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Phytochemical Analysis of n- Hexane and Ethylacetate Extracts of *Diodia scandens* Sw and Spectroscopic Identification of an Omega- 6 Fatty Acid and a Glyceryl Trilinoleoate

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors MUA and JCC designed the study, wrote the protocol and performed the isolations and the chromatographic analysis. Author JCC wrote the initial draft of manuscript and author MUA wrote the final manuscript. Author JOI directed the fractionations of crude products, ran the spectroscopic analyses and the interpretation of the spectra. All authors managed the literature searches, read and approved the final manuscript.

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ABSTRACT

Traditional Medicine Practitioners in South East Nigeria use *Diodia scandens* Swartz for the treatment of different diseases. Cold maceration of n- hexane and ethyl acetate solvents extracts for 48 hours yielded brownish oily solids which were subjected to the phytochemical analysis. Column chromatography of the crude extracts using n-hexane-ethyl acetate yielded different

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fractions. These were subjected to spectroscopic analysis. Phytochemical analysis result of the crude n- hexane extract showed the presence of steroids, glycoside, alkaloids, and saponins. Crude ethyl acetate extract showed the presence of steroid and saponin. The column chromatographic fraction CHDS11, was identified as cis, cis-9, 12 - octadecadienioc acid. The ^{1}H NMR chemical shift of 5.05(t) confirmed that the hydrogens are attached to the double bonds at C-9 and 10; C-12 and 13, CHDS12 was identified as 1, 2, 3-propanetriyltris (cis, cis - 9, 12-octadecadienoate), with the chemical shifts 5.28 ppm showing the vinyl protons at C-9 and 10; C-12 and 13; 5.19 ppm showed protons in between trilinoleoate chains. The presence of these compounds justified the use of this plant for treating arthritis and other diseases. This is the first report regarding these compounds which are isolated from this plant.

Keywords: Diodia scandens; n-hexane; ethylacetate; phytochemical analysis; ^{1}H NMR.

1. INTRODUCTION

Human beings from antiquity depended on plants either directly for foods and beverages, or indirectly as feed for animals or the flavouring of foods. Plants are also the source of beverages produced either by infusions, such as coffee and tea; by fermentation, such as beer and wine; or by distillation, such as whisky, vodka, rum, and other alcoholic spirits [1].

Plants are the source of many natural products such as essential oils, natural dyes, pigments, waxes, resins, tannins, alkaloids, amber and cork. Products derived from plants include soaps, shampoos, perfumes, cosmetics, paint, varnish, turpentine, rubber, latex, lubricants, linoleum, plastics, inks, and gums. Renewable fuels from plants include firewood, peat and many other biofuels. Coal and petroleum are the fossil fuels derived from the remains of plants. Olive oil has been used in lamps for centuries to provide illumination [1].

The man has been engaged in the use of the plants and their different parts for the treatment of various ailments. Generally, plants have been used throughout the world in folk medicine and as a local cure for common ailments. Medicinal plants, in particular, have been in use for centuries as remedies for human diseases like malaria, dysentery, diarrhoea, typhoid, arthritis, infertility etc. because they contain components of therapeutic value [2]. Folk medicine gave rise to the traditional system of medicine in various diseases.

Medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, a sesquiterpene, lactones and oils (essential and fixed). India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. According to an estimate, 120 or so plant-based drugs prescribed throughout the world come from just 95 plant species [3]. Natural antimicrobials can be derived from plants, animal tissues and microorganisms. The shortcoming of the drugs available today propelled the discovery of new pharmacotherapeutic agents from medicinal plant research [3]. The research aims to carry out phytochemical analysis of n-hexane and ethyl acetate extracts of *Diodia scandens* Sw and spectroscopic identification of an omega-6 fatty acid and a glyceryl trilinoleoate.

1.1 Description of Plant

Diodia scandens Sw (Rubiaceae) is an evergreen perennial herb, which has an alternate leaf arrangement, and the petiole is present. It has compound leaves, ovate to lanceolate in shape, reticulate venation, margin entire, acute apex, cuneate base, with glabrous surface, and its texture is chartaceous. *Diodia scandens* Sw has a dark green colouration, tasteless, odourless and has solitary inflorescence. It is a straggling herb, which has been in use in the West African system of medicine. It has enormous usefulness and importance. The whole parts of the plants are useful in curing various ailments [4].

The plant's medicinal value includes its use as antidotes, painkiller, treatment of venereal diseases and cutaneous and subcutaneous fungal infections. The different parts of the plants- sap, leaf, stem and root, are used for various medical purposes. The leaf is used for treating arthritis, rheumatism, cutaneous and subcutaneous parasitic infection, diarrhoea, dysentery and anti- abortifacients. The leaf and roots are used for dropsy, swellings, oedema, and gout and as lactation stimulants; while the sap is used for treating ear infections, paralysis, epilepsy, convulsions, spasm and pulmonary troubles [4]. The whole plant of *D. scandens Sw* is used for treating fibroid and uterine disorder [5].

