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Comparative Studies of the Antimicrobial Activity of Components of Different Polarities from the Leaves of *Nauclea latifolia*

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ABSTRACT

The aim of this study is to obtain the 50% methanol extract from the dried leaves of *Nauclea latifolia*, fractionate it and investigate the antimicrobial activity of the resulting components. The aqueous solution of the 50% methanol extract obtained from the dried leaves of *Nauclea latifolia* was partitioned sequentially with hexane, ethyl acetate and butanol to yield fractions of different polarities which were separated by preparative thin layer chromatography to give components. The extract, fractions and components were tested for antimicrobial activity against *Bacillus subtilis, Citrobacter trendi, Enterobacter faecalis, Escherichia coli* ATCC 25922, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus albus, Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* and *Candida albicans* using the dish diffusion method. The results show that phytomedicinal preparations targeted for the management of bacterial and/or fungal infections could be obtained from the extract, fractions and components.

Key words: *Nauclea latifolia*, antimicrobial activity, aqueous methanolic extract, chromatography, solvent polarity

INTRODUCTION

Nauclea latifolia, commonly called Nauclea or pincushion tree or African peach, belongs to the Rubiaceae family. Various parts of the plant including the inner bark, stem, sap, roots, fruits and bark of root have been found useful in the management of sleeping sickness, cough, febrile conditions, thrush, jaundice, piles, stomach and menstrual disorders as well as sores (Gbile *et al*., 1984; Odugbemi, 2008). The stem bark is used as dentifrices and for the management of toothache, dental caries, septic mouth, malaria, diarrhea and dysentery, thus suggesting some antimicrobial activity (Kokwaro, 1976; Akabue and Mittal, 1982; Falodun *et al*., 2007).

The roots of *N. latifolia* combined with those of *Anthocleista djalonensis* and *Uvaria afzalii* are used as phytomedicine for the treatment of Sexually-Transmitted Diseases (STD) (Okoli and Iroegbu, 2004). *N. latifolia* root decoction has been alleged to possess antidepressant, myorelaxant and anti-anxiety-like effects (Taiwe *et al*., 2010).

The aqueous extract of the leaves has been used as a remedy for diabetes mellitus in Northern Nigeria (Gidado *et al*., 2005) and hypertension (Akpanabiatu *et al*., 2005). The antihelmintic activity of the aqueous extract of the stem bark has been demonstrated (Onyeyili *et al*., 2001). The extracts of the roots and stem have been found by Rotimi *et al*. (1988) to exhibit significant antimicrobial activity against a strain of *Bacteriodies melaninogenicus* as well as a strain of *B. gingivalis*. *B. gingivalis* is one of the most frequently isolated oral pathogens in periodontal

diseases (Slots, 1982; Loesche *et al.*, 1985). Okoli and Iroegbu (2004) also reported the antimicrobial activity of the ethanolic and cold water root extract of the plant.

Nauclea latifolia has been found to contain terpenes, alkaloids and glycoalkaloids (Hotellier *et al.*, 1979; Morah, 1995). The leaves have been found extremely useful in the treatment of skin diseases in South-Western Nigeria. The aim of investigating the antimicrobial activity of various extracts from the leaves, namely, 50% methanol extract, non-polar, medium polar and polar fractions obtained by sequential extraction of the aqueous solution of the 50% methanol extract with hexane, ethyl acetate and butanol as well as the components isolated from the fractions by thin layer chromatography (TLC) is to assess the alleged efficacy of the plant in the treatment of various skin diseases.

