Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2011, 1 (3):16-22

ISSN : 2249 – 7412

Phytochemical analysis and the chemotherapeutics of leaves and stem-bark of *Nauclea latifolia* grown in Hong, Adamawa State Nigeria

O.N. Maitera, M. E. Khan* and T. F. James

Department of Chemistry Adamawa State University Mubi, P. M. B. 25 Mubi, Nigeria

---

ABSTRACT

Methanol and aqueous extracts of leaves, stem-bark and roots of the indigenous medicinal plant Nauclea latifolia (Rubiaceae), grown in Hong Local Government Area, Adamawa State, Nigeria, were analyzed and found to confirm to their folkloric claims of malaria, jaundice, diarrhea, hypertension, and tuberculosis treatment. Phytochemical screening of the different parts of the plant confirmed the presence of metabolites like saponins, alkaloids, glycosides, tannins, flavonoids and anthraquinones. Steroids were absent in all plant parts. Antimicrobial studies of the methanol, n-hexane and aqueous extracts showed inhibitory activity on Escherichia coli, Streptococcus pneumonia, Shigella, dysenteriae, and Staphylococcus aureus, with zones of inhibitions of 10 -25mm.This invariably confirms the folkloric usage of the various plant parts in the treatment of the listed diseases with careful selection of extracting solvent.

Keywords: Phytochemical, malaria, diarrhea/dysentery, *Nauclea latifolia*, Hong, anthraquinones.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years –such extensive dependence of human being on "*Mother Nature*" has invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with potentials for multipurpose uses (Cragg and Newman, 2001, Brahmachari, 2006).

Due to its multidirectional promising aspects, the interest in natural product continues to this day (Barron and Vanscoy, 1993; Chan, 1995; Koh and Woo, 2000; Kaul and Joshi, 2001; Kroll, 2001; Marriott, 2001; Bhattaram *et al*., 2002; Holt and Chandra, 2002).The use of herbal drugs is once more escalating in the form of complementary and alternative medicine (CAM) (Cooper, 2004). This phenomenon has been mirrored by an increasing attention to phytomedicines as a form of alternative therapy by the health professions; in many developing countries there is still a

major reliance on crude drugs prepared from plants used in traditional medicines, for primary healthcare (Chang and But, 1986; Dev, 1999; Kapoor, 1990; Schultes and Raffaut, 1990). The World Health Organization (WHO) estimates that approximately 80% of the world`s population relies mainly on traditional medicine, predominantly originated from plants, for their primary healthcare (Farnsworth *et al*, 1985; Arvigo and Balick1993).

Although about 200,000 natural compounds are currently known, this figure is just a drop of water in the ocean considering the wideness of natural resources. Despite the availability of a vast range of territorial flora, it is estimated that only 5 -15 % of the approximately 250,000 species of higher plants have been investigated chemically and pharmacologically so far. Hence, the potential of large areas of tropical rainforests remains virtually untapped (Balandrin *et al*., 1993). The world's oceans, covering more than 70% of the earth`s surface and the potential of which has barely been tapped, stand as an enormous resource for the discovery of potential chemotherapeutic agents primarily from invertebrates (Mc Connell, *et al*.,1994; Newman *et al*., 2000; Donia and Hamann, 2003).Another vast unexplored area is the microbial world (Colwell, 1997); it has been reported that "less than 1% bacterial species and less than 5 % of fungal species are currently known and recent evidences indicate that millions of microbial species remain undiscovered (Yong, 1997).

The continuing treat to biodiversity thorough the destruction of terrestrial and marine ecosystems lends urgency to the need to expand the exploration of these resources as a source of novel bioactive chemotypes and pharmacophores which would eventually serve the purpose of the pharmacy industries for the search of "lead" for new effective drugs against variety of disease types. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Much of the exploration and utilization of natural products as antimicrobials arise from microbial sources. It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin. (Trease 1989).

