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Chemical Fingerprinting of *Nauclea latifolia*, an Antidiabetic Plant, Using GC-MS

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Authors' contributions

This work was carried out in collaboration between both authors. Author BIAM managed the project conception and design, coordination, interpretation of data and preparation of manuscript. Author CA managed the experimentation, acquisition and analysis of data, statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Earlier studies have established the antidiabetic activity of *Nauclea latifolia.* The aim of this study was to obtain chemical fingerprints of the ethanolic extracts of *Nauclea latifolia* leaf using Gas chromatography-Mass spectrometry (GC-MS), in order to assure proper identification of the plant and to evaluate its phytochemical composition using GC-MS. It was also aimed at unravelling the phytochemical constituents responsible for its antidiabetic activities.
Methodology: The phytochemicals in the plant leaves were extracted by cold maceration in ethanol and subjected to GC-MS analysis.
Results: Chromatograms showed 51 peaks of identified phytochemical compounds. The GC-MS analysis also revealed sixteen (16) active antidiabetic phytochemicals namely: Pentanoic acid 4-oxo- ethyl ester, 2-Methoxy-4-vinylphenol, Triethyl citrate, Quinic acid, 3-O-Methyl-d-glucose, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, Phytol, Hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, Octadecanoic acid, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester, Vitamin E, Campesterol, Stigmasterol, Gamma-sitosterol.

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Conclusion: The GC-MS profile provides a suitable chemical fingerprint to assure proper identification of the *Nauclea latifolia* plant. Sixteen (16) active phytochemicals of the plant leaf are known to have antidiabetic activities; these could be used as a basis for standardization of the plant preparations for diabetes therapy.

Keywords: Nauclea latifolia; antidiabetic; chemical fingerprinting; gas chromatography; mass spectroscopy.

1. INTRODUCTION

Medicinal plants constitute a source of raw materials for both traditional systems of medicine and modern medicine. Most rural populations, especially in the developing world, depend on medicinal plants as their main source of primary healthcare ostensibly because of its relatively low cost, availability and limited access to modern medicine [1,2]. It is estimated that about 80% of the world's population living in Africa, Asia and Latin-America, use herbal medicine as their source of primary health care [2-6]. Over the years there has been increasing acceptance and public interest in natural therapies in developed countries because of the belief that it will result in healthier living. Herbal medicines are also often viewed as a balanced and moderate approach to healing [7] more so as adverse effects of most herbal drugs are minimal and relatively less frequent, when the drugs are used properly.

However, the increasing popularity of the use of medicinal plants globally has brought concerns and fears over quality, efficacy and safety of the products from medicinal plants used in health care [8-11]. These fears has limited the rational use of herbal medicine and have been responsible for the rather slow integration into western medicine [9,10].

An important issue in the Quality Assurance of medicinal plant products is the improper plant identification. Correct identification of the starting material is an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy [5,12-14]. Wrongly identified species, subspecies or species varieties are either therapeutically less active or inactive, or may even contain poisonous ingredients [9]. This is particularly pertinent as many medicinal plants are harvested in the wild, where conditions for growth and cultivation have not been optimized [15].

To ensure proper identification of medicinal plants the classical botanical identification of the plant is now being supplemented with chemical tests and chromatographic techniques as well as DNA based technologies which produce unique chemical and DNA fingerprint respectively for the plant. A chemical fingerprint can be defined as a characteristic profile reflecting the complex chemical composition of the analyzed plant sample that indicates the presence of multiple chemical markers within a sample and can be obtained by spectroscopic, chromatographic or electrophoretic techniques [5,9,16]. Chemical fingerprinting has been demonstrated to be a powerful technique for the quality control of medicinal plant extracts. Chromatographic fingerprinting analysis (chemo profiling) are known to disclose the detectable ingredients composition and concentration distribution [16-18]. Apart from qualitative and quantitative data, chemical fingerprints enable the screening of plant samples for the presence of active compounds from a myriad of compounds present in herbal samples [19]. Fingerprint analysis has been accepted by the World Health Organization (WHO) as a methodology for the quality control of herbal samples [3,4,16].