MATERIALS AND METHODS

Collection, identification and extraction of plant materials: Several batches of the leaves of *N. latifolia* were purchased at Mushin and Oyingbo markets in Lagos, Nigeria, between June and August 2007. They were identified by Dr. A.A. Adekunle of the Department of Botany and Microbiology of the University of Lagos and Mr. Felix Usang of the Forestry Research Institute of Nigeria, Ibadan (FRIN) where a voucher, number 107159, was deposited at the Herbarium of the Botany Department. The fresh leaves were cut into small pieces and air-dried at room temperature in a dust-free environment for about three weeks and powdered in a blender equipped with stainless steel blades. In a typical experiment, 2.65 kg of fresh leaves gave 1.12 kg of dried material. Batches of 400 g of powdered leaves were extracted with 3 L, 50% aqueous methanol at room temperature with intermittent and gentle stirring over a period of 72 h. The residue obtained after filtration was further extracted with 500 mL of the solvent as described over a period of 24 h. The combined filtrate was concentrated in a vacuum oven at 55 °C and freeze-dried giving 75 g of a brownish extract which was sticky and in form of a gel. This is the 50% methanol extract. It was dissolved in 50 mL of distilled water and sequentially extracted as described in a previous report (Okiei *et al.*, 2009) with hexane (6×10 cm^3), ethyl acetate (9×10 cm^3) and butanol (7×10 cm^3) to give a greenish yellow hexane fraction (57 mg), a brown ethyl acetate fraction (1.02 g) and an orange butanol fraction (1.08 g) after evaporation of solvents.

Isolation of components by Thin Layer Chromatography (TLC): Small quantities of each of the various fractions were chromatographed on gel-coated aluminum plates to determine the suitable solvent mixture for the preparative TLC. The hexane fraction was separated into two components labeled Hex a and Hex b using dichloromethane-hexane-methanol (50:40:10) solvent mixture. The ethyl acetate fraction was separated into four components labeled Et- a, b, c, d using ethyl acetate-methanol-water-hexane (70:15:10:1) solvent mixture. The butanol fraction was separated into five components labeled But-a, b, c, d, e using ethyl acetate-methanol-water-dichloromethane (7:2:1:1) solvent mixture.

The various fractions were applied on silica gel (UV lambda at 254 nm with fluorescent indicator) spread on glass plates and fractionated using the corresponding solvent systems. The individual bands were identified with the aid of UV light, scraped into clean beakers and the constituents were eluted using methanol-chloroform (1:1) solvent mixture. In a typical experiment, 57 mg of the hexane fraction gave a yield of component Hex a (19 mg; colourless) and Hex b (18 mg; colourless). 250 mg of ethyl acetate fraction gave a yield of Et a (13 mg; light brown), Et b (12 mg; deep brown), Et c (30 mg; deep brown) and Et d (20 mg; brown). Two hundred

milligram of the butanol fraction gave a yield of But a (12 mg; deep orange), But b (12 mg; light orange), But c (11 mg; light yellow), But d (10 mg; light orange) and But e (10 mg; light yellow).

Preparation of 50% methanol extract, fractions and components for antimicrobial screening: The solutions used for assessment of antimicrobial activity consisted of 50% methanol extract (250 mg cm^{-3}) dissolved in dimethyl sulphoxide (DMSO), the hexane fraction (68 mg cm^{-3}) and hexane components Hex a and Hex b (18 mg cm^{-3}) each dissolved in hexane, the ethyl acetate fraction (70 mg cm^{-3}) and the components Et a, Et b, Et c, Et d (10 mg cm^{-3}), each dissolved in ethyl acetate and the butanol fraction (13 mg cm^{-3}) and the components But a, But b, But c, But d and But e (10 mg cm^{-3}) each dissolved in DMSO. The control was 0.05% ciprofloxacin suspension. All the neat solvents used were tested for antimicrobial activity.

Antimicrobial assay: The test microorganisms were obtained from the collection in the Department of Medical Microbiology and Parasitology of the College of Medicine, University of Lagos and are listed as follows: *Bacillus subtilis, Citrobacter trendi, Enterobacter faecalis, Escherichia coli* ATCC 25922, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus albus, Staphylococcus aureus* ATCC 25923, *Staphylococccus aureus* and *Candida albicans*.

The assay was carried out according to the method of Sharhidi-Bonjar (2004) as adopted in similar assays of extracts from some medicinal plants (Okiei *et al*., 2009). Fifty microliters of the test materials were used for the assay. Clear zones of inhibition around the wells were measured in millimeter and were used as an assessment of positive antimicrobial activity.

