Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 116-120
Received: 06-11-2020
Accepted: 15-12-2020

Okwute SK
Department of Chemistry, University of Abuja, P.M.B. 117, Gwagwalada, F.C.T. Abuja, Nigeria

Ohiakwu CS
Department of Chemistry, University of Abuja, P.M.B. 117, Gwagwalada, F.C.T. Abuja, Nigeria

Corresponding Author:
Okwute SK
Department of Chemistry, University of Abuja, P.M.B. 117, Gwagwalada, F.C.T. Abuja, Nigeria

Phytochemical and GCMS analysis of the leaves extracts of *Nauclea Latifolia*

Okwute SK and Ohiakwu CS

Abstract
Nauclea latifolia (Rubiaceae), found in Gauraka Tafa L.G.A., Niger State, Nigeria, is used in folkloric medicine to manage malaria, jaundice, diarrhea, hypertension, cancer and tuberculosis. The aim of the present study is to evaluate the chemical and biological characteristics of the leaf to confirm its traditional medicinal uses. The leaf was extracted with methanol to obtain the crude extract. Phytochemical screening of the crude extract revealed the presence of the following metabolites: saponins, alkaloids, glycosides, tannins, flavonoids and anthraquinones. The crude extract was also fractionated into petroleum ether, ethyl acetate and methanol solubles. Antimicrobial studies of the crude and fractions showed inhibitory activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Pseudomonas aeroginosa, Klebsiella pneumoniae, Candida albicans, and Aspergillus niger* with *ciprofloxacin* serving as the control antibiotic. The zones of inhibition ranged from 11mm to 28mm. The moderately polar and antimicrobial ethyl acetate fraction was partially purified using column chromatography and the sub-fractions were then subjected to GC-MS analysis to identify some of its chemical constituents. The GC-MS analysis led to the identification of a number of fatty compounds including open and cyclic hydrocarbons, alkyl benzenes, fatty acid derivatives and other oxygenated compounds. Some of the compounds are known to possess a number of bioactivities including antimicrobial, anti-malaria and anti-inflammatory which may account for the ethno-medicinal uses of the plant.

Keywords: *Nauclea latifolia*; leaf, phytochemicals; fractionation, GC-MS analysis, volatile components

1. 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, 2005) [12] 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) [11]. 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 (Arvigo and Balick, 1993) [5].
Nauclea latifolia (Rubiaceae), common name "Bishop`s head", "Mbom-mbog" in AkwaIbom and Cross River States in Nigeria, "Ubuluilu" in Eastern part, "Agbaseagbase" in Yoruba, "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 *et al.,* 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 and Hussain 1999) [13]. In Ivory Coast infusions and decoctions are used to cure malaria (Benoit-Vical *et al.*,1998) [9].
Pharmacological investigations of various parts of the plant found that the plant has antioxidant (Mordi *et al.,* 2014) [33], antidiabetic (Asanga *et al.*,2013, Effiong and Akpan, 2015) [15, 17], anti-cholesterol and antihypertension (Omale and Ugede, 2011) [34], anti-malarial (Ettenbong *et al.,* (2015) [17], anthelmintic (Ademola *et al.,* 2007) [1], anti-viral (Manuela *et al.,* 2013) [30], antibacterial (Bamidele *et al.,* 2014, Fadipe *et al.,* 2013) [8, 20].
Some chemical investigations of the plant led to the isolation of six known compounds, including three alkaloids and glycosides with anti-GST and anti-fungal properties from the crude ethanolic extract (Ata *et al.*,2009) [6] and the determination of the phytochemicals and vitamins composition as well as the proximate analysis of the root and stem bark (Egbung *et al.,* 2013) [16]. It appears not much of chemical studies have been done on the leaves.

The present investigation is therefore on the antimicrobial activity and volatile constituents of the leaves of the plant in view of its wide uses in ethno-medicine.

2. Materials and Methods

2.1 Materials

Fresh leaves of *Nauclea latifolia* were collected from Gauraka village, Tafa Local Government Area, Niger State, in April, 2019. They were authenticated at the Herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, where a Voucher specimen (Number NIPRD/H/7019) was deposited. The leaves were air-dried at room temperature for three weeks and then pulverized into a fine powder using a piston and a mortar. The powdered plant material was stored in a tightly closed polythene bag.

The chemicals used were of analytical grade manufactured by BDH Chemicals Poole, London. All the organic solvents used were redistilled before use to remove impurities.