2. MATERIALS AND METHODS

2.1 Materials

The solid compounds used in this work were purified and solvents redistilled before use. The spectroscopic equipment was;

Infrared spectrometer, transmittance 4000-650.
Ultraviolet/ Visible spectrometer, 4.20(468).
Nuclear Magnetic Resonance, 400 MHz

2.2 Methods

2.2.1 Sample collection and preparation

Diodia scandens Sw was collected at St. Mary's Pro-Cathedral Parish Udi and the plant was identified by a qualified taxonomist, Prof. J C. Okafor, at No. 7 Dona drive, Off Ihiala Street, Independence Layout Enugu, Enugu State, Nigeria.

The plant leaves were washed and air dried. The dried leaves were ground with a mechanical grinder. It was stored in a large sample bottle and labelled.

2.2.2 Extraction of the plant material

Using cold maceration method n-hexane and ethyl acetate were used. The pulverised sample (200 g) was macerated in 500 mL of n-hexane, stirred vigorously and was left for 48 hours. It was filtered and the filtrate was allowed to dry at room temperature. This was repeated using ethyl acetate.

2.2.3 Preparation of reagents

Meyer's reagent: Meyer's reagent was prepared by adding 1.3 g of mercuric iodide in 10 mL of water and 5.0 g of potassium iodide in 20 mL of water and making it up to 100 mL solution in a flask [6].

Marquis reagent: Marquis reagent was prepared by mixing concentrated tetraoxosulphate (VI) acid and formalin in the ratio of 10:1 v/v respectively [6].

Draggendorf's reagent: Draggendorf's reagent was prepared by dissolving 0.6 g bismuth subnitrate in 2 mL concentrated hydrochloric acid and 10 mL of water and was mixed with 6 g of potassium iodide in 10 mL of water. Then 7 mL of concentrated hydrochloric acid 15 mL of water was added, and the whole was diluted with 400 mL of water [6].

2.3 Phytochemical Analysis [6,7]

Phytochemical analyses were carried out using standard procedures.

2.4 Column Chromatography

The crude n- hexane extract was column chromatographed on silica gel adsorbent using n-hexane: ethyl acetate v/v in the ratio of 95:05, 90:10, 80:20, and 70:30 up to 100 mL of ethyl acetate. Fractions with same or similar R_f values were pooled together. Fractions CHDS11 and CHDS12 were collected from 95:05 n-hexane: ethyl acetate.

3. RESULTS AND DISCUSSION

3.1 Mass and Percentage Yield of Crude Extracts

Table .1 shows the mass and percentage yield of the crude extracts from the solvents. Ethyl acetate had a mass yield of 7.15 g (3.58%), and n-hexane had weight 3.18 g (1.59%). From the result, a polar solvent ethyl acetate was extracted more than a non- polar solvent. This shows that polar solvents are better extraction solvent than non-polar solvents.

3.2 Phytochemical Analysis of the Crude Extracts of *D. scandens Sw*

Table 2. shows the phytochemical result for the crude extract of *D. scandens Sw*. The phytochemical analysis of n-Hexane crude extract showed that glycoside, steroid, saponin and alkaloid were present. In the ethyl acetate, steroid and saponin were present. Properties of saponin- containing herbs are many and varied and may include diuretic, expectorant, anti-catarrhal, anti-inflammatory, antispasmodic, aphrodisiac, antioxidant, emmenagogue, cardiac stimulant, hormone modulating, hepatoprotective, and adrenal adaptogenic effects [8]. Steroids have been reported to have antibacterial properties, and they are very

important compounds especially due to their relationship with compounds such as sex hormones. Alkaloids have been associated with medicinal uses for centuries, and one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports [8]. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents, and this plant proves to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

3.3 Spectroscopic Analysis of the Pure Samples

Table 3 Pure samples were analysed for structural elucidation using ^{1}H NMR (400 MHz, $CDCl_3$).

Compounds extracted were: CHDS11, and CHDS12.

The compound CHDS11 was oily solid and has a wine red colour. Fraction CHDS12 was an oily solid.

3.4 ^{1}H NMR Spectrum of CHDS11 as (cis, cis-9,12-octadecadienoic acid) in $CDCl_3$ Compared to Literature

Table 3 shows the experimental chemical shifts and multiplicity of CHDS11 compared to literature values.

The chemical shift at 0.8(qd) showed the presence of methyl group at C-18; 5 .05(t) confirming the attachment of hydrogens to the 2 double bonds at C – 9 and 10; C-12 and 13 of the compound.

