

ANTIMICROBIAL PROPERTIES OF THE CHLOROFORM EXTRACT OF THE STEM BARK OF NAUCLEA LATIFOLIA

CHINEDU FREDRICK ANOWI¹, NNABUIFE CHINEDU CARDINAL², C.O.EZUGWU³, UCHECHUKWU ANASTASIA UTOH-NEDOSA⁴

¹Department of Pharmacognosy and Traditional medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, ²Department of Pharmaceutical Chemistry, Madonna University, Elele, Rivers State, ³Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, ⁴Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.

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ABSTRACT

Objective – *Nauclea latifolia* had been claimed to have antimicrobial properties by the people of Ogidi in Idemili North Local Government Area of Anambra State of Nigeria who use it to treat wound infections. This study is therefore aimed at determining the correctness of this claim.

The stem bark of *Nauclea latifolia* was used for this investigation and the results of the study will also serve as a criteria to recommend the Ethnopharmacological uses of the plant

Methodology – The stem bark was dried, powdered and extracted by cold maceration with chloroform for 48hours. This was concentrated using rotary evaporator. Phytochemical evaluation revealed the presence of alkaloids, tannins, glycosides and saponin in varying degrees. The antimicrobial activities (sensitivity test) of the crude extracts and the standards (augmentin; an antibacterial agent and ketoconazole; an antifungal agent) were evaluated against Gram positive and Gram negative bacteria and fungi using agar diffusion method at the concentration of 100mg/ml for extracts and 50µg/ml for standards. The same method was used to evaluate the minimum inhibitory concentration.

Result - results show that chloroform extract of the stem bark exhibited activity against *E.coli*, *S. dysenteriae*, *S. aureus*, *B. subtilis* and *A. niger*, with minimum inhibitory concentration (MIC) ranging from 2.97mg/ml- 19.95mg/ml. On *Pseudomonas aeruginosa*, the chloroform extract of stem bark also showed significant effectiveness when compared to the standard. The percentage susceptibility test indicated that the effect of ethyl chloroform extract of stem bark on *S. dysenteriae* is very high (81.6%).

Conclusion - From the results obtained, it could therefore be concluded that the stem bark of *Nauclea latifolia* possesses broad spectrum antimicrobial activities. *Nauclea latifolia* may be useful in the formulation of antimicrobial agent that could be used for the treatment of microbial infections of different origins.

Keywords: *Nauclea latifolia*, Ketoconazole, Minimum bactericidal activity

INTRODUCTION

Nauclea latifolia Smith (family: Rubiaceae) is a straggling, evergreen, multi-stemmed shrub or small tree native to tropical Africa and Asia. It grows up to an altitude of 200 m. It is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. It grows rarely over 20ft high, bole crooked; or a larger tree over 100ft high and 8ft girth, in closed forest. The plant has rough bark, leaves are 7 by 4-5 inches and are glabrous obovate. Flower head is up to 2mm in diameter, sweet scented and sought by bees. Three other related species *Nauclea pobeguini*, *N. diderichii*, and *N. vanderghuchtii* are forest trees. *N. Diderichii* is planted in Omo forest reserve, Nigeria.

In folk medicine, the species *N. diderichii* and *N. orientalis* are used in the same way as *N. latifolia*. *Nauclea latifolia* has an open canopy and terminal spherical head lined cymes of white flowers. The flowers are joined with their calyces. The fruit is syncarp, up to 3 inches in diameter. The tree flowers from April to June. The fruits ripen from July to September. Baboons eat them and disperse the seeds. Livestock eat shoots and leaves. The fruits are edible too.

Plant Taxonomy

Family: RUBIACEAE

Genus: *Nauclea*

Species: *latifolia*

Accepted name: *Nauclea latifolia* Sm.

Synonyms: *Sarcocephalus latifolius* (Sm.) E.A.Bruce

Common names: English; African peach, Pin cushion tree, Guinea peach.

Igbo; Ubulinu.

French; Scille maritime, oignon marine.

Hausa; Tafashiya, tafiyaigia.