The culture media used for the antimicrobial screening were Mueller Hinton Agar (MHA), Potato Dextrose Agar (PDA), Nutrient Agar (NA) and Mueller Hinton Broth (MHB). All the media were prepared according to the manufacturer's instructions against the following micro-organisms: *Staphylococcus aureus* (*Sa), Bacillus subtilis (Bs), Escherichia coli (Ec), Salmonella typhi (St) and Candida albicans (Ca).* The test organisms used were clinical isolates obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Thin layer chromatography (TLC) was carried out on pre-coated Merck Millipore classical silica gel 60 F_{245} aluminum plates with thickness of 0.25 mm. The spots were visualized by exposure to UV light at the wavelength of 254/366 nm and to iodine vapour. Column chromatography was carried out over silica gel (200-400 mesh from Qingdao Marine Chemical Inc., China.

The GC-MS analysis of the samples was performed using Agilent Technologies 7890A GC System coupled with MS of model Agilent Technologies 5975C VLMSD. The injector used was Agilent Technologies 7683B Series Injector. The operating conditions were as follows: Column HP5MS with length 30 m, internal diameter of 0.320 mm and thickness 0.25 µm. The oven conditions were: initial temperature was 30^{o}C which held for 2 minutes; it increased by 10^{o}C per minute to a final temperature of 240^{o}C which held for 6 minutes. The injection temperature was 250^{o}C and the volume of the sample injected was 1 µL. Helium was used as the carrier gas at the flow rate of 1 mL/minute.

2.2 Methods

2.2.1 Extraction of plant material

The air-dried pulverized leaves (1kg) was extracted with methanol (4.5L) by maceration (one week). The extract obtained was filtered using sterile Whatmann No. 1 filter paper and concentrated with Rotary evaporator to obtain the crude extract (51.06 g, 5.11%).

2.2.2 Fractionation of the crude extract

The crude methanol extract (37g) was dissolved in methanol (200 ml) and extracted successively and exhaustively with petroleum ether and ethyl acetate (300 mL x 3 each). The petroleum ether, ethyl acetate fractions and the residual methanol solution were each evaporated to dryness to give 2.05g (4.01%), 5.80g (11.36%) and 16.70g (32.71%) of the residues, respectively.

2.2.3 Preliminary phytochemical screening of crude extract

The crude methanol extract was subjected to preliminary qualitative phytochemical screening for secondary metabolites using procedures described by Harborne (1984) [22], Sofowora, (1993) [38] and Trease and Evans (2002) [18].

2.2.4 Antimicrobial activity tests

The crude extract and fractions were tested for antimicrobial activity, minimum inhibitory concentration(MIC) and minimum bactericidal concentration(MBC) using standard procedures (Mitscher *et al.,* 1972) [32]. The positive controls used were ciprofloxacin (10 µg) for the bacteria and ketoconazole (250 mg) for the fungus, *Candida albicans.*

2.2.5 Column chromatography (CC) of ethyl acetate fraction

The moderately polar and antimicrobial ethyl acetate fraction (2.5g) was subjected to column chromatography on silica gel, using 100% petroleum ether initially, then mixtures of petroleum ether and ethyl acetate(9:1, 8:2, 7:3 6:4, and 1:1 and then reversed with ethyl acetate: petroleum ether (6:4), (7:3), (8:2), (9:1) and finally 100% ethyl acetate. The column was eluted with 200 mL of each solvent system, collecting 20 ml portions to a total volume of 260 ml. Each collection was concentrated and subjected to TLC on the basis of which similar fractions were combined. The fractions 110-120, 130-140, 150-160, 230-240 and 250-260 were separately pulled together, evaporated and coded NL1, NL2, NL3, NL4, NL5, NL6, respectively.

2.2.6 Gas chromatography-mass spectrometry (GC-MS)

The fractions (NL1-NL6) obtained from column chromatography of ethyl acetate fraction were subjected to GC-MS analysis.

3. Results and Discussion

3.1 Results

The results of the preliminary phytochemical screening of crude methanolic extract of the dried powdered leaves of *Nauclea latifolia* are presented in Table **1.**

Table 1: Phytochemical screening of the crude methanol extract

Parameter	Result
Saponins	+
Glycosides	+
Tannins	+
Flavonoids	+
Anthraquinones	+
Steroids	_
Alkaloids	+
Terpenoids	_
Quinones	_

Key: + = Present; - = Absent

The results of the antibacterial screening of crude methanolic extract and fractions of *Nauclea latifolia* leaves against selected organisms are in Tables 2 and 3.