One of those ailments for which plants are continuously being scrutinized for its remedy is diabetes mellitus [20,21] a metabolic disorder characterized by hyperglycaemia and resulting from defects in insulin secretion or decreased sensitivity of tissues to insulin or both [22]. One of the medicinal plants that have been used in ethno medicine for the treatment of diabetes is *Nauclea latifolia* [23,24]. It is in ensuring the proper identification of this medicinal plant preparation by providing its chemical profiles, as part of assuring the quality of the plant preparation that this work is being carried out. It will also enable the screening of the plant preparation for the presence of antidiabetic compounds from the myriad of compounds present in the plant preparation.

Nauclea latifolia, commonly known as Pin cushion tree, belongs to the family Rubiaceae. It is a deciduous flowering, straggling evergreen, multi-stemmed shrub or small tree possessing an open canopy and not exceeding a height of 10 m

(32 ft). It grows well in tropical environments and is native to tropical Africa and Asia [25]. Its antidiabetic activity has been established in our laboratory [24] and elsewhere [26-28]. It has also been shown to have cardiovascular [29], anti-hypertensive [30], anticonvulsant, anxiolytic and sedative [31] antiviral [32], antimalarial [33], analgesic [34] and antiulcer [35] activities. Other ailments that have been proven to be improved by extracts of *Nauclea latifolia* include anxiety, destruction of intestinal parasites, boost of male libido and potency as well as the management of menstrual issues [36].

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh mature leaves of *Nauclea latifolia,* were harvested from the University of Calabar botanical farm, Calabar, Cross River State, Nigeria. The plant was authenticated at the Department of Botany, University of Calabar and was in accordance with voucher specimen Herb/Bot/ucc/017 already deposited in the department's herbarium.

2.2 Preparation of Plant Extracts

The harvested *Nauclea latifolia* leaves were thoroughly washed with clean water, rinsed with distilled water and allowed to drain. The leaves where then dried away from direct sunlight for seven (7) days, after which they were grounded into powder with a manual blender. The pulverised leaves were weighed and then soaked in 80% ethanol solution in the ratio 1:4 (sample: ethanol) for 48 hours with intermittent agitation. The plant homogenate was doubly filtered using cheese cloth followed by Whatman filter paper and the filtrates concentrated in a water bath at a temperature of 40ºC. The dark brown gummy residual mass was stored at a temperature of 4ºC in a refrigerator until ready for use.

2.3 Determination of Chromatographic Chemical Profile Using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was performed as previously described [21]. A Shimadzu GC-MS QP-2010 comprising a gas chromatograph hyphenated to a mass spectrophotometer (MS) and an auto sampler was used for this analysis. A 25 m x 0.25 mm fused silica capillary column coated with CP-Sil5 and film thickness at 0.15 µm was fitted to the gas chromatograph. The carrier gas was helium at 1.2 ml/min. Operating conditions of the MS was: ion source temperature 230°C, ionization voltage 70ev. The MS data obtained was processed online with desktop computer fitted with disk memory. The identification of the components was accomplished by comparison of the retention indices, fragmentation pattern and mass spectra with spectrum of known components stored in the database of National Institute of Standard and Technology (NIST) library (http://chemdata.nist.gov).

3. RESULTS AND DISCUSSION

3.1 Gas Chromatography - Mass Spectroscopy (GC-MS) Profiling

The GC-MS chromatograms of ethanol leaf extract of *Nauclea latifolia* is shown in Fig. 1. A complete list of the identified compounds, retention times and percentage peak areas are shown in Table 1. The chromatogram revealed the presences of fifty-one (51) phytochemical compounds. Hexadecanoic acid, ethyl ester was the most abundant compound (15.80%) and 2-Hexanethiol the least abundant (0.06%).

Similar GC-MS analysis have been carried out on *Nauclea latifolia* leaf, by other workers, after steam distillation [37], cold maceration in purified re-distilled methanol [37], ethanol and aqueous extraction [38]. GC-MS analysis have also been performed on the methanol extract of the stem [37], chloroform extract of the pulverized ripe fruits [39] and the dry water extract of the root of the plant [40]. The GC-MS profile of the ethanol leaf extract by Iheagwam et al. [38] showed the presence of 47 peaks while the same profiling in this study resulted in 51 peaks. Even more striking was that only fourteen (14) of the phytochemicals (taking into account the derivatives) were common to both profiles emphasizing the need for chemical profiling as a means of unambiguously identifying plant preparations. The cause of the variability in the profile of the two ethanol extract could be Intrinsic factors like difference in subspecies or species varieties, age of the plant, genetic variability and/or extrinsic factors like environment (climate, altitude, temperature, light exposure, water and nutrient availability), geographic location, diurnal and seasonal variations and method of cultivation [11,14,41].