Traditional uses of *Nauclea latifolia*

Nearly all plant parts are useful in treatment of diseases. Infusions and decoctions of the stem bark and leaves of *Nauclea latifolia* are used for the treatment of stomach pain, constipation, fever, and diarrhea. In kano (Nigeria) the plant is used as a chewing stick and as a remedy against stomach ache and tuberculosis (Deeni & Hussain 1991). In Ivory Coast infusions and decoctions from stems and roots of *N. latifolia* are used against malaria by traditional healers (Benoil-vical, Valentin, counal et al. 1998). In Kinshasa, extracts and preparations together with other plants are applied against diarrhoea (Tona, Kambu, Ngimbi et al. 2000). Abbiw (1990) stated that root infusion of *N. latifolia* is used in Sudan for the treatment of gonorrhoea its roots and leaves are used in Ghana for treating sores. In Nigerian folklore medicine, the fruits are sometimes used in the treatment of piles and dysentery. Because of its reported anti-malarial activity, the plant has been known as 'African cinchona' or 'African quinine'. (Abbiw 1990; Gidado, Ameh, & Atawodi, 2004), demonstrated that aqueous extract of the leaves of *N. latifolia* possess hypoglycaemic activity in alloxan-induced diabetic rats. The plant is also used in hypertension (Akabue and Mittal, 1982), gastrointestinal tract disorders (Maduabunyi, 1995), sleeping sickness (Kerharo, 1974), and to prolong menstrual flow (Elujoba, 1995).

MATERIALS AND METHODS

Materials

❖ Standard Drugs

- Amoxicillin/ potassium clavulanate (Augmentin[®]).
- Ketoconazole; (Korlyns).

❖ Microorganisms

- Gram positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis*
- Gram negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella dysenteriae*.
- Fungi; *Candida albicans* and *Aspergillus niger*.

❖ Reagents/ Chemicals

chloroform, Dragendorff's reagent, Hager's reagent, Concentrated sulphuric acid (H₂SO₄) (BDH Chemicals), concentrated hydrochloric acid, ferric chloride hexahydrate, copper sulphate pentahydrate, sodium tartarate, sodium hydroxide,

❖ Biological Media

- Nutrient Agar (Fluka)
- Nutrient Broth (Fluka)
- Saubaraud dextrose agar (Biochemika)

❖ Equipment

Electrical weighing balance (OHAUS Model 2610), Laboratory Incubator (Sanfa-Moodel No DNP-9022A), wooden mortar and pestle, laboratory oven (Surgienfield Instrument, England), digital water bath (Sanfa-Model No DK420), autoclave (Health team instrument, England), Refrigerator (LG), Bunsen burner.

❖ Apparatus/Glass wares

Petri-dishes, Pasteur pipettes, swab sticks, calibrated syringes, measuring cylinders, beakers, test tubes, reagents bottles, stirrer, inoculating loop, cork borer.

Methods

Plant Collection and Identification and Preparation

The fresh stem bark of *Nauclea Latifolia* was collected from a bush at Ogidi, Idemili North Local Govt. Area Anambra State, Nigeria in November 2010 and was identified as *Nauclea latifolia* by Dr Ezugwu of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The stem bark was dried under the shade for seven days. The dried materials were powdered using locally made mortar and pestle, weighed in an electronic weighing balance and stored in an air-tight container.

Extraction Procedure

500gm of the pulverised powder of the stem bark of *Nauclea latifolia* was macerated in 1500ml (1.5L) of chloroform (AnalaR grade), in about 3L capacity glass ware and was made air-tight and left for two days with intermittent shaking. The mixture was filtered using Whatman's filter paper No. 1 to obtain a solution devoid of solids. The chloroform extract was evaporated using rotary evaporator to get dried concentrate.

Phytochemical Screening

Qualitative assay for the presence of secondary plant metabolites were carried out on methanolic extract of the stem bark of *Nauclea latifolia* using the standard procedures (Harborne 1991), (Trease and Evans, 1989).

Tests for alkaloids

Being bases, a 100mg of the crude was boiled for about 2 minutes with 5ml of 2M HCl on a steam bath. The mixtures were filtered and to 1ml of filtrate was added 2 drops of Dragendorff's reagent (bismuth potassium iodine solution), (brick red colouration test). To another 1ml of each filtrate, 2 drops of Hagers (saturated solution of picric acid) reagent was also added. The test tubes were observed for coloured precipitates.

Tests for tannins

Preparation of ferric chloride test solution;

Ferric chloride hexahydrate.....7.50g.

Concentrated hydrochloric acid.....1.0ml.

Water to.....100mls.

A few drops of ferric chloride were added to the test tubes containing about 2ml of extract. The test tubes were observed for greenish-black precipitates.

Another 2ml each of the extract was diluted to 10ml and few drops of lead acetate solution added. A redish colour indicated the presence of tannins.

Test for Saponins

Frothing test; 5ml of the extract was diluted with 10ml of distilled water; the solutions were then vigorously shaken and observed on standing to obtain persistent of foam.

Test for Sterols

Preparation of Liebermann-Burchard's reagent;

Acetic anhydride.....1.0g.

Concentrated sulphuric acid.....2.0g.

Chloroform.....30ml.

Liebermann Burchard's reagent was added to the sample and was observed for bluish green colour reaction.

Tests for Glycosides

Preparation of Fehling's solution;

Copper sulphate pentahydrate.....3.46g.

