* and are almost half of that of *Nauclea latifolia. P. aeruginosa* isolates showed less susceptibility to the activity of the crude extract than the other organism. The MIC of gentamicin was less than the crude extracts and ranged between 12.5 to 25 $µgml^{-1}$.

Providing effective health care is a challenge under the best of economic circumstances. In the world's poorest countries, where infectious diseases are rife and resources limited, that challenge can assume overwhelming proportions, hence the resurgence in the use of herbal preparations to treat diseases. In this study, the phytochemical properties of two popular African medicinal trees; *D. oliveri* and *Nauclea latifolia* have been assessed for their effects against some clinical isolates of *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. dysenteriae*, obtained from the FMC Yola specialist hospital, a 700 bed tertiary hospital. Each of the 100 g of the dried powdered rootbark yielded a semi solid brown powdery substance of 15 g for the aqueous and 8.6 g for the methanolic extracts. The leaves, barks and roots of *N. latifolia* were found to contain alkaloids, tannins and glycosides and traces of saponins, while those of *D. oliveri* contained alkaloids, tannins, saponins and glycosides as shown in Table 1. The presence of some of the phytochemical components like saponins, tannins and phenolic compounds have been attributed to the antibacterial activity of the crude drugs observed (De and James, 2002). Extracts from the leaves of *Euginia uriflora* were found to contain tannins, glycosides and alkaloids and were effective against some strains of *E. coli*, *P.aeruginosa* and *K. pneumoniae* (Adebayo et al., 2001). Extracts of the leaves of *Parkia biglobosa* were reported

Table 2. Diameters (mm) of zones of inhibitions produced by the aqueous and ethanolic extracts of *N. latifolia* (NL) and *D. oliveri* (DO).

Organisms	Zone diameter (mm)*											
	Leave				Bark				Root			
	Aqueous		Ethanolic		Aqueous		Ethanolic		Aqueous		Ethanolic	
	NL	DO	NL	DO	NL	DO	NL	DO	NL	DO	NL	DO
P. aerugenosa	8.0	9.0	9.0	9.0	7.0	8.8	7.0	8.0	5.0	5.0	6.0	5.0
E. coli	9.0	10.0	10.0	10.0	9.0	8.0	7.0	8.0	5.0	5.0	6.0	6.0
S. aureus	10.0	11.0	11.0	10.0	9.0	10.0	10.0	9.0	7.0	8.0	7.0	6.0
K. pneumoniae	11.0	11.0	11.0	12.0	9.0	9.0	9.0	10.0	6.0	7.0	7.0	7.0
S. dysenteriae	12.0	11.0	11.0	11.0	10.0	11.0	8.0	9.0	6.0	7.0	8.0	7.0

* 12.5 µg gentamicin zone diameters = *P. aeruginosa* 5 mm; *E. coli* 5 mm; S. *aureus* 6 mm; *K pneumoniae* 6 mm; *S. dysentriae* 8 mm.

Table 3. Minimum Inhibitory concentration (MIC) ($mgml^{-1}$) *N. latifolia* (NL) and *D. oliveri* (DO).

Organisms	*MIC ($mgml^{-1}$)											
	Leave				Bark				Root			
	Aqueous		Ethanolic		Aqueous		Aqueous		Ethanolic		Aqueous	
	NL	DO	NL	DO	NL	DO	NL	DO	NL	DO	NL	DO
P. aerugenosa	-	-	100	100	-	100	100	100	100	50	100	50
E. coli	100	100	100	50	50	100	50	50	50	50	50	50
S. aureus	100	50	50	25	50	50	50	25	25	25	12.5	12.5
K. pneumoniae	50	50	50	25	25	25	25	12.5	12.5	12.5	12.5	6.25
S. dysenteriae	50	25	25	25	50	50	25	25	12.5	12.5	6.25	6.25