Table 1. Phytochemical compounds identified for various peaks in *Nauclea latifolia* ethanolic leaf extract (GC-MS)

Peak	Retention time	Area%	Height%	Name
1	3.048	0.06	0.13	2-Hexanethiol
2	3.157	0.11	0.11	2-Decanynoic acid
3	3.324	1.42	2.53	Pentanoic acid, 4-oxo-, ethyl ester
4	4.431	0.25	0.33	Butanedioic acid, diethyl ester
5	4.685	0.23	0.15	Pentanoic acid, 3-hydroxy-, ethyl ester
6	4.825	0.11	0.10	Isosorbide
7	5.083	0.74	0.78	Pentanoic acid, 4,4-dimethoxy-, ethyl ester
8	5.583	0.63	0.79	Butanedioic acid, hydroxy-, diethyl ester
9	6.206	0.38	0.17	Cyclohexanone, 2-(hydroxymethyl)
10	6.442	0.32	0.18	2-Methoxy-4-vinylphenol
11	6.880	0.13	0.13	2-Ethyl-3-hydroxy-2-methyl-succinic acid, 1-ethyl ester
12	7.593	0.06	0.09	Nonanoic acid, 9-oxo-, ethyl ester
13	7.853	0.18	0.17	alpha.-D-Glucopyranoside, O-.alpha.-D-glu
14	8.117	0.43	0.27	l-Pyrrolid-2-one, N-carboxyhydrazide
15	8.635	0.55	0.65	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo
16	8.829	0.76	0.45	alpha.-D-Glucopyranoside, O-.alpha.-D-glu
17	9.072	0.16	0.18	9-Octadecenoic acid (Z)-, methyl ester
18	9.479	0.21	0.17	3-Decenoic acid, (E)-
19	9.745	0.62	0.70	Ethyl tridecanoate
20	10.011	1.03	0.59	Decanoic acid, silver(1+) salt
21	10.314	3.66	1.54	Triethyl citrate
22	10.453	4.96	2.04	Quinic acid
23	10.526	1.92	1.29	Azelaic acid
24	11.394	9.79	1.78	3-O-Methyl-d-glucose
25	11.757	0.59	0.87	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
26	11.811	0.38	0.27	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl
27	11.931	0.84	0.91	Hexadecanal
28	12.062	0.63	0.91	Phytol, acetate
29	12.118	0.20	0.36	Hexadecanoic acid, ethyl ester
30	12.218	0.39	0.66	3,7,11-Trimethyl-2,4-dodecadiene
31	12.342	1.77	2.99	Hexadecanoic acid, methyl ester
32	12.657	1.24	1.02	Ethyl 9-hexadecenoate
33	12.753	1.22	1.46	Ethyl 9-hexadecenoate
34	12.823	15.80	20.29	Hexadecanoic acid, ethyl ester
35	13.444	1.37	1.83	Ethyl 14-methyl-hexadecanoate
36	13.488	1.62	2.66	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethyl cyclo propyl)
37	13.642	0.74	1.05	Methyl stearate
38	13.781	2.41	1.30	cis-9-Hexadecenal
39	13.871	6.46	7.69	9,12-Octadecadienoic acid, ethyl ester
40	13.922	13.62	18.26	9,12,15-Octadecatrienoic acid, ethyl ester,
41	14.055	4.93	7.19	Octadecanoic acid, ethyl ester
42	14.627	0.55	0.56	Ethyl 9-hexadecenoate
43	15.183	1.41	2.05	Hexadecanoic acid, ethyl ester
44	15.795	1.54	1.28	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
45	17.803	1.04	1.04	Methyl 2-hydroxy-pentacosanoate
46	18.816	1.53	1.27	Stigmast-5-en-3-ol, oleate
47	18.898	4.32	3.45	Vitamin E
48	19.594	1.48	1.09	Campesterol
49	19.807	0.68	0.64	Stigmasterol
50	20.239	4.11	3.39	gamma.-Sitosterol
51	20.348	0.41	0.21	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β…)
		100.00	100.00	