RESULTS

The colours of the fractions and components, the masses of the fractions used for the preparative TLC, the yield of the components and their R_f values are presented in Table 1. The results of the sensitivity tests are presented in Table 2. The various solvents used to dissolve the extracts and components, namely hexane, ethyl acetate and DMSO did not inhibit the growth of any of the microorganisms. The 50% methanol extract exhibited strong activity, comparable to that of the control, 0.05% ciprofloxacin, against *B. subtilis*, *E. coli* ATCC 25922,

Table 1: Solvent systems, R_f values and colours of the fractions and components obtained from the 50% methanol extract from the dried leaves of *Nauclea latifolia*

Fraction	Mass fractionated (mg)	Colours of fractions	Solvent systems used for fractionation	R_f values of components	Colours of components	Masses of components (mg)
Hexane fraction	57	Faint greenish yellow	Dichloromethane-hexane-methanol 50:40:10	Hex a 0.69	Colourless	19
				Hex b 0.85	Colourless	18
Ethyl acetate fraction	250	Brown	Ethyl acetate-methanol-water-hexane 70:15:10:1	Et a 0.38	Light brown	13
				Et b 0.72	Deep brown	12
				Et c 0.86	Deep brown	30
				Et e 0.91	Brown	20
Butanol fraction	200	Orange	Ethyl acetate-methanol-water dichloromethane 7:2:1:1	But a 0.24	Deep orange	12
				But b 0.32	Light orange	12
				But c 0.45	Light yellow	11
				But d 0.60	Light orange	10
				But e 0.70	Light yellow	10

Table 2: The results of antimicrobial screening of the various fractions and components obtained from the 50% methanol extract of the dried leaves of *Nauclea latifolia*

Microorganisms	50% methanol extract	Butanol fraction	Butanol Comp. a	Butanol Comp. b	Butanol Comp. c	Butanol Comp. d	Butanol Comp. e
	(mm)						
Bacillus subtilis	20	12	-	-	-	10	-
Citrobacter trendi	-	-	-	-	-	-	-
Enterobacter faecalis	7	-	-	-	-	-	-
Escherichia coli ATCC 25922	20	16	-	-	-	12	-
Escherichia coli	20	12	-	-	-	-	14
Klebsiella pneumoniae	-	11	-	-	-	-	-
Proteus mirabilis	21	15	20	-	11	-	12
Pseudomonas aeruginosa	25	-	-	-	16	-	20
Staphylococcus albus	-	12	-	-	20	-	-
Staphylococcus aureus ATCC 25923	25	12	-	-	-	-	-
Staphylococcus aureus	25	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	14	-

Microorganisms	Ethyl acetate fraction	Ethyl acetate Comp. a	Ethyl acetate Comp. b	Ethyl acetate Comp. c	Ethyl acetate Comp. d	Hexane fraction	Hexane Comp. a	Hexane Comp. b	Cipro-floxacin
	(mm)								
Bacillus subtilis	16	-	10	16	-	-	-	-	23
Citrobacter trendi	-	-	23	14	-	-	-	-	23
Enterobacter faecalis	-	-	-	-	-	-	-	-	-
Escherichia coli ATCC 25922	12	-	-	-	-	-	-	-	20
Escherichia coli	-	22	-	-	-	13	-	-	22
Klebsiella pneumoniae	15	-	-	-	-	-	-	-	24
Proteus mirabilis	13	12	-	-	-	-	-	-	25
Pseudomonas aeruginosa	14	-	-	-	-	-	-	-	20
Staphylococcus albus	-	-	25	22	-	-	-	-	25
Staphylococcus aureus ATCC 25923	-	-	22	-	-	-	-	-	24
Staphylococcus aureus	-	-	13	14	-	-	11	10	19
Candida albicans	12	-	15	14	15	10	15	17	-

E. coli, *P. mirabilis*, *P. aeruginosa*, *S. aureus* ATCC 25923 and *S. aureus*, weak activity against *E. faecalis* and no activity against *C. trendi*, *K. pneumoniae*, *S. albus* and *C. albicans*. It is significant that its activity against *P. aeruginosa* exceeded that of the control.