Sodium potassium tatarate.....17.3g.

Sodium hydroxide.....6.0g.

Water to100ml.

5ml of the extract was mixed separately with 2ml of Fehling's solution (freshly prepared) and then boiled in water bath for 15 minutes. The sample was observed for a brick red precipitate which indicated the presence of reducing sugars and is also an indirect determinant of presence of glycosides.

A 3ml of dilute sulphuric acid was added to the mixture (above) and was boiled for another 15min. and cooled. The sample was observed for an increase in the amount of precipitate previously formed. Theoretically, more precipitate indirectly confirms the presence of glycosides since the reducing sugars are obtained from the hydrolysis of glycosides present.

Antimicrobial Activity Screening

Preparation of inoculums

The bacteria used were *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *acillus subtilis* and *Staphylococcus aureus*. The fungi used were *Aspergillus niger* and *Candida albicans*. All microorganisms were isolated from clinical specimens obtained from the Medical Microbiology department of Madonna University Elele, Rivers State Nigeria. The test organisms were separately prepared by subculturing the pure isolates in nutrient agar and incubated at 37°C for 24 hours for bacteria and in sabaraud agar for 72 hours for fungi. Two loopfuls of the microbial culture were collected using sterilized (heat fixed) inoculating loop into 10mls nutrient broth contained in sterilized universal bottles and then incubated at 37° C overnight for subsequent use. 0.2ml of the overnight cultures of different organisms was then diluted with 20ml nutrient broth to give 1 in 100 dilution equivalents to 1x 10⁶cfu/ml which were then used for the study.

Sterilisation of working materials

Petri dishes and pipettes, were washed with detergents, rinsed with distilled water and wrapped with aluminium foil before they were sterilised in hot air oven at a temperature of about 100°C for about one hour. The laboratory benches were cleaned with 70% alcohol before and after each experiment.

The tests carried out were;

- Sensitivity test.
- Minimum inhibitory concentration (MIC).
- Minimum bactericidal concentration (MBC)

Preparation of the culture media

Culture media are commercially available in the dehydrated form. The nutrient agar medium was prepared by suspending 28g of the nutrient agar in one litre of distilled water. The suspension was then dissolved completely. It was then sterilised by autoclaving at 121°C for 15 minutes.

Sensitivity Test

The method used here was agar diffusion method. 20ml of nutrient agar was melted in water bath at 100°C for thirty minutes after which they were stabilised at 45°C for 15 minutes. Each molten agar inoculated with 0.2ml (containing 10⁶CFU) of a 24-hour culture of the test organisms. The dishes were rotated to homogenize with the microorganism. The seeded agar were poured into separate sterile petri-dishes and allowed to set. Using a sterile cork borer, four cups were bored in the set agar. The plates were turned upside down, divided into four and cups were labelled appropriately. The extracts were reconstituted by dissolving 1gm of each in 1ml of water. Two cups in each petri dish were filled with 100mg equivalent of the chloroform extracts from the stem bark of *Nauclea latifolia*. The remaining cups were filled with 25µg/ml equivalent of standard drug. The stem bark chloroform extract was tested against five bacteria (*Escherichia Coli*, *Pseudomonas auriginosa*, *Neisseria gonorrhoea*, *Staphylococcus aureus*, *Bacillus subtilis* and *Shigella dysenteriae*). 25µg/ml of amoxicillin/potassium clavulanate solution was used as comparative standard drug for the bacteria.

Sabouraud dextrose agar was used as the culture medium for the fungi and the above procedure repeated. The extract was tested against two fungi (*Aspergillus niger* and *Candida albicans*). The comparative drug used was ketoconazole (25µg/ml).

The plates were allowed to stand for one hour to allow adequate diffusion of the extracts and the drugs. The plates seeded with bacteria were then incubated at 37°C for 24 hours and other plates seeded with fungi were incubated at 28°C for 5days. The zones of inhibition were measured in millimetre (mm) and the average found and recorded (Collin et al. 1995).

Sensitivity Test Result

Table 2: Sensitivity results of the extracts and standards

Mean inhibition zone diameter							
Extracts	P.A	E.C	S.A	S.D	B.S	C.A	A.N
CE / SB	10.6±0.9	17.8±0.9	15.8±0.5	15.5±0.2	17.0±0.5	15.1±1.1	-
AUG.	15.0±1.0	28.3±1.1	25.4±0.4	19.0±1.2	27.2±0.8	-	-
KET.	-	-	-	-	-	27.1±0.05	18.0±0.8

KEY:

P.A = *Pseudomonas aeruginosa*. E.C. = *Escherichia coli*. S. A. = *Staphylococcus aureus*

S.D. = *Shigella dysenteriae* B. S. = *Bacillus subtilis*. C. A. = *Candida albicans* A.N. = *Aspergillus niger* AUG. = augmentin[®] KET. = ketoconazole.