In Hong LGAs of Adamawa State Northeastern, Nigeria, malaria, diarrhea and dysentery are health challenges (Emokpae *et al*., 2006) and is believed that *Nauclea latifolia* possesses medicinal properties effective in their management since concoctions and decoctions of this plant are used for their cure.

Nauclea latifolia (Rubiaceae), common name "Bishop`s head", *"Mbom-mbog"* in Akwa Ibom and Cross River States in Nigeria, *"Tabashiya"*in most parts of the north and *"molsa*" in Kilba, is an ever green multi-stemmed shrub that grows up to an altitude of 200m, with flowers joined to the calyces. The fruits are syncarpous (Edet, 2005).

Traditionally, in West and South Africa infusions and decoctions of the stem bark and leaves of the plant are used for the treatment of malaria, stomach ache, fever, diarrhea and nematodes infections in human and animals. In Kano, Nigeria, it is used as a chewing stick and as a remedy against stomach ache and tuberculosis (Deeni, 1999).In Hong Adamawa State, concoctions, infusions and decoctions from stem bark and roots are used against jaundice, fever, stomach

ache, and dysentery (Per. Com). The Fulani in Mubi use the leaves to de-worm their cattle. In Ivory Coast infusions and decoctions are used to cure malaria (Benoit, 1998).

The present investigation seeks to examine this valuable indigenous medicinal plant based on its local uses as a treatment for jaundice, fever, dysentery, and malaria with the mind to determining the physicochemical composition of the compounds that are present and to carry out activity tests so as to see whether the growth of organisms known to be the causative agents of the above ailments can be inhibited.

MATERIALS AND METHODS

Collection of plant materials:

Fresh samples of the stem bark, leaves and roots of the "Bishop`s head", were collected in Hong Adamawa State and were identified in the Biological Sciences Department of Adamawa State University Mubi. The FHI number is 0242 and a specimen of the plant was deposited in the herbarium. The samples (1.00kg) each were air dried in the Chemistry laboratory before pounding to a fine powder using pestle and mortar to about 70 mesh sizes and then stored in a dry container.

Extraction

100g each of the powdered samples were accurately weighed and percolated with 2.0L each of methanol, water and N-hexane for 48hrs. After which there was decantation, filtration, and concentration using rotary evaporator (NYC R-205D) at 35^0C to obtain methanol, water and N-hexane soluble fractions, labeled (F_M^L), (F_W^L), and (F_H^L) for methanol, water (aqueous) and N hexane fractions of the leaves, F_M^S F_W^S and F_H^S for methanol, water and N-hexane fractions of the stem bark and (F_M^R), (F_W^R), and (F_H^R) for methanol, water and N-hexane fractions of the roots respectively. These fractions were weighed and stored in airtight containers for further use.

Qualitative Chemical test

The extracts were evaluated using standard methods described by (Sofowora 1993, Evans 2000, Abulude *et al*., 2001, Isaac and Chinwe 2001 and Abulude 2007) for the presence of phytochemical compound(s) [Alkaloids, Tannin, Saponins, Glycosides, Steroids, Terpenoids, Flavonoids and anthraquinones].

Antibacterial Screening

Kirby Bauer (Duguid et al. 1989) disc diffusion method was used to determine the antibacterial activity of the extracts.

Preparation of medium (Salmonella Shigella Agar [SSA] Medium)

0.6g of powder was weighed and dissolved in 20mL of distilled water. It was allowed to soak for 10 minutes before swirling to mix. The solution was allowed to boil and then cooled to 47^OC before pouring into sterilized Petri dishes.

MacConkey Agar Medium
MacConkey Agar medium was prepared by dissolving 0.5g of powder in 20mL of distilled water. The solution was sterilized in an autoclave at 121^{O}C for 15 minutes. It was cooled and poured into sterile Petri-dishes to solidify.

Blood base Agar Medium
This was prepared by weighing 0.74g of nutrient agar which was dissolved in 20mL of distilled water. The solution was sterilized by autoclaving at 121^{O}C for 15mins and allowed to cool. 5mL defabrinated blood was added, mixed and poured in sterile Petri-dishes to solidify.