The butanol fraction showed appreciable activity against *E. coli* ATCC 25922 and *P. mirabilis* (16 and 15 mm inhibitory zone respectively), but lower activity, 11-12 mm inhibitory zone, against *B. subtilis*, *E. coli*, *K. pneumoniae*, *S. albus* and *S. aureus* ATCC 25923 while no activity was observed against *C. trendi*, *E. faecalis*, *P. aeruginosa*, *S. aureus* and *C. albicans*. Component But a exhibited strong activity against only *P. mirabilis* and no activity against the other test microorganisms. Component But b was not active against any of the microorganisms tested. Component But c exhibited strong activity against *S. albus* but lower activity against *P. aeruginosa* and *P. mirabilis* and no activity against the remaining nine microorganisms. Component But d was significantly active with 14 mm inhibitory zone against *C. albicans*, but exhibited lower activity against *E. coli* ATCC 25922 and *B. subtilis* and no activity on the remaining nine microorganisms. But e exhibited strong activity, equal to that of the control, against *P. aeruginosa*, weaker activity against *E. coli* and *P. mirabilis* and no activity against the other microorganisms tested.

The ethyl acetate fraction exhibited significant activity against *B. subtilis, K. pneumoniae and P. aeruginosa* with 16, 15 and 14 mm inhibitory zone respectively, lower activity against *E. coli* ATCC 25922, *P. mirabilis* and *C. albicans* and no activity on the remaining six microorganisms. Ethyl acetate component Et a showed significant activity comparable to that of the control against *E. coli*, lower activity against *P. mirabilis* and no activity against the other microorganisms. Hence component Et a may suffice for the management of infection arising from *E. coli* rather than using the 50% methanol extract. Ethyl acetate component Et b exhibited strong activity of about the same magnitude as the control against *C. trendi, S. albus* and *S. aureus* ATCC 25923. The ethyl acetate fraction did not exhibit activity against any of these three microorganisms. Component Et b was also active, though to a lower degree, against *B. subtilis, S. aureus* and *C. albicans*. Thus component Et b may suffice for the management of infections arising from *C. trendi, S. albus, S. aureus* ATCC 25923 and *C.albicans*. However it did not inhibit the growth of the remaining six microorganisms.

Ethyl acetate component Et c exhibited strong activity, comparable in magnitude to the control, against *S. albus* but exhibited lower activity against *B. subtilis, C. trendi, S. aureus* and *C. albicans* and did not inhibit the growth of the remaining seven microorganisms.

Ethyl acetate component Et d was active against only *C. albicans* and to the same extent as components Et b and Et c but did not inhibit the growth of any of the remaining eleven microorganisms. It is noteworthy that neither the ethyl acetate fraction nor any of its components was active against *E. faecalis*.

The hexane fraction was active against *E. coli,* 13 mm inhibitory zone and exhibited lower activity against *C. albicans* but no activity on the remaining ten microorganisms. Hexane component Hex a was significantly active against *C. albicans*, 15 mm inhibitory zone, but less active against *S. aureus* while hexane component Hex b out of all the test extracts, fractions and components exhibited the highest activity against *C. albicans* though lower activity against *S. aureus*. The two hexane components exhibited activity on only these two microorganisms.

DISCUSSION

The 50% methanol extract was active against eight microorganisms namely *B. subtilis, E. faecalis, E. coli* ATCC 25922, *E. coli, P. mirabilis, P. aeruginosa, S. areus* ATCC 25923 and *S. aureus* all of which with the exception of *E. faecalis* exhibited antimicrobial activity of comparable magnitude to ciprofloxacin. The butanol fraction was active against seven microorganisms, the ethyl acetate fraction against six and the hexane fraction against two and all exhibited lower activity than the 50% methanol extract with respect to the eight microorganisms listed. However, an inspection of the results shows that the butanol fraction was active against *K. pneumoniae* and *S. albus,* the ethyl acetate fraction was also active against *K. pneumoniae* and both the ethyl acetate and hexane fractions were active against *C. albicans*. The 50% methanol extract was inactive against these microorganisms. Thus fractionation of the 50% methanol extract to the three fractions has therefore been of great benefit in that the three fractions obtained when considered in totality exhibited antimicrobial activity against a broader spectrum of microorganisms.