SB. = stem bark CE. Chloroform extract. - no activity

Determination of minimum Inhibitory Concentration (MIC)

The back of the agar plate was divided into 5 parts. 0.2 ml of a standard suspension of each microorganism was placed into 2 separate petri dishes for each microorganism. The prepared sterile molten agar was poured into each of the plates and mixed by rotating each plate to homogenise the microorganism. The agar was allowed to set on a flat surface for 10min.

A cup was made up on the agar using a sterile cork borer of 9mm in diameter at the center of each section. The cups were labelled to indicate the concentration of the extract to be introduced into each cup. After boring the cups the extract was introduced into the corresponding cups and allowed for diffusion for about 15 minutes and incubated at 37°C for 24 hours. The fungi dishes were incubated at 28°C for four days. The zones of inhibition were measured and the mean recorded. The inhibition distance was determined by subtracting the diameter of the cup (9mm) from the zone of inhibition. A graph of the square of the corresponding mean inhibition distance was plotted against the log concentration of the extracts and of the drugs. A straight line of best fit was drawn and extrapolated to the log concentration axis. The resultant intercept was recorded as the log minimum inhibitory concentration (MIC) against that organism. The antilog of this is the minimum inhibitory concentration.

RESULTS

Statistical Analysis

The results were expressed as means ± standard error of mean (S.E.M). Percentage susceptibility of the extract was evaluated using the standards as 100%. The significance difference between the mean values were measured using analysis of variance (ANOVA) at P<0.05.

Secondary metabolites present in the extracts:

Secondary plant metabolites found in ethyl acetate extract of *Nauclea latifolia*.

Table 1: Secondary plant metabolites found in extracts of *Nauclea latifolia*.

Secondary	Stem bark
Metabolites	CE
Alkaloids	++
Glycoside	++
Saponins	++
Sterols	-
Tanins	++

KEY:

CE = chloroform extract.

++ = presence of the compound.

_ = compound not detected.

+ = trace amount of compound.

Table 3: Result of percent susceptibility of the extracts and standards

Microorganisms	Chloroform extract		Standards	
	SB		AUG	KET.
<i>P. aeruginosa</i>	70.7		100	-
<i>E. coli</i>	62.9		100	-
<i>S. aureus</i>	62.2		100	-
<i>S. dysenteriae</i>	81.6		100	-
<i>B. subtilis</i>	62.5		100	-
<i>C. albicans</i>	55.7		-	100
<i>A. niger</i>	-		-	100

KEY:

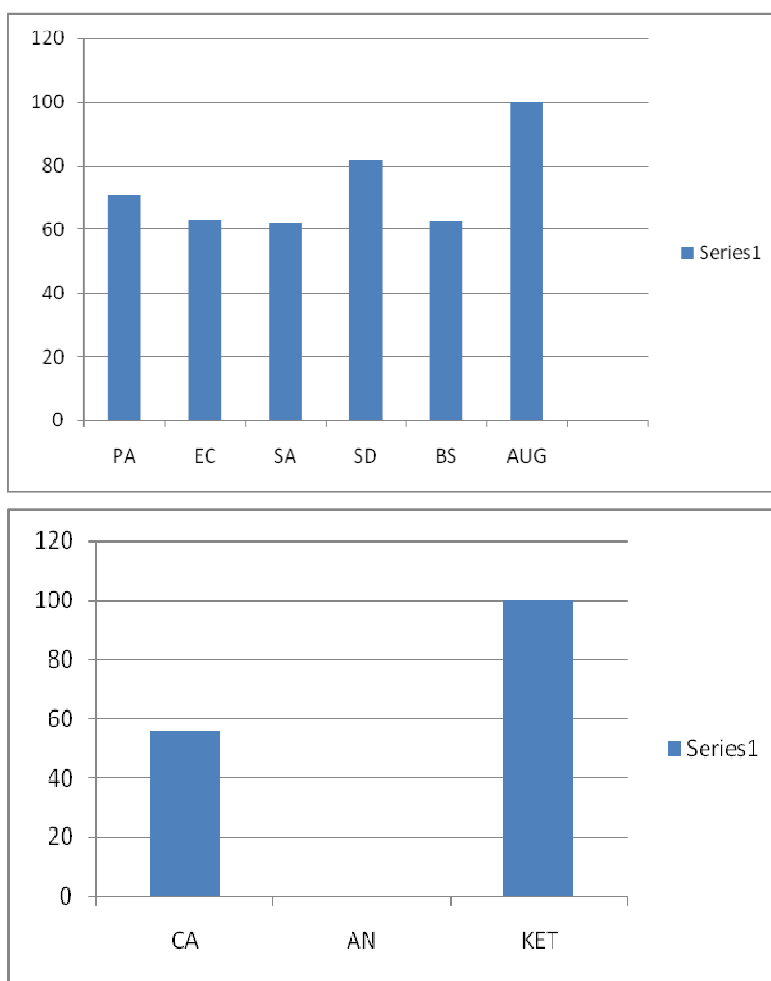
- No activity AUG. = augmentin[®] KET. = ketoconazole. SB. = stem bark