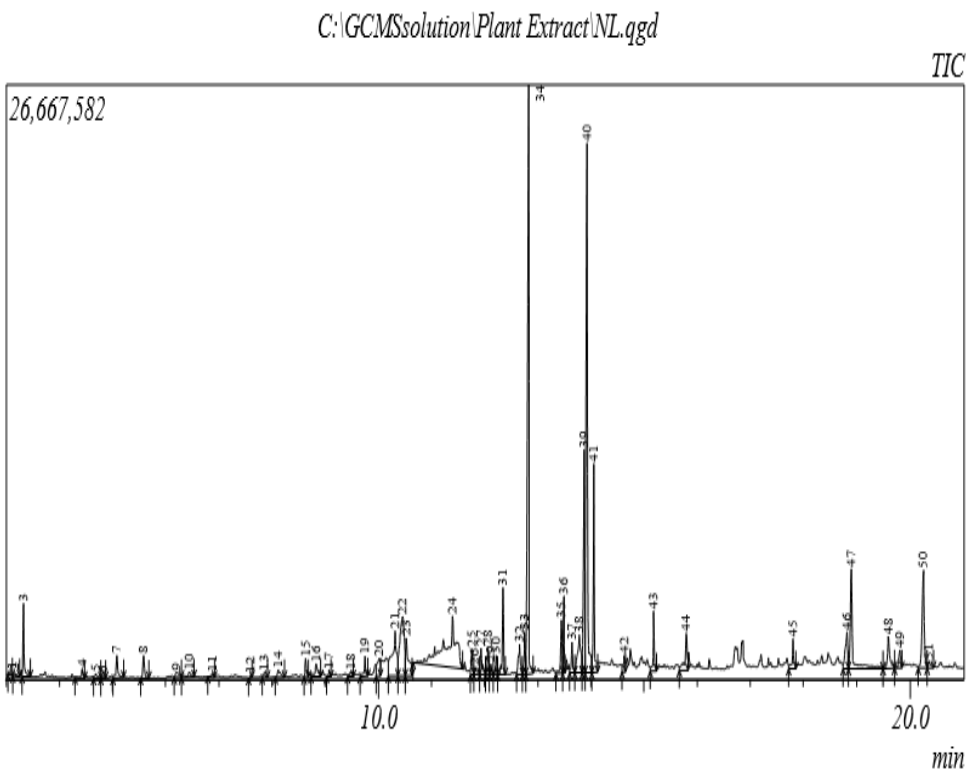

Fig. 1. GC-MS full spectrum analysis of *Nauclea latifolia* ethanolic leaf extract

Of the 51 phytocomponents in the ethanol extract of *Nauclea latifolia*, at least sixteen (16) phytochemicals namely: Pentanoic acid 4-oxo-ethyl ester, 2-Methoxy-4-vinylphenol, Triethyl citrate, Quinic acid, 3-O-Methyl-d-glucose, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, Phytol, Hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, Octadecanoic acid, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester, Vitamin E, Campesterol, Stigmasterol and Gamma-sitosterol are known to have anti-diabetic properties. These 16 components will be discussed further only as they affect diabetes.

Pentanoic acid, 4-oxo-, ethyl ester is Hepatoprotective [42]. *2-Methoxy-4-vinylphenol* has anti-inflammatory and antioxidant activities [43-45] while *Triethyl citrate* is an anti-inflammatory agent [46]. *Quinic acid* has been shown to reduce hyperglycemia and oxidation by increasing c-peptide as well as insulin levels [47]. *3-O-Methyl-d-glucose* inhibits glucose-stimulated insulin release under experimental condition [48]. *3,7,11,15-tetramethyl-2-hexadecen-1-ol* has been reported to have, among other properties, insulin/antidiabetic, antiarthritic [49] and anti-inflammatory [50] properties. *Phytol* is important in the processing of glucose and can activate enzymes within the body that have strong positive effects on insulin level implying that it could possibly help restore the metabolic functions of those with type-2 diabetes [21,51]. Phytanic acid (PA) a chlorophyll metabolite from phytol has potentials in regulating glucose metabolism by regulating hepatic glucose homeostasis [52]. *Hexadecanoic acid*, otherwise called palmitic acid, has anti-inflammatory [53], antioxidant, hypocholesterolemic, anti-androgenic [54] activities. It has also been shown to stimulate glucose uptake in skeletal muscle cells [55].