Table 2: Results of Inhibitory Activity (Sensitivity Test) of crude extract and fractions

Organisms	Zone of Inhibition (mm)/ Concentration (mg/ml)																
	PE				ETA				ME				Crude				
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	Cp= 10µg/ml Ke=250mg/ml
Sa	19	16	14	0	16	14	12	0	22	20	18	16	16	14	12	10	35cp
Bs	20	18	16	14	18	15	13	11	23	21	19	16	18	16	12	10	32cp
Ec	16	13	10	0	14	12	10	0	16	14	11	0	14	11	0	0	37cp
St	17	15	13	10	12	10	0	0	12	0	0	0	13	10	0	0	38cp
Pa	22	18	16	14	19	16	14	12	18	15	13	10	16	13	11	0	36cp
Ca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42ke
An	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45ke

Key: *Sa=Staphylococuss aureus, Bs=Bacilus subtilis, Ec=Escherica coli, St=Staphylococuss typhi, Pa=Pseudomonas aeroginosa, Ca=Candida albicans, An=Aspergerus niger*, PE=petroleum ether, ETA= ethyl acetate, ME= methanol, Cp=ciprofloxacin, Ke=ketoconazole.

Table 3: Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against the test organisms.

Organisms	MIC(mg/mL)				MBC(mg/mL)			
	PE	ETA	ME	Crude	PE	ETA	ME	Crude
Sa	25	50	12.5	25	50	*	25	*
Bs	12.5	25	6.125	25	25	50	12.5	50
Ec	50	50	50	100 *		*	*	*
St	25	50	100	50	50	*	*	*
Pa	12.5	25	25	50	25	50	50	*
Ca	-	-	-	-	--	-	-	-
An	-	-	-	-	-	-	-	-

Key: Key:*Sa=Staphylococuss aureus, Bs=Bacilus subtilis, Ec=Escherica coli, St=Staphylococuss typhi, Pa=Pseudomonas aeroginosa, Ca=Candida albicans, An=Aspergerus niger*, PE=petroleum ether, ETA= ethyl acetate, ME= methanol. **(-)** = Not determined, **(***)= Bacteriostatic

The ethyl acetate fraction was subjected to column choromatography, and the six fractions (NL1-NL6) obtained were subjected to GC-MS. The major volatile compounds are found in Tables **4.**.

Table 4: GCMS analyis data of ethyl acetate fraction of *Nauclea latifolia* leaves

	S/N Compound	RT(mins)	M.Wt	Mol. Formula
1.	Benzene-1-ethyl-3-methyl	4.111	120	C_9H_{12}
2.	Mesitylene-7- hexadecene, (z)	4.315	120	$C_9 H_{12}$
3.	Benzene-1-2,4-trimethyl	4.635	120	$C_9 H_{12}$
4.	Benzaldehyde-3-phenoxy	12.289	198	$C_{13} H_{10} O_2$
5.	Ethyl-4-t-butybenzoate	12.292	206	$C_{13} H_{18} O_2$
6.	Pentadecanoic acid, 14-methyl-, methyl ester	14.573	270	$C_{17}H_{34}O_2$
7.	Dibutyl phthalate	14.654	286	$C_{16}H_{30}O_4$
8.	Undec-10-ynoic acid, dodecyl ester	16.011	350	$C_{23}H_{42}O_2$
9.	Tert-hexadecanethiol	17.773	256	$C_{16}H_{34}S$
10	Di (Z)- hex-z-enyl phthalate	18.973	330	$C_{30} H_{26} O_4$
11	(E)-4-hexenoic acid, 2-acetyl-2-(1-buten-3-yl)-, ethyl ester	20.425	237	$C_{14}H_{21}O_3$

3.2 Discussion

The results of the preliminary phytochemical screening of the crude extract (Figure **1**) showed that the leaves were very rich in glycosides, tannins, flavonoids, alkaloids and anthraquinones. These secondary metabolites reported from this investigation are known for their broad spectrum of pharmacological and physiological properties in medicinal applications (Ezekiel, *et al.,* 2010) [19].