Fig. 1 (above); Fig. 2 (below) Bar Charts of % susceptibility of the extract

Key; PA=*Pseudomonas aeruginosa*, EC= *Escherichia coli*, SA=*S. aureus* SD= *S.dysenteriae*, BC= *B.subtilis*, CA= *C.albicans*, KET= Ketoconazole AUG= Augmentin

Table 4: Result of inhibition zone diameter (mm) of the chloroform extract of the stem bark of *Nauclea latifolia*;

Organisms	Inhibition zone diameter (mm)				
	Concentration (mg/ml)				
	200	100	50	25	12.5
<i>P. aeruginosa</i>	12.5	11.6	10.0	9.0	5.0
<i>E. coli</i>	18.0	16.2	13.5	12	9.1
<i>S. aureus</i>	20.1	15.9	15.4	14	11.5
<i>S. dysenteriae</i>	19.5	17.6	16.0	15.1	8.7
<i>B. subtilis</i>	22.2	19.0	15.7	11.6	7.1
<i>C. albicans</i>	15.0	13.9	10.3	10.0	8.2
<i>A. niger</i>	6.3	4.4	0	0	0

Table 5: Result of inhibition zone diameter squared (mm²) and the Log concentration of the chloroform extract of stem bark of *Nauclea latifolia*

Organisms	Inhibition Zone Diameter squared				
	Log concentration (200mg/ml-12.5mg/ml)				
	2.30103	2.00000	1.69897	1.39794	1.09691
<i>P. aeruginosa</i>	156.25	134.56	100	81	25
<i>E. coli</i>	324	262.44	182.25	144	82.81
<i>S. aureus</i>	404.01	252.81	237.16	196	132.25
<i>S. dysenteriae</i>	380.25	309.76	256	228	75.69
<i>B. subtilis</i>	492.84	361	246.49	134.56	50.41
<i>C. albicans</i>	225	193.21	106.09	100	67.24
<i>A. niger</i>	39.69	19.36	0	0	0

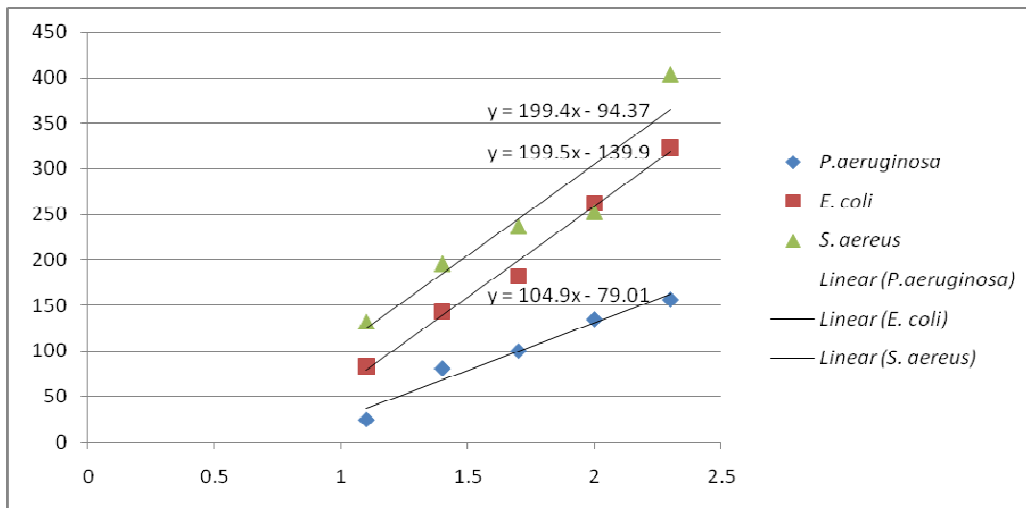


Fig. 3: Fi Plot of square of inhibition zone diameter (IZD) against the log concentration of chloroform extract of stem bark of *Nauclea latifolia*