*Gentamycin (MIC) values for the test organisms = *P. aeruginosa* 25 μgml^{-1}; *K. pneumonia* 12.5 μgml^{-1}; *E. coli*; 12.5 μgml^{-1}; *S. aureus* 12.5 μgml^{-1}; *S. dysenteriae* 12.5 μgml^{-1}.

to contain tannins, alkaloids, steroids and cardiac glycolside and were demonstrated to inhibit the growth of *B. cereus* and *S. aureus* (Ajaiyeoba, 2002; Isuzu and Harvey, 2003). The presence of these bioactive components in the crude drugs have been linked to their activeties against disease causing microorganisms (Farnsworth, 1990) and also offering the plants them-selves protection against infection by pathogenic microorganisms (De and Ifeoma, 2002).

The antibacterial properties of the extracts expressed in forms of diameter of zones of inhibition are given in Table 2. At a concentration of 100 gml^{-1}, the extracts, though less effective than 12.5 µg gentamicin, inhibited the growth of the pathogens, with *P. aeruginosa* and *E. coli* less susceptible similar to the findings of Ajaiyeoba (2002). The lack of susceptibility of *P. aeruginosa* and *E. coli* to the extracts could be attributed to the fact that these bacteria are inherently resistant to many antibiotics and non-antibiotic antimicrobial agents due to the permeability barrier afforded by their outer membranes (Lino and Deogracious, 2006). The aqueous extracts of the plants displayed smaller zones of inhibitions and the higher MIC values against all test organisms compared to ethanolic extract.

The MIC of the extracts obtained by doubling dilutions showed that *P. aeruginosa* has the highest MIC and therefore less susceptible. Contrary to the data presented by Lino and Degracious (2006), the ethanolic extracts are more potent in terms of activity than the aqueous extracts probably because the bioactive components are less soluble in water leading to incomplete extraction of the components than ethanol (De and Ifeoma, 2002). The higher solubility of tannins and phenols in ethanol might have contributed for the higher activity observed (Wolinsky and Sote 1982). Extracts from the roots are more effective than those from the bark and leaves, similar to the findings of Banso and Olatimayin (2001). The test organisms *E. coli*, *K. pneumonia*, S. *dysentriae* and *P. aeruginosa* are associated with both nosocomial and community acquired infections and is susceptible to the crude plant extracts. These pathogens are known to cause majority of community and hospital acquired infections and are capable of elaborating several virulence factors including the formation of biofilms on colonized surfaces (Indrayan et al. 2002; De and James, 2002). This may explain why the leaves, bark, and stem are used as herbal recipe in the treatment of diarrhea and dysentery urinary tract infections and infections involving the buccal cavity. In an earlier study, Banso and Olutimayin (2001) demonstrated the activity of *D. oliveri*

S. aureus, Streptococcus spp, *E. coli* and *Clostridium perfringenes*. Gentamicin exhibited a higher activity than ethanolic extracts from both plants which in turn exhibited higher activity than aqueous extracts. This could be attributed to the fact that unlike conventional antibiotics and other pharmaceutical products which are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from materials of plant origin, which may be subjected to contamination and deterioration (Babu et al., 2002). The storage of extracts may require special condition of humidity, temperature and or protection from light. The plants extract might contain little of the active ingredient. The extracts which were inactive *in vitro* may have properties similar to pro-drugs which are administered in an inactive form, but their metabolites could be active in-vitro (Lino and Deogracious, 2006).

In conclusion, water and ethanolic extracts of *Nauclea latifolia* and *D. oliveri* were assessed in this study. The results seem to justify their continued use in the treatment of microbial infections. The inhibition of growth of the test organisms that are known to cause nosocomial infections and displaying multidrug resistance to most antibiotics and non-antibiotic antimicrobial agents justify the continued use of these plants in folk and traditional medical practice. Studies should therefore be done in order to identify the active phytochemical constituents and evaluate their effectiveness *in vitro* so that they can be synthesized and commercial production begins in earnest.

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