Nutrient Agar medium
Nutrient Agar medium was prepared by dissolving 0.56g of nutrient agar in 20mL of distilled water for 15 min. This was sterilized in an autoclaved at 121^{O}C for 15 min and allowed to cool and poured in to Petri dishes to solidify.

Preparation of Cultures and Inoculation
Sputum, Mucus and Stool samples collected from patients in General Hospital Mubi, Adamawa State, Nigeria were separately used to inoculate the different media. Stool samples were used for *Escherichia* and *Shigella* media while mucus and sputum were used for Macconkey and blood base Agar media respectively. The plates were incubated for 24h at 37^{O}C. Pure cultures of *Escherichia coli, Shigella dysenteriae, Staplococcus aureus* and *Streptococcus pneumonia* clinical isolates obtained from Yola Specialist Hospital, Adamawa State, Nigeria were used. These pure cultures were used separately to inoculate nutrient agar media Petri-dishes, by streaking the surfaces of the plates in a zigzag fashion until the entire surfaces were covered. The inoculated plates were incubated at 37^{O}C for 24 hr

Assay of extracts
The extracts were dissolved in 5mLof distilled water to produce 0.05 % m/v dilution to give 0.05 %. A standard drug levofloxacine with 0.01mg /mL dilution was used as the positive control while distilled water as the negative control.

Wells (3mm in diameter) were punched into the nutrient agar containing bacterial suspension using a sterile borer and filled with 0.1mL of the extracts. The controls were filled with 0.1mL distilled water and drug for negative and positive control respectively. The plates were incubated for 24hr at 37^{O}C and examined for zones of inhibition. Presence of these zones indicates activity. Observation comprising of diameter of disk and zone of inhibition was made for proper evaluation.

RESULTS AND DISCUSSION

Phytochemical and antimicrobial investigation: The phytochemical analysis of the various parts of the indigenous plant *N. latifolia* is shown in Table (1). Table (2), the inhibition of the bacterial growth by the plant parts

Table (1): Phytochemical screening of extracts of: *N latifolia*

Chemicals constituents	Plant parts					
	Leaves		Stem bark		Roots	
	M	A	M	A	M	A
Saponins	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	-	-	-	-
Anthraquinones	+	-	-	-	-	-
Steroids	-	-	-	-	-	-

+ = present - = absent
M = Methanol A = Aqueous

Table (II): Antimicrobial activity of the extracts

Microorganisms	Mean Diameter of zones of inhibition (mm) of various plant parts							
	Leaves			Control		Stem Bark		
	M	A	H	DC	WC	M	A	H
Escherichia coli	13.3±0.2	-	-	16.4±0.2	-	15.3±0.1	-	-
Streptococcus pneumoniae	11.1±0.4	10.8±0.4	-	18.1±0.1	-	12.3±0.0	-	-
Shigella dysentryae	14.0±0.2	-	-	18.0±0.0	-	10.8±0.2	-	-
Staphylococcus aureus	8.6±0.1	7.2±0.2	-	19.3±0.1	-	13.7±0.2	-	-

Results are mean of three trials ± Standard error.
Key: M =Methanol extract, A = Aqueous extract, H = n-Hexane, DC = Drug as positive control
WC = Water as negative control, - = Not Sensitive.
Concentration of the extract used = 0.05mg/ml
Concentration of the levofloxacine =0.01mg/ml

Table (1) shows the phytochemical screening of the extracts. It reveals that alkaloids, saponnins glycosides, tannins were all present in methanol and water extracts of all the plant parts. Steroids were also seen to be absent in all the plant parts. Flavovoids were found in all leaf extracts. Anthraquinones were only present in the methanol extracts of the leaves. Literature from previous studies confirms the presence of tannins, alkaloids, glycosides and saponins in this class of plants, (El-Mahmood *et al*, 2008). This could be used as an acid test to attest to the fact that the indigenes could use this plant for the cure of antiviral, anti-allergic, anti-inflammatory and as an anti-oxidant agent (Zhang *et al*., 2002 and Ramassamy 2006).