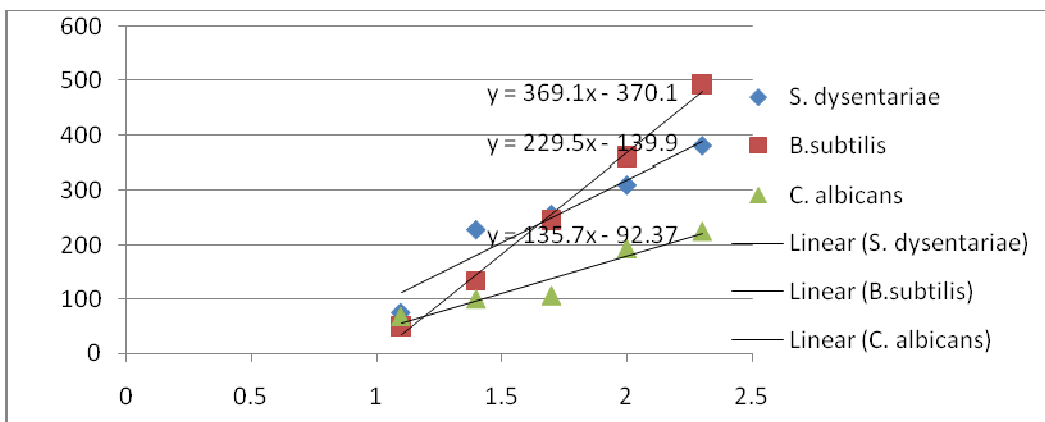


Fig. 4: Plot of square of inhibition zone diameter (IZD) against the log concentration of chloroform extract of stem bark of *Nauclea latifolia*

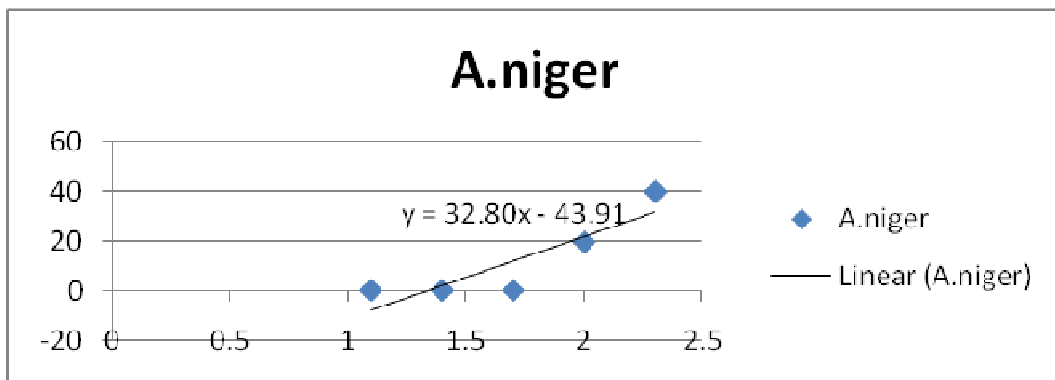


Fig. 5: Results of log conc. (mg/ml) of the extracts of leaves and stem bark of *Nauclea latifolia* and the standards (µg/ml)

Table 6: The no activity log of concentrations were extrapolated from the x-axis of the graph and then converted to antilogarithm which gave the minimum inhibitory concentration (MIC) of each of the clinical isolates in mg/ml for the extracts and µg/ml for the standards.

Test Organisms.	CE/SB [(mg/ml)-]	AUG. [---- (µg/ml)----]	KET.
<i>P. aeruginosa</i>	0.753	0.697	-
<i>E. coli</i>	0.70	0.260	-
<i>S. aureus</i>	0.473	0.160	-
<i>S. dysenteriae</i>	0.609	0.186	-
<i>B. subtilis</i>	1.002	0.380	-
<i>C. albicans</i>	0.680	-	0.087
<i>A. niger</i>	1.300	-	0.722

Key;

Aug. = augmentin^R ket. = ketoconazole. SB. = stem bark. CE. Chloroform extract –

The no activity log of concentrations were extrapolated from the x-axis of the graph and then converted to antilogarithm which gave the minimum inhibitory concentration (MIC) of each of the clinical isolates in mg/ml for the extracts and µg/ml for the standards.