9,12-octadecadienoic acid: *(linoleic acid)* has, among other attributes, been shown to have hypocholesterolemic, hepatoprotective, anti-androgenic [54] and antioxidant [56] properties. *9,12,15-octadecatrienoic acid* also called α-linolenic acid (ALA) possesses anti-cardiovascular, anti-inflammatory, hypocholesterolemic, hepatoprotective and antiarthritic properties [57-59] via inhibition of reactive oxygen species (ROS) generation and

modulation of NF-kB signal transduction [57]. *Octadecanoic acid (Stearic acid)*, as it concerns diabetes, has been shown to lower LDL cholesterol, the ratio of total to HDL cholesterol [60] and to dramatically reduce visceral fat as well as lowering blood glucose and leptin concentrations [61]. Reducing excess visceral fat may be very beneficial for type 2 diabetes, and possibly other disease states. *Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester* has antioxidant and anti-inflammatory activities [46,62]. *Vitamin E* is a potent antioxidant, hypocholesterolemic, anti-inflammatory, hepatoprotective and antidiabetic agent [50,59,63]. *Campesterol* has antioxidant and hypocholesterolemic activity [63,64]. The campesterol molecules are thought to compete with cholesterol, thus reducing the absorption of cholesterol in the human intestine [64]. *Stigmasterol* has been shown to have anti-hepatotoxic, antioxidant, Cancer preventive, hypocholesterolemic [63] and antidiabetic [65] activity. *Gamma-sitosterol*: has anti-inflammatory, antidiabetic, properties [66,67]. Administration of Gamma-sitosterol resulted in significant decreases in glycosylated hemoglobin and blood glucose with a significant improvement in plasma insulin levels, body weight and also food intake and further showed antihyperlipidemic and hepatoprotective activities [66].

4. CONCLUSION AND SUMMARY

The GC-MS profile provides a suitable chemical fingerprint that will ensure proper identification of the *Nauclea latifolia* plant. Sixteen (16) active phytochemicals of the plant leaf are known to have antidiabetic activities; these could be used as a basis for standardization of the plant preparations for diabetes therapy.

In summary earlier studies has validated the use of *Nauclea latifolia* leaf extract for treatment of diabetes. To assure property identification of the plant, a GC-MS chemical profile has been established for the plant. Of the fifty-one (51) phytochemical constituents of the plant, as revealed by GC-MS analysis, at least sixteen (16) are known to have antidiabetic activities. This work represents a step towards “true” standardization of the plant preparation, based on constituents of known therapeutic or pharmacological activity, for presentation in diabetes therapy. It also is a step towards isolating the active diabetic principles in the plant.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. World Health Organ. 1985;63:965.
2. Odugbemi TA. A textbook of medicinal plants from Nigeria. Lagos: University of Lagos Press; 2008.
3. WHO. Guidelines for the assessment of herbal medicines, World Health Organization, Geneva, Switzerland; 1991.
   Available:http://whqlibdoc.who.int/HQ/1991/WHO.TRM.91.4.pdf
   (Accessed October 28, 2019)
4. WHO. General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization, Geneva, Switzerland; 2000.
5. Rasheed NMA, Nagaiah K, Goud PR, Sharma VUM. Chemical marker compounds and their essential role in quality control of herbal Medicines. Annals of Phytomedicine. 2012;1(1):1-8.
6. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation. 2012;4(3): 101-112.
7. Bandaranayake WM. Quality control, screening, toxicity, and regulation of herbal drugs. In Modern Phytomedicine. Turning Medicinal Plants into Drugs. Edited by I. Ahmad, F. Aqil, and M. Owais. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. 2006;25-57.
8. Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine. 2006;27(1):1–93.
9. Efferth T, Greten HJ. Quality control for medicinal plants. Medicinal and Aromatic Plants. 2012;1:7-9.
10. Padalia RC. Medicinal and aromatic plants: Chemical goldmines. Medicinal Aromatic Plants. 2012;1:e113.