Flavonoids are known for their health related properties which are based on their antioxidant activities. These properties have been found to include: anti-cancer, anti-viral, anti-allergic and anti-inflammatory activities (Mahato & Sen, 1997; Valsaraj *et al.*, 1997) [29, 39]. Apart from industrial applications, herbal preparations containing tannins are used for the treatment of small hemorrhage, sore mouth, bronchitis, burns, and scars of the skin, wounds and many others. They are also used for the treatment of diarrhea. Tannins are considered antioxidants and they prevent the onset of degenerative diseases such as cancer and cardiovascular disease. They have been also reported to have anti-viral (Lin *et al.*, 2004) [28], antibacterial (Akiyama *et al.*, 2001) [3] and antiparasitic effects. Extracts of cardiac glycosides have been reported for their use as diuretics and emetics, as heart tonics for the treatment of congestive heart failure and cardiac arrhythmia (Zhang *et al.*, 2006) [41]. Alkaloids, on the other hand, are popular for their use as stimulants, anti-malarials, analgesics, muscle relaxants and anti-tumor agents.

Saponins exhibit a variety of biological activities, and have been investigated toward the development of new natural medicines (Waller & Yamasaki, 1995) [40]. Other interesting biological applications of various specific saponins include their uses as anti-inflammatory (Balandrin, 1996) [7], hypocholesterolemic (Oakenfull, 1996) [35] and immune-stimulating (Klausner, 1988) [24] agents. The primary function

of carbohydrates is to provide energy for the body, especially the brain and the nervous system (Rockville, 2010, Leotério *et al.*, 2015) [37, 27].

From the results of the antimicrobial screening (Tables **2** and **3)** the crude extract and fractions exhibited characteristic strong concentration-dependent activity (CDA) against the test organisms, with zones of inhibition ranging from 10-22 mm at various concentrations. Both the crude extract and fractions were completely inactive against the two fungi (Ca *and An*). Both the crude extract and the fractions were however active against the Gram-negative bacteria (*Ec*, *St*, and *Pa*) and the Gram-positive bacteria (*St* and *Bs*). The results of this study agree with the documented records of the ethno-medicinal uses of *N. latifolia* (Hutchings *et al.*, 1996; Lemenih *et al*., 2003) [23, 26].

The results of the GC-MS analysis of the column fractions of the ethyl acetate fraction(Table **4)** showed that the ethyl acetate fraction contains about 12 major components, belonging to aromatics, open and cyclic hydrocarbons and long chain fatty acids and their oxygenated derivatives. They were identified based on direct comparison with NIST Computer MS analysis data as seen in Table **4**. The constituents identified belong to various classes of phytochemicals which are known to possess a wide range of pharmacological activities (Handecoeur *et al*, 2018) [21].

Thus, the anti-inflammatory, anti-oxidant, hypocholesteromic, antibacterial, activities reported for phthalates, aromatic hydrocarbons and fatty acids such as pentadecanoic acid may suggest the rationale for the traditional uses of *N. latifolia* (Aparna *et al*, 2012, Kumar *et al*, 2010, Rahuman *et al*, 2000) [4, 25, 36]. Pentadecanoic acid methyl ester and other fatty acid derivatives have antimicrobial and antifungal properties (Chandrasekaran *et al*., 2011, Mishra *et al*., 2007) [10, 31]. 1, 2-benzenedicarboxylic acid monobutyl ester has been reported to possess antimicrobial and antifungal activities (Adeyemi and Okwute, 2009) [2].

4. Conclusion

The present study has investigated the chemical and biological activities of *Nauclea latifolia* methanol leaves extract and revealed that it is rich in phytochemicals. The reported bioactivities of the plant, including the results of antimicrobial tests in this work, may largely be due to the presence of these secondary metabolites. GC-MS analysis data led to the identification of some of the volatile compounds in the extracts which have previously been known to possess pharmacological activities. Therefore, the leaves extracts can be harnessed as potential sources of new therapeutics. Thus, the findings in this study support the ethno-medicinal uses of the plant.