Table 7: Results of MIC (antilog.) of the extracts and standards

Test Organisms.	CE/SB [(Mg/ml)]	AUG. [---- (µg/ml) --]	KET.
<i>P. aeruginosa</i>	5.66	4.98	-
<i>E. coli</i>	5.01	1.82	-
<i>S. aureus</i>	2.97	1.45	-
<i>S. dysenteriae</i>	4.06	1.53	-
<i>B. subtilis</i>	10.00	2.40	-
<i>C. albicans</i>	4.79	-	1.22
<i>A. niger</i>	19.95	-	5.23

Key;

Aug. = augmentin^R ket. = ketoconazole. SB. = stem bark. CE. Chloroform extract. – no activity'

DISCUSSIONS

There is increased research on the medicinal effects of plant extracts as traditional medicinal plants are seen as future source of new drugs (Soumya Prakash Rout et al, 2009). For example, *Portulaca oleracea* was found to have anti-oxidant properties (S> D. Sanja et al, 2009; *Solanum surattense* demonstrated anti-helminthic properties Bhabani S. Nayak et al, 2009; the latex of *Caltropis gigantea* demonstrated wound healing effects (Narendra Nalwaya et al, 2009 and aqueous extract of aerial parts of *Phyla nodiflora* Linn demonstrated diuretic effects (Sangita Shukla et al, 2009).

The present study was carried out to evaluate the antimicrobial activity of chloroform extract of stem bark of *Nauclea latifolia*, and their phytochemical constituents.

The phytochemical analysis revealed the presence of tannins, saponins, alkaloids, and glycosides in varying degrees in all the extracts of the stem bark. These secondary metabolites have been reported to exhibit varied biochemical and pharmacological effects in animals and microorganisms when ingested (Trease and Evans 2008). The high content of saponins and tannins, could be the basis for its antimicrobial activity. Tannins act by coagulating the cell wall proteins (Trease and Evans, 1983), while saponin causes the lysis of the bacterial cells (Robinson, 1975). This may therefore explain the demonstration of antimicrobial activity by chloroform extract of stem bark of *Nauclea latifolia*.

De and Ifeoma (2002), reported that the phytochemical components also offer plants themselves protection against infection by pathogenic microorganisms.

These results obtained in the phytochemical screening correlate with the work done by Hotellier et al., (1979) and Morah (1995) who reported that *Nauclea latifolia* contains terpenes, alkaloids, glycoalkaloids and tannins.

The emerging antimicrobial resistance to diseases have compromised chemotherapy of patients suffering from severe infectious diseases especially in Nigeria. These infectious diseases include urinary tract infection and severe diarrhoea caused by *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae*. Wound infections, otitis media, genital tract infections, periodontal diseases

and osteomyelitis in children are mainly caused by *Streptococcus spp.* and *B. subtilis* (Walker and Whittlesea, 2007). The antimicrobial evaluation results of chloroform extract of the stem bark of *Nauclea latifolia* on the selected clinical isolates: Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa*) and fungi (*Aspergillus niger*, *Candida albicans*) revealed the antimicrobial efficacy of stem bark of this plant. The observed inhibitory properties of the extract against these clinical bacteria and fungi isolates using agar diffusion method indicated that *Nauclea latifolia* possesses a broad spectrum of antimicrobial activity (with reference to the standard antimicrobials used). The percentage susceptibility observed were *Escherichia coli* 64%, *S. aureus* 64%, *S. dysenteriae* 82%, *B. subtilis* 64% and *C. Albicans* 58%. *S. dysenteriae* was most susceptible. *Pseudomonas aeruginosa* 70% is susceptible and *A. niger* was resistant.

On comparing the activity of the extract with that of the standard, there was no significant difference (p>0.05) between the activity of the extract and that of *S. dysenteriae*; a significant difference (p<0.05) between the activity of the extract and that of the standard against *P. aeruginosa* and a very significant difference (p<0.01) between the activity of the extract and that of the standard on the other clinical isolates used in this study.

CONCLUSION

The present study shows *Nauclea latifolia* as a medicinal plant with lot of potential as antimicrobial agent. The observed antimicrobial effects in the present study justifies the ethnomedicinal use of the plant as chemotherapy for infectious diseases.

Recommendations

In view of the present findings, it seems important to recommend for further bioassay targeted technique studies on the crude extracts. That is purification and identification of the constituents with antimicrobial properties as well as its effect on more pathogenic organisms are recommended as this would produce more specific and effective results.

It would also make a great impact in the health care system and the populace if this work on in vitro studies is extended to in vivo

determination of antimicrobial effects using animals. This is suggested because the extracts which were inactive in vitro may have properties similar to prodrugs which are administered in an inactive form, but their metabolites could be active in vivo (Lino and Deogracios, 2006).

Finally, Health foundations should increase their efforts on the plant research as it is a potential source of broad spectrum antimicrobial agent that could be helpful in reducing the emergence and spread of antimicrobial resistance.