The results show that the butanol fraction exhibited higher activity than any of its components against *B. subtilis, E. coli* ATCC 25922 and both *K. pneumoniae* and *S. aureus* 25923 against which none of the components was active, but the fraction exhibited lower activity against *E. coli* (compared to But e), *P. mirabilis* (compared to But a) and *S. albus* (compared to But c) but no activity against *C. trendi, E. faecalis, S. aureus* (against which none of the components was active),

P. aeruginosa and *C. albicans*. But a was active against only one microorganism, namely *P. mirabilis*, though with increased activity compared to the butanol fraction and the other butanol components. But b was not active against any of the test microorganisms. But c was strongly active against *S. albus*, fairly close in magnitude to the control. But c, But d and But e were each active against three microorganisms. But d was active against *C. albicans* while the butanol fraction and the other butanol components were not. Hence component But d will be effective against infections caused by *C. albicans*. Thus the fractionation of the butanol fraction into components is useful in identifying components which are active against specific microorganisms. For example, components But c and But e were strongly active against *P. aeruginosa* hence more useful than the butanol fraction when the infection is due to *P. aeruginosa*. It should, however, be noted that the butanol fraction was active against *S. aureus* ATCC 29523 but none on the components exhibited such activity.

The results show that while the butanol fraction and its components were active against the test microorganisms with the exception of *C. trendi, E. faecalis* and *S. aureus*, the ethyl acetate fraction and its components in totality exhibited significant activity against all the test microorganisms except *E. faecalis*, while the hexane fraction and its components in totality were active against only three microorganisms, namely, *E. coli*, *S. aureus* and *C. albicans*.

The fractionation of the ethyl acetate fraction resulted in the isolation of components of significant antimicrobial activity; for example Et a, was of comparable activity as the control against *E. coli*, Et b exhibited equal activity as the control against *C. trendi* and *S. albus* and activity close to the control against *S. aureus* ATCC 25923; Et c exhibited activity close to the control against *S. albus* while the ethyl acetate fraction did not show any activity against these three microorganisms. However, the ethyl acetate fraction exhibited activity against *E. coli* ATCC 25922, *K. pneumoniae* and *P. aeruginosa* for which none of the components was active. Thus while fractionation has been useful in producing components of strong activity against some microorganisms the ethyl acetate fraction was found useful against some other microorganisms namely, *E. coli* ATCC 25922, *K. pneumoniae* and *P. aeruginosa* which were not inhibited by its components, thus suggesting some synergy. The ethyl acetate fraction and components Et b, Et c and Et d were all effective against *C. albicans*.

The fractionation of the hexane fraction gave components Hex a and Hex b which were both active against *S. aureus* while the hexane fraction was not and these components were also more significantly active than the fraction against *C. albicans*. However, the hexane fraction was active against *E. coli* while none of the components exhibited such activity. The hexane fraction and components exhibited weaker antimicrobial activity compared to the butanol and ethyl acetate fractions and their components.

In a study by Agyare *et al.* (2006), the methanolic extract of the leaves as well as the methanolic extract of the bark exhibited strong activity against six microorganisms, namely, *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *Candida albicans* and *Aspergillus niger*. The petroleum ether extract of the leaves was active against four microorganisms, namely, *E. coli*, *S. aureus*, *B. subtilis* and *C. albicans* while that of the bark was active against these four and *P. aeruginosa*. The results of the study indicate that the methanolic extracts of both the leaves and bark exhibited appreciable inhibition against all the tested microorganisms but the petroleum ether extracts did not inhibit all the microorganisms.

In our study, *E. coli, P. aeruginosa, S. aureus* and *B. subtilis* were inhibited by 50% methanol extract as well as some of the fractions and components as discussed earlier. The hexane fraction

and components in our study inhibited only *E. coli, S. aureus* and *C. albicans* while the petroleum ether leaf extract (Okoli and Iroegbu, 2004) which is a non-polar extract like the hexane extract and components demonstrated activity against these three microorganisms in addition to *B. subtilis*.