5. References

1. Ademola IO, Fagbemi BO, Idowu SO. Antihelminthic efficacy of Nauclea latifolia Extract against gastrointestinal nematodes of sheep *in vitro* and *in vivo* studies. African Journal of Traditional, Complementary and Alternative Medicine 2007;4(2):148-156.
2. Adeyemi GO, Okwute SK. Isolation and characterization of lup-20(29)-en-3-one and diisonylphthalate from antimicrobial *Pterocarpus erinaceaus* (Poir) stem bark. J. Chem. Soc. Nigeria 2009;34(2):156-161.
3. Akiyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against Staphylococcus aureus. Journal of Antimicrobial Chemother 2001;48:487-491.
4. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasvan C, Haridas M, *et al*. Anti-inflammatory property of n-hexadecanoic acid:Structural evidence and kinetic assessment. Chem. Biol. Drug Des 2012;80:434-439.
5. Arvigo R, Balick M. Rain forest Remedies. *Lotus Press*, Twin Lakes, WI. Asanga, E.E., Ebong, E.P. and Eseyin, A.O. (2013). Haematological parameters of alloxan-induced diabetic rats treated with ethanol extracts and fractions of Nauclea latifolia leaf. European Scientific Journal 1993;9(27):2003-2010.
6. Ata A, Udenigwe C, Matochko W, Holloway P, Eze M, Uzoegwu PN, *et al*. Chemical constituents of *Nauclea latifolia* and their anti-GST and anti-fungal activities. Natural Products Communications 2009;4(9):1185-1188.
7. Balandrin MF. Commercial utilization of plant-derived saponins: An overview of medicinal, pharmaceutical and industrial applications. In:Waller GR and Yamasaki K. (eds) Saponins used in food and agriculture: Plenum Press 1996, 1-14.
8. Bamidele FA, Ogundipe FO, Shogeyinbo UA. Determination of antibacterial activity and phytochemistry of three herbal plants on clinical isolates. International Journal of Scientific & Technology Research 2014;3(12):355-360.
9. Benoit-Vical F, Valentin A, Cournac V, Pelissier Y, Mallie M, Bastide JM, *et al*. *In vitro* antiplasmodial activity of stem and root extracts of Nauclea latifolia S.M (Rubiaceae). Journal of Ethnopharmacology 1998;61(3):173-8.
10. Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of Sesvurium portulacastrum L. European Rev.Med. Pharmacol. Sci 2011;15:775.
11. Chang HM, But PP. Pharmacology and Applications of Chinese Materi Medica 1986.
12. Cragg GM, Newman DJ. Plants as a source of anticancer agents. Journal of Ethnopharmacology 2005;100:72-79.
13. Deeni Y, Hussain H. Screening for antimicrobial activity and for alkaloids of Nauclea latifolia. Journal Ethnopharmacology 1991;35:91-96.
14. Edet A, Ebong P, Eseyin O, Udoh I, Eyo R, Effiong G, *et al*. Lipid profile of alloxan-induced diabetic albino Wistar rats treated with ethanol whole extract and fractions of *Nauclea latifolia* leaves. Journal of Science and Technology 2013;3(10):1009-1013.
15. Effiong GS, Akpan HD. The effect of Nauclea latifolia leaf extract on some biochemical parameters in streptozotocin diabetic rat models. Journal of Medicine and Medical Sciences 2015;6(3):47-52.
16. Egbung GE, Attangwho IJ, Iwara IA, Odey MO, Ebong PE. Chemical composition of root and stem bark extracts of Nauclea latifolia. Arch. Appl. Science Research 2013;5(3):193-196.
17. Ettebong EO, Ubulom PM, Edwin Ekpenyong CE, Ekong US, Akpan OE, Tambari V, *et al*. *In vivo* antiplasmodial activities of Nauclea latifolia Asian Journal of Medical Sciences 2015;6(3):6-11.
18. Evans WC. Trease and Evans Pharmacology; 15th ed., London: Saunders Publisher 2002, 585.
19. Ezekiel I, Mabrouk MA, Ayo JO. Study of the effect of hydro-ethanolic extract of Commiphora africana (Stem-bark) on sleeping time and convulsion in mice. Asian Journal of Medical Sciences 2010;2(3): 81-84.
20. Fadipe LA, Haruna K, Mohammed I, Ibikunle GF. Phytochemical and in-vitro antibacterial evaluation of the