REFERENCES

- Abbiw K D, (1990). Useful Plants of Ghana, West Africa; uses of wild and cultivated plants, Intermediate Technology Publication, London pp 98- 212.
- Aguiyi J C (2006), Antifungal Agents. In Akubue PI (ed.), Textbook of Pharmacology. Africana First Publishers Ltd Onitsha, pp 496-500.
- Akabue P, Mittal H C (1982). Clinical Evaluation of a Traditional Herbal Practice in Nigeria: A Preliminary Rep. J. Ethnopharmacol. 6(3):355-359.
- Akubue P I (2006). Textbook of Pharmacology, 1st Ed. Africa First Publishers Ltd Onitsha.
- Banso A and Adeyemo S O, (2007). Comparative studies of antimicrobial properties of *Cardiospermum gradiflorum* and *Cardiospermum halicacabum*. Nigerian journal of Health and Biomedical science. Pp 31-34.
- Benoit-Vical F, Valentin A, Cournac V et al. (1998) In vitro antiplasmodial activity of stem and root extracts of *Nauclea latifolia* S.M. (Rubiaceae) J Ethnopharmacol 61: 173-8
- Collins C H, Patricia N L, Lyme M And Grange J M (1995), Microbial Methods 7th Ed. Butler-Swirth Heinnemann.
- David A L, Robin R, Alastair D B, David H And Stewart F (2008). Muir's Textbook of Pathology, 14th Ed. Power Publisher, India pp 508-509
- De N and Ifeoma E (2002), Antimicrobial effects of components of the bark extract of neeru (*Azadirachta indica* A. Juss. Tech. Dev. (8):23-28.
- Deeni Y Y, Hussain H S N (1991) Screening for antimicrobial activity and for alkaloids of *Nauclea latifolia* J Ethnopharmacol 35: 91-6.
- Elujoba A A A (1995). Female Infertility in the Hands of Traditional Birth Attendants in South-West Nigeria. Fitoterapia 66(3):239 – 248.
- Gidado A, Ameh D A, Atawodi S E, (2005). Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. African J. Biotech. 4(1): 91-93.
- Harborne J A, (1991). Phytochemical Methods, A guide to common techniques of plant analysis
- Hotellier F, Delaveau P, Pousset J L (1979). Alkaloids and Glycoalkaloids from Leaves of *Nauclea latifolia*. Planta Medica. 35:242 – 250
- Lino A, Deogracios O (2006). The in-vitro antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steanotaenia araliacea*- Ugandan Medicinal plants. Afr. Health Sci. 6(1):31-35.
- Madubunyi I I, (1995). Anti- Hepatotoxic and Trypanocidal Activities of the Ethanolic Extract of *Nauclea latifolia* Root Bark. J. Herbs Spices Med Plants. 3 (2):23 – 53.
- Morah F N I (1995). Naucleal and Epinaucleal from an Antiviral Preparation from *Nauclea latifolia*. Global J. Pure Appl. Sci. 1(1 – 2):59 – 62.
- Ohadoma S C (2008). The Pharmacology Made Easy. 1st Ed. Publishers by Reverend print, Nigeria. 367-370, 402
- Rang H P, Dale M M, Ritter J M, Flower RJ, (2007). Rang and Dale's Pharmacology, 6th Ed. USA, Elsevier pp:47
- Trease G E. and Evans W C, (1983). Pharmacognosy. 11th Ed. Balliere and Tindall East Bourne, London. 243-551
- Trease G E and Evans W C, (2008). Pharmacognosy. 5th Ed. Elsevier publishers, India.
- World Health Organisation (2002). Epidemiology of Nosocomial Infections. In: Prevention of Hospital Acquired Infections. A Practical Guide, 2nd eds (Ducel G, Fabry J, Nicolle L, eds Malta. pp. 4-16.
- Soumya Prakash Rout, K. A> Coudary, D. M. Kar, Lopamudra Das/ Avijet Jain (2009) Plants in Traditional Medicinal System-future source of new drugs, International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1 (issue 1) July-Sept 2009
- S. D. Sanja, N. R. SHeth, N. K. Patel, Dhavel Patel, Biraju Patel (2009) Characterisation and evaluation of anti-oxidant activity of portulaka oleracea , International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1 (issue 1) July –Sept 2009.
- Bhabani S. Nayak, Prabhat K Jena, Nigam P Sahu, Udaya K Nayak, K Balakrishna Patro. Comparative study of antihelminthic activity between Aqueous and ethanolic extract of *Solanum surattense* Linn; International Journal of Pharmacy and Pharmaceutical sciences, Vol. 1 (issue 1) July –Sept 2009.
- Narendra Nalwaya, Gauray Pokharna, Lokesh Deb, Naveen Kumarjain. Wound healing activity of the latex of *Calotropis gigantea*; International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1 (issue 1) July –Sept 2009.
- Sangita Shukla, Rashmika Patel, Rajiv Kukkar; study of phytochemical and diuretic potential of methanol and aqueous extracts of aerial parts of *Phyla Nodiflora* Linn; International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1 (issue 1) July –Sept 2009.