11. Zhang J, Wider B, Shang H, Li X, Ernst E. Quality of herbal medicines: Challenges and solutions. Complement Ther Med. 2012;20(1-2):100-6.
12. De Smet PAGM. Herbal remedies. N. Engl. J. Med. 2002;347:2046-56.
13. Straus SE. Herbal medicines - What's in the bottle? N. Engl. J. Med. 2002;347: 1997-1998.
14. Mgbeje BIA. Quality and safety of medicinal plants in phytomedicine: Panacea for Primary Health Care Delivery in Nigeria (P. E. Ebong Ed.). Calabar, University of Calabar Press. 2015;293-310.
15. Briskin DP. Medicinal plants and phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. Plant Physiol. 2000;124:507-514. Available:www.plantphysiol.org
(October 19, 2015)
16. Tistaert C, Dejaegher B, Heyden YV. Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. Analytica Chimica Acta. 2011;690(2):148–161.
17. Liang X, Zhang L, Zhang X, Dai W, Li H, Hu L, Liu H, Su J, Zhang W. Qualitative and quantitative analysis of traditional Chinese medicine Niu Huang Jie Du Pill using ultra performance liquid chromatography coupled with tunable UV detector and rapid resolution liquid chromatography coupled with time-of-flight tandem mass spectrometry. J. Pharm. Biomed. Anal. 2010;51(3):565–571.
18. Yang H, Zhao C, Wang X, Liang Y, Zeng Y, Wu H, Xu Q, Lv H. Chromatographic fingerprint investigation for quality evaluation and control of Shengui hair-growth tincture. Planta Med. 2010;76:372-377.
19. Ciesla L. Biological fingerprinting of herbal samples by means of liquid chromato-graphy. Chromatography Research International. 2012;1-9.
20. Tiwari AK, Rao JM. Diabetic mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Current Science. 2002;83:30-37.
21. Mgbeje BIA, Ofem-Otu J, Ugoanyanwu FO. Phytochemical components of butanol fraction of *Vernonia amygdalina* leaf extract using GC-MS analysis. Merit Research Journal of Medicine & Medical Sciences. 2020;8(4):74-79.
22. ADA (American Diabetes Association). Diagnosis and classification of diabetes mellitus. Diabetes Care. 2005;28(Suppl. 1): S37–S43.
23. Iwu MM. Handbook of African medicinal plants. 1st Ed. Florida: CRC Press. 1993;221-222.
24. Effiong GS, Mgbeje BI, Igile GO, Atangwho JI, Eyong EU, Ebong PE. Antioxidant enzymes activity and hormonal changes following administration of ethanolic leaves extracts of *Nauclea latifolia* and *Gongronema latifolium* in streptozotocin induced-diabetic rats. Eur. J. Med. Plants. 2013;3:297–309.
25. Mabberley D. Mabberley's plant book: Dictionary of plants, their classification and uses. Oxford: Cambridge University Press; 2008.
26. Gidado A, Ameh DA, Atawodi SE, Ibrahim S. A preliminary study of the mechanism of hypoglycaemic activity of *Nauclea latifolia* leaf ethanolic extract. J. Complement. Integr. Med. 2012;9:16.
27. Antia BS, Okokon JE. Phytochemical composition and antidiabetic activity of ethanol root extract of *Nauclea latifolia*. J. Phytopharmacol. 2014;3:52–56.
28. Boucherle B, Haudecoeur R, Queiroz EF, De Waard M, Wolfender JL, Robins RJ, Boumendjel A. *Nauclea latifolia*: Biological activity and alkaloid phytochemistry of a West African tree. Nat. Prod. Rep. 2016;33:1034–1043.
29. Akpanabiatu MI, Umoh IB, Udosen EO, Udoh AE, Edet EE. Rat serum electrolytes, lipid profile and cardiovascular activity on *Nauclea latifola* leaf extract administration. Indian Journal of Clinical Biochemistry. 2005;20(2):29-34.
30. Nworgu ZA, Ejerakeya AE, Onwukaeme DN, Afolayan AJ, Ameachina FC, Ayinde BA. Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. African Journal of Pharmacy and Pharmacology. 2008;2(2):37-41.
31. Ngo B, Taiwe E, Moto F, Ngoupaye G, Nkantchoua G, Pelanken M, Rakotonirina S, Rakotonirina A. Antioconvulsant, anxiolytic and sedative properties of the roots of *Nauclea latifolia* Smith in mice. Epilepsy Behavior. 2009;15(4):434-440.