The results of antibacterial screening, (**Table 2**), showed that the methanol extract of the leaves and stem bark exhibited anti-microbial activity against all the microorganisms tested. The most susceptible organism was *E. coli* and the least susceptible, *S. aureus*, comparing their zones of inhibition.

Water extract of leaves was found to have anti-microbial activity against *S. peumoniae* and *S. aureus*. All the tested microorganisms were not sensitive to the water extract of the stem bark and the n-Hexane extract. The low zone of inhibition by water extract and relatively non inhibition by N-Hexane may be due to their inability to extract the major bioactive compounds present in the plant part. This shows that this indigenous medicinal plant possesses the

phytochemical (chemotherapeutic agents) for the treatment of various ailments (diarrhea, typhoid, fever and as an antiseptic agent), but the method and solvent of extraction should be carefully selected (Sofowara 1993; Okoli *et al*., 2004).

CONCLUSION

The results of this research have shown that the different plant parts posses a wide range of antimicrobial activity against the microorganisms tested. Methanol seems to be the best solvent for extracting the constituents responsible for the inhibitory activity of the leaves and stem bark, since it gave highest zones of inhibition. I.e. the microorganisms were more sensitive to the extract obtained using methanol. The inhibitory activity of these extracts confirmed the potential use of the plant parts in the treatment of microbial induced ailments. Aqueous extract of the leaves was sensitive on *S. pneumonia*, and *S. aureus* as used by the practitioners. Alkaloids, saponins, and tannins are reported to have antimicrobial activity (Evans 1999). These chemicals constituents might be responsible for the antimicrobial activity observed. The organisms tested are known to cause typhoid, diarrhea, tuberculosis, skin infection, malaria, and other ailments. The results are a good indication that the plant could be utilized for possible antimicrobial agents. The practitioners of traditional medicine can serve as an additional source of health care to mankind. Little wonders that N. *latifolia* attend to many diseases. Thus this result justifies the use of the plant parts in ethno medicine.

It is recommended that the active constituents of the plant parts be isolated and characterized. Clinical studies be carried out to know the toxic effect (s) of the plant on the human system and lastly if the indigenes could be advised to use methanol instead of water for the extraction, it may increase the efficacy of the extracts.

REFERENCES

[1] Abulude, F. O; Akajagbor, C. and Dafiehware, B. H; (**2001**); *Adv, Food Sci*., 23 P3-116.
[2] Abulude, F. 0; (**2007**); *Research journal of Phytochemistry* 1(1) 33-39
[3] Arvigo R and Balick M (**1993**): Rain forest Remedies. Lotus Press, Twin Lakes, WI.
[4] Balandrin M. F, Kinghorn A. D and Farnsworth N. R (**1993**) plant –derive d natural products in
[5] Drug discovery and development: an overview. In Human medicinal Agents from plants, American Chemical Society Symposium Series, No 534 AD Kinghorn & MF Balandrin, Eds.:2-12. American Chemical Society Washington D.C
[6] Barron R.L and vanscoy G.J (**1993**): *Ann pharmacother* 27 (5), 607
[7] Benoit-Vical F; Valentin A; Coumac V (**1998**): *J. Ethnopharmacol* 61: 173 -178.
[8] Bhattaram V.A; Graefe U; Veit m and Derendorf H (**2002**): *Phytomedicine* 9 (Suppl.3), 1
[9] Brahmachari G (**2006):** Prospects of natural products research in the 21st century: a sketch.
[10] In Chemistry of Natural products; recent trends and developments (ed, G. Brahmachari), Research Signpost, Kerala, India pp. 1 -22
[11] Chan K (**1995**): *Trends Pharmacol. Sci.* **16** (6) 182
[12] Chang H. M and But P.P (**1986**): Pharmacology and Applications of Chinese *Materia Medica*.
[13] *World Scientific* Publishing, Singapore Cooper E.L. (**2004**): eCAM **1**(2), 207