extracts, portions and sub-portions of the ripe and unripe fruits of *Nauclea latifolia.* Journal of Medicinal Plants Research 2013;7(11):629-636.
21. Handecouer R, Peuchmaur M, Peres B, Rome M, Tauve GS, Boumendjel A, *et al*. Traditional uses, phytochemistry and pharmacological properties of African Nauclea species: A review. J. Ethnopharmacol 2018;212:106-136 [CrossRef] [PubMed].
22. Harborne JB. Phytochemical Methods, A Guide to Modern Techniques in Plant Analysis. New York: Chapman and Hall 1993.
23. Hutchings MR, Athanasiadou S, Kyriazakis I, Gordon IJ. Can animals use foraging behaviour to combat parasites? Proceed. Nutr. Soc 2003;62:361-370.
24. Klausner A. Adjuvants: a real shot in the arm for recombinant vaccines. Bio/Technology 1988;6(7):773-777.
25. Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afr. J. Biochem. Res 2010;4:191-195.
26. Lemenih M, Abebe T, Olsson M. Gum and resin resources from some *Acacia*, *Boswellia* and *Commiphora* species and their economic contributions in Liban, South-East Ethiopia. Journal of Arid Environments 2003;55:465-482.
27. Leotério Dilmo MS, Silva Paulo, Souza Gustavo, Alves Aline de A, Belian Mônica. Galembeck André. "Copper–4,4′- dipyridyl coordination compound as solid reagent for spectrophotometric determination of reducing sugar employing a multicommutation approach". Food Control (European Federation of Food Science and Technology; International Union of Food Science and Technology) 2015;57:225-231.
28. Lin LU, Shu-wen L, Shi-bo J, Shu-guang W. Tannin inhibits HIV-1 entry by targeting gp 41. Acta Pharmacol Sin 2004;25(2):213-218.
29. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochemistry, 1997;44:1185-236.
30. Manuela D, Huguette NN, Rosalie Annie NN, Donatien G, Alembert TT, Roberta R, *et al*. *In vitro* anti-Herpes simplex virus activity of crude extract of the roots of Nauclea latifolia Smith (Rubiaceae). Complementary and Alternative medicine 2013;13:266
31. Mishra PM, Sree A. Antibacterial activity and GCMS analysis of the leaves of *Finlaysonia obovata*(a mangrove plant). Asian J. Plant Sci 2007;6(2007)168-172.
32. Mitscher LA, Leu RP, Bathala MS, Wu WN, Beal JL, White R. Antibiotics from higher plants.1.Introduction, rationale, and methodology. J. Nat. Products 1972;32:157.
33. Mordi JC, Ojieh AE, Uzuegbu UE, Onyesom I, Onokurafe F. Changes in ocular oxidative indices in Plasmodium berghei infected mice treated with aqueous leaf extract of Nauclea latifolia. Bioscience Biotechnology Research Communications 2014;7(1):1-6.
34. Omale J, Ugbede HH. Hypocholesterolemic effects of Nauclea latifolia (Smith) fruit studied in albino rats. American Journal of Tropical Medicine & Public Health 2011;1(1):11-21.
35. Oakenfull D. Saponins in the treatment of hypercholesterolemia. In:Spiller GA (ed.). *Handbook of Lipids in Human Nutrition*. CRC Press 1999, 107-112.
36. Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B. Effects of *Feronia limonia* on mosquito larvae. Filoterapia 2000;71:553-555.
37. Rockville MD. United States Department of Health and Human Services and United States Department of Agriculture. Dietary Guidelines for Americans (7th ed.) 2010.
38. Sofowora EA, Isaac-Sodeye WA, Ogunkoya LO. Isolation and characterisation of an antisickling agent from Fagara zanthoxyloides root. Llyodia 1975;38(2):169-71.
39. Valsaraj R, Pushpangadan P, Smitt UW. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol 1997;58:75-83.
40. Waller GR, Yamasaki K. Proceedings of an American Chemical Society. Symposium on saponins: August 20-24, Chicago, Illinois 1995.
41. Zhang Y, Li HZ, Zhang YJ, Jacob MR, Khan SI, Li XC, *et al*. Steroids 2006;71:712.
42. Odey MO, Johnson JT, Iwara IA, Gauje B, Akpan NS, Luke UO, *et al*. Effect of antihypertensive treatment with root and stem bark extracts of Nauclea latifolia on serum lipid profile. G. J. P&A Sc and Tech 2012;2(4):78-84.
43. Gamaniel K, Wambebe C, Amupitan J, Hussaini IM, Amos S, Awodogan A, *et al*. Active column fractions of Nauclea latifolia on Plasmodium berghei and rabbit ileum. Journal of Pharmaceutical Research and Development 1997;2:44-47.
44. Gidado AA, Ameh DE, Atawodi S, Ibrahim S. Antidiabetic Effect of Nauclea latifolia Leaf Ethanolic Extract in Streptozotocin-induced diabetic rats. Pharmacognosy Research 2009;1(6):392-395.
45. Eze SO, Obinwa E. Phytochemical and Nutrient Evaluation of the Leaves and Fruits of Nauclea Latifolia (Uvuru-ilu). Communications in Applied Sciences 2014;2:8-24.