32. Donalisio M, Nana HM, Ngane RA, Gatsing D, Tchinda AT, Rovito R, Cagno V, Cagliero C, Boyom FF, Rubiolo P, Bicchi C, Lembo D. *In vitro* anti-herpes simplex virus activity of crude extract of the roots of *Nauclea latifolia* Smith (Rubiaceae). BMC Complement. Altern. Med. 2013;13:266–274.
33. Onyesom I, Osioma E, Okereke PC. *Nauclea latifolia* aqueous leaf extract eliminates hepatic and cerebral *Plasmodium berghei* parasite in experimental mice. Asian Pac. J. Trop. Biomed. 2015;5:546–551.
34. Taïwe GS, Ngo Bum E, Talla E, Dimo T, Dawe A, Sinniger V, Bonaz B, Boumendjel A, De Waard M. *Nauclea latifolia* Smith (Rubiaceae) exerts antinociceptive effects in neuropathic pain induced by chronic constriction injury of the sciatic nerve. J. Ethnopharmacol. 2014;151:445–451.
35. Balogun ME, Jeje SO, Salami SA, Onwe PE, Folawiyo MA. Anti-ulcerogenic and gastric anti-secretory effects of *Nauclea latifolia* extract in male albino rats. Eur. J. Exp. Biol. 2015;5:74–80.
36. Khan M, Ugbede O, James T. Phytochemical analysis and the chemotherapeutics of leaves and stem bark of *Nauclea latifolia* grown in Hong, Adamawa State. Nigeria. Asian Journal of Plant Science and Research. 2011;1(3): 16-22.
37. Morah FNI, Emehige EP, Mowang MM. Chemical composition and antimicrobial activity of *Nauclea latifolia* leaf essential oil. International Journal of Chemical and Biochemical Sciences. 2017;11:44-50. Oluremi B, Oloche JJ, Fasusi ET, Lawal MA. Evaluation of phytochemical constituents and antimicrobial activity of leaves and stem bark extracts of *Sarcocephalus latifolius*. Microbiology Research Journal International. 2018; 24(2):1-10.
38. Iheagwam FN, Ogunlana OO, Chinedu SW. Model optimization and *in silico* analysis of potential dipeptidyl peptidase IV antagonists from GC-MS identified compounds in *Nauclea latifolia* leaf extracts. Int. J. Mol. Sci. 2019;20:5913 - 5347.
39. Fadipe AL. Some fatty acid esters of the ripe fruits of *Nauclea latifolia* (family Rubiaceae). International Journal of Research in Pharmacy and Chemistry. 2014;4(4):783-788.
40. Ameh SJ, Ibekwe N, Ambi A, Obodozie O, Abubakar M, Garba M, Cocker H, Gamaniel KS. Standardization of *Andrographis paniculata*, *Mitracarpus scaber* and *Nauclea latifolia* herbal preparations as per European and Nigerian drug regulations. European Journal of Medicinal Plants. 2014;4(4):413-443.
41. Fong HH. Integration of herbal medicine into modern medical practices: Issues and prospects. Integr Cancer Ther. 2002;1(3): 287-93.
42. Ueno O, Ohmi T, Yamamoto M, Kato N, Moriguchi Y, Kojima M, Shimozono R, Suzuki S, Matsuura T, Eda H. Orally-administered caspase inhibitor PF-03491390 is retained in the liver for prolonged periods with low systemic exposure, exerting a hepatoprotective effect against a-Fas-induced liver injury in a mouse model. Journal of Pharmacological Sciences. 2007;105:201-205.
43. Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of Ethnopharmacology. 2005;99(1):69-73.
44. Fukai S, Tanimoto S, Maeda A, Fukuda H, Okada Y, Nomura M. Pharmacological activity of compounds extracted from persimmon peel (*Diospyros kaki* Thunb.). Journal of Olea Science. 2009;58(4):213-219.
45. Jeong JB, Hong SC, Jeong HJ, Koo JS. Anti-inflammatory effect of 2-methoxy-4-vinylphenol via the suppression of NF-κB and MAPK activation and acetylation of Histone H3. Archives of Pharmacal Research. 2011;34(12):2109–2116.
46. Al-Marzoqi AH, Hameed IH, Idan SA. Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). African Journal of Biotechnology. 2015;14(40):2812-2830.
47. Arya A, Al-Obaidi M, Shahid N, Bin N, Loo C, Wong W, Khaing S, Mustafa M. Synergistic effect of quercetin and quinic acid by alleviating structural degeneration in the liver, kidney and pancreas tissues of

STZ-induced diabetic rats: A mechanistic study. Food and Chemical Toxicology. 2014;71:183-196.