[14] Cragg G. M. and Newman D. J (**2001**): *Ann N.Y Acad. Sci*. **953**, 3
[15] Deeni Y; Hussain H (**1999**): *J. Ethnopharmacol.* 35: 91 -96
[16] Dev S (**1999**): *Environ Health Prospect* **107**, 783
[17] Donia M, Hamann M.T (**2003**): *Lancet. Infect. Dis*. **3** (6), 338
[18] Duquid J. P; Marmoid B. P and Swain R. H (**1989**): Mackie and McCartney's medical microbiology, vol. 1 13th edition. Churchill Livingston, London, Pp 163.
[19] Edet E.E; Akppanabiatu M. I; Umoh I. B; Udosen E O (**2005**): *Inter. J. Clinical biochemistry*, **20**:29 -34.
[20] El-Mahmood A.M; Doughari J.H and Chagi F. J. (**2008**): Scientific Research and Essay vol.3 (3): 102 -105.
[21] Emokpae, M. A; Nwokedi, E. E; Dutse, A. I; (**2006**): *Highland Medical Research Journal* 4(**1**): 15-21.
[22] Evans, W, C, (**2000**): Trease and Evans Pharmacology, 4th Edn. WB Saunders Company Ltd., Pp: 224 – 239.
[23] Evans, W, C, (**1999**): "Trease and Evans Pharmacognosy", 13th Edn. English Language Book Society/Bail ere Tindall . London. Pp: 537 – 536.
[24] Farnsworth N.H, Akerele A. S, Soejarto DD and Guo Z (**1985**): Medicinal plants in therapy. Bull WHO **63**, 965
[25] Holt G. A and Chandra A (**2002**): *Clin. Res*. *Regulatory Affairs* (USA) **19**, 83
[26] Isaac O and Chinwe J (**2001**): *J. Chem. Society fo Nig*. 26: 53 -55.
[27] Kapoor L. D (**1990**): CRC Handbook of Ayurvedic Medicinal plants. *CRC Press*, Boca Raton, FL.
[28] Kaul P. N and Joshi B. S (**2001**): *Prog. Drug Res* **57**, 1
[29] Koh H and Woo S (**2000**): *Drug safety* **23** (5), 351
[30] Kroll D. J (**2000**): *J. Herbal Phamrcother* **1**(2)3.
[31] Marriott B.M (**2001**): *Adv. Exper Med Biol* **492** (Nutrition and Cancer Prevention), 203
[32] Mc Connell O, Longlley R. E and Koehn F. E (**1994**): The discovery of marine natural products with therapeutic potential. In The Discovery of Natural Products with Therapeutic potential. VP Gullo (Ed), pp. 109 -174. Butterworth –Heinemann. Boston.
[33] Newman D. J, Cragg G. M and Snader K.M (**2000**): *Nat. Prod Rep* **17** , 215
[34] Okoli A. S, and Iroegbu C.U (**2004**): *J Ethnopharmaco.* 92, 91): 135 -144.
[35] Ramassamy E (**2006**): *Eur J. Pharmacol*., 245 (1): 51 – 64.
[36] Schultes R. E and Raffauf R. F (**1990**): the Healing Forest. *Dioscorides Press*, Portland, OR.
[37] Sofowara, A. (**1993**). Medicinal plants and Traditional medicine in Africa Spectrum Books Ltd, Ibadan: Nigeria.p.289.
[38] Trease G. E and Evans M. D (**1989**): A text book of Pharmacognosy. 13th Edn. Baillier, Tindal and Caussel; London, Pp: 144 -148.
[39] Yong P (**1997**): *ASM News* **63**, 417
[40] Zhang H, Qui S, Tamez P, Tan, G. T Aydogmus Z, Hung N. M, Angerhofer C, Soejarto D.D. Pezzuto J.M. Fong, H. H. S (**2002**): *Phaem. Biol*. **40**, 221 -224