In a study by El-Mahmood *et al*. (2008) aqueous and ethanolic extracts of the leaf, bark and roots were tested against pathogenic bacteria including *P. aeruginosa, Klebsiella pneumoniae, E. coli, S. aureus* and *Shigella dysenteriae*. The aqueous and ethanolic leaf extracts exhibited moderate activity against each of the microorganisms. In the assessment using the two leaf extracts, *K. pneumoniae* and *S. aureus* exhibited similar degree of susceptiblity while *P. aeruginosa* and *E. coli* were of comparable but lower susceptibility. The aqueous and ethanolic root extracts exhibited lower activity against the microorganisms compared to the leaf extracts.

Present study shows that *P. aeruginosa* was susceptible to four test plant items, namely 50% methanol extract which showed higher activity than the ciprofloxacin control, butanol components But c and But e as well as the ethyl acetate fraction. The results indicate the susceptibility of the microorganism to polar and medium polar materials. *E. coli* ATCC 25922 was inhibited by four test plant items, namely 50% methanol extract, butanol fraction, butanol component But d and ethyl acetate fraction while *E. coli* was inhibited by five test plant items, namely 50% methanol extract, butanol fraction, butanol component But e, ethyl acetate component Et a, which had comparable activity as ciprofloxacin, as well as hexane fraction. These results indicate that the *E. coli* ATCC 25922 was susceptible to polar (50% methanol extract, butanol fraction and component) and medium polar (ethyl acetate fraction) materials while *E. coli* was in addition susceptible to the non-polar hexane fraction. Table 2 shows that *K. pneumoniae* was susceptible to only two test plant items, namely the butanol and the ethyl acetate fractions, the latter, that is, the medium polar material, exhibiting more inhibitory activity. The results in Table 2 seem to suggest that of these three microorganisms, *K. pneumoniae* was the least susceptible to the test plant items. The differences in our results and that of El-Mahmood *et al*. (2008) in the degree of susceptibility of *K. pneumoniae* to the test plant items may be due to the differences in the polarity of the extractive media used; the 50% aqueous methanol used in our study being of higher polarity than the aqueous or ethanolic medium used by El-Mahmood *et al*. (2008).

Okwori *et al*. (2008) also evaluated the antibacterial potentials of the hot water, cold water and chloroform extracts of the roots and leaves, diether extract of the roots and petroleum ether extract of the leaves of *N. latifolia* against four clinical bacterial isolates, namely *S. aureus, E. coli, Salmonella typhi* and *P. aeruginosa*. The various extracts of the roots and leaves did not inhibit *S. typhi*. *P. aeruginosa* was largely more susceptible than *S. aureus* to the various leaf extracts.

In our study, *S. aureus* and *P. aeruginosa* exhibited the same susceptibility to the 50% methanol extract. *P. aeruginosa* was susceptible to But c and But e while *S. aureus* was resistant. However *S. aureus* showed appreciable susceptibility to Et b and Et c to which *P. aeruginosa* was resistant while the later showed susceptibility to ethyl acetate fraction to which *S. aureus* was resistant. In addition, *S. aureus* showed susceptibility to Hex a and Hex b which are non-polar in nature while *P. aeruginosa* was resistant. It is pertinent to note that both hexane components which are essentially non-polar in nature exhibited bioactivity against *S. aureus* as was observed for the chloroform and petroleum ether leaf extracts in the study by Okwori *et al*. (2008). However, it must be appreciated that the extractive media are different in the various studies.

It is pertinent to note that the essential oil from the stem of *Cissus populnea*, another plant which has been found useful in the management of skin diseases (Kone *et al*., 2004), has been

investigated for antimicrobial activity and was found to exhibit a significant activity against several of the microorganisms used in this study (Osibote *et al*., 2010).

CONCLUSION

The results of this study indicate that the 50% aqueous methanol extract and several of the fractions and components obtained from the leaves of *N. latifolia* exhibit significant antimicrobial activity against several microorganisms including Gram-positive and Gram-negative bacteria as well as a fungal organism.

The results show that by careful selection of extract, fractions and components, a medication that would be useful for the management of infections arising from all the microorganisms listed could be obtained though with low activity against *E. faecalis*.

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