48. Sener A, Scruel O, Louchami K, Jijakli H, Malaisse WJ. Inhibition of glucose-induced insulin release by3-O-methyl-D-glucose: Enzymatic, metabolic and cationic determinants. Molecular and Cellular Biochemistry. 1999;194(1/2):133–145.
49. Elmazar M, El-Abhar H, Schaalen M, Farag N. Phytol/phytanic acid and insulin resistance: Potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. PLoS One. 2013;8(1):45-48.
50. Padmashree MS, Ashwathanarayana R, Naika R, Roopa B. Antioxidant, cytotoxic and nutritive properties of *Ipomoea staphylina* Roem & Schult. plant extracts with preliminary phytochemical and GCMS analysis. Asian Journal of Pharmacy and Pharmacology. 2018;4(4):473-492.
51. Venkata Raman B, Samuel LA, Pardha Saradhi M, Narashimha Rao B, Naga Vamsi Krishna A, Sudhakar M, Radhakrishnan TM. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. Asian J Pharm Clin Res. 2012;5(2):99-106.
52. Che BN, Oksbjerg N, Hellgren LI, Nielsen JH, Young JF. Phytanic acid stimulates glucose uptake in a model of skeletal muscles, the primary porcine myotubes. Lipids in Health and Disease. 2013;12: 14.
53. Aparna V, Dileep KV, Pradeep K, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of *n*-hexadecanoic acid: Structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80:434–439.
54. Sivagurunathan A, Xavier Innocent B. GC-MS evaluation of bioactive compounds of *Marsilea quadrifolia* Linn (Aquatic Fern). International Journal for Pharmaceutical Research Scholars (IJPRS). 2014;3(I-1): 164-174.
55. Jing P, Gong P, Linghai L, Huimin N, Yanbo L, Pingsheng L. Palmitic acid acutely stimulates glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells. Journal of Lipid Research. 2011;52(7):133-146.
56. Umeno A, Yoshino K, Hashimoto Y, Shichiri M, Kataoka M, Yoshida Y. Multiple biomarkers for early detection of type 2 diabetes, including 10 and 12 (Z-E)-hydroxyoctadecadienoic acids, insulin, leptin and adiponectin. PLoS ONE. 2015;10(7):130-137.
57. Erdinest N, Shmueli O, Grossman Y, Ovadia H, Solomon A. Anti-inflammatory effects of alpha linolenic acid on human corneal epithelial cells. Invest. Ophthalmol. Vis. Sci. 2012;53:4396–4406.
58. Kim K-B, Nam YA, Kim HS, Hayes AW, Lee B-M. α-linolenic acid: Nutraceutical, pharmacological and toxicological evaluation. Food and Chemical Toxicology. 2014;70:163–178.
DOI: 10.1016/j.fct.2014.05.009
59. Srinivasan K, Sivasubramanian S, Kumaravel S. Phytochemical profiling and GC-MS study of *Adhatoda vasica* leaves. International Journal of Pharma and Bio Sciences. 2014;5(1):714–720.
60. Hunter JE, Zhang J, Kris-Etherton PM. Cardiovascular disease risk of dietary stearic acid compared with *trans*, other saturated and unsaturated fatty acids: A systematic review. Am J Clin Nutr. 2010;91(1):46-63.
61. Shen MC, Zhao X, Siegal GP, Desmond R, Hardy RW. Dietary stearic acid leads to a reduction of visceral adipose tissue in athymic nude mice. PLoS One. 2014;9(9)15.
DOI: 10.1371/journal.pone.0104083
62. Gnanavel V, Saral M. GC-MS analysis of petroleum ether and ethanol leaf extracts from *Abrus precatorius* Linn. International Journal of Pharmacology and Biosciences. 2013;4(3):37-44.
63. Ponnamma SU, Manjunath K. GC-MS analysis of phytocomponents in the methanolic extract of Justicia wynaadensis (nees) t. anders. International Journal of Pharma and Bio Sciences. 2012;3(3):570–576.
64. Choudhary SP, Tran LS. Phytosterols: Perspectives in human nutrition and clinical therapy. Current Medicinal Chemistry. 2011;18(29):4557–67.
65. Wang J, Huang M, Yang J, Ma X, Zheng S, Deng S, Huang Y, Yang X, Zhao P. Antidiabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. Food & Nutrition Research. 2017;61(1):610.

66. Balamurugan R, Ignacimuthu S, Duraipandiyan V. Antidiabetic activity of gamma-sitosterol from *Lippia nodiflora* in streptozotocin induced diabetic rats. European Journal of Pharmacology. 2011;1(3):410-418.

67. Makoto K, Koji H, Motoei K, Masumi H, Yakata Y, Tamio T, Kazuhisa T. Sitosterol prevents obesity-related chronic inflammation. Biochemica et Biophysica Acta. 2018;1863(2):191-198.

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