The chemical shift 2.3 (t) at C-11 shows the methylene hydrogen linking the two double bonds, 1.97 (t) at C-2 indicating the attachment of methylene hydrogen to the carboxylic acid group. The chemical shift 1.61(s) at C-14 shows methylene hydrogen preceding the vinyl carbons. The chemical shift 1.20 (m) indicates methylene

Table 1. MASS and percentage yield of the crude extracts from the solvents

Extracting solvents	Mass of crude extract (gram)	Percentage yield
Ds ethyl acetate	7.15	3.58
Ds n-hexane	3.18	1.59

Ethyl acetate extracted a higher percentage mass than n-hexane

Table 2. Phytochemical result for crude extract of *Diodia scandens Sw*

Glycoside	Ds n-Hexane	Ds ethylacetate
	+	–
Steroid		
i) Salkowski test	+	+
ii) Lieberman-Buchard test	+	+
iii) Formaldehyde test	+	+
Saponin		
i) Emulsion test	+	+
ii) Frothing test	–	+
Alkaloids		
i) Maquis test	+	–
ii) Meyer’s test	+	–
iii) Draggendorff’s test	–	–
Tannins		
i) Test with 5% $Fecl_3$	–	–
ii) Test with 10% K0H	–	–
Phlobatanintest	–	–
Anthraquinonetest	–	–
Flavanoids		
Test with concentrated H_2SO_4	–	–
Test with 10% NaOH	–	–
Test with Mg - HCl	–	–

***Key:* + =** *present, - = absent.*

Table 3. Spectroscopic analysis of the pure samples

Fraction	Colour and nature
CH DS_{11}	Wine red oil
CH DS_{12}	Orange oil

Isolated fractions and their colours

groups in between the CH_2 following the vinyl carbon and the carboxylic acid group and the one (3(CH_2)) in between the methyl group and the – CH_2– group attached to vinyl carbon, given a total of 14 hydrogens. These chemical shifts as compared with literature confirm the presence of linoleic acid in the sample from *D. scandens Sw*.

The presence of fatty acids such as, linoleic acid which balances female reproductive hormones and also helps to lubricate the mucous membrane was confirmed.

Growing evidence indicates that the design and delivery of supplemental fatty acids to the lower gut may target reproductive tissues to improve reproductive function and fertility. Improvement in embryo survival may be associated with suppression of uterine prostaglandin secretion via linoleic acid or another longer unsaturated fatty acid chain. Changes in follicular dynamics can be affected by fat supplementation and may lead to a more fertile ovulation. This improvement may be due to the alterations in metabolic hormones and growth hormone or hormonal clearance [9]. Thus, the fatty acid present in *D. scandens Sw* must be responsible for the reduction of swelling and nourishing of the uterine wall.

Table 4: shows the experimental chemical shift and multiplicity of CHDS12. It was orange oil and gave an R_f value of 0.68.

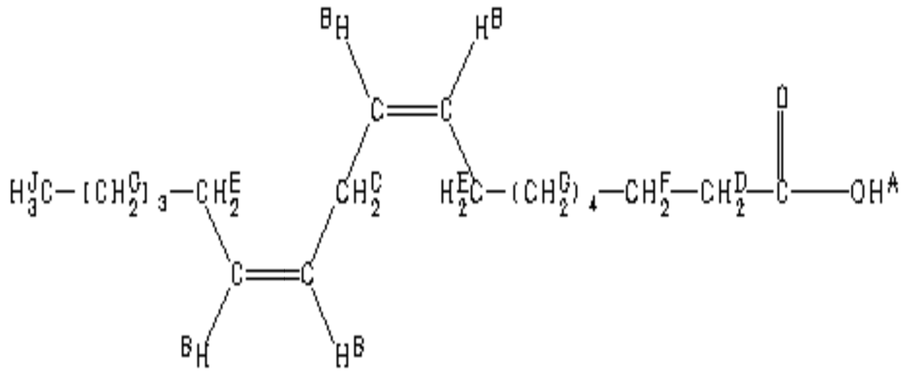
The chemical shift 5.28(m) indicates vinyl protons at C – 9, 12 in the compound. The chemical shift 5.19(t) shows protons in between trilinoleoate chains. The chemical shifts 4.22 (dd) and 4.07 (dd) indicate the proton glyceride group. The chemical shift 2.72 (dq) shows methylene protons in between the double bonds. The chemical shift 2.24 (td) shows – CH_2–group following the ester group. The three chemical shifts 1.99 (m), 1.54 (d) and 1.21 (m) are methylene group in between the vinyl carbon and the – CH_2– group attached to the ester carbon. 0.81 (td) shows a methyl group. The chemical shifts as compared to literature confirm the presence of triglyceride, 1, 2, 3 – propanetriyltris (cis, cis – 9, 12 – octadecadienoate) is present in *D. scandens* Sw.

This compound 1,2,3-propanetriyltris(cis,cis-9,12-octadecadienoate) increases the blood-permeability in the brain. The blood brain barrier forms a structural and functional barrier between the blood circulation and brain parenchyma, regulates the transport of molecules, and prevents blood cells from accessing brain tissues. Triolein emulsion transiently increased vascular permeability with interstitial oedema in the cat brain when administrated via the carotid artery [10]. Triglycerides are esters of glycerol combined with three chains of fatty acids. Elevated triglyceride is a strong indicator of biliary function, fat metabolism, liver function and hereditary. There is generally a sugar-handling issue with elevated triglyceride or adult- onset diabetes. Decrease triglyceride suggests a poor release of fatty acid, endocrine hyperfunction, and immune problem [11]. Triglyceride insulates the body from extreme temperature changes. They absorb and transport vitamin A, D, E and K through the blood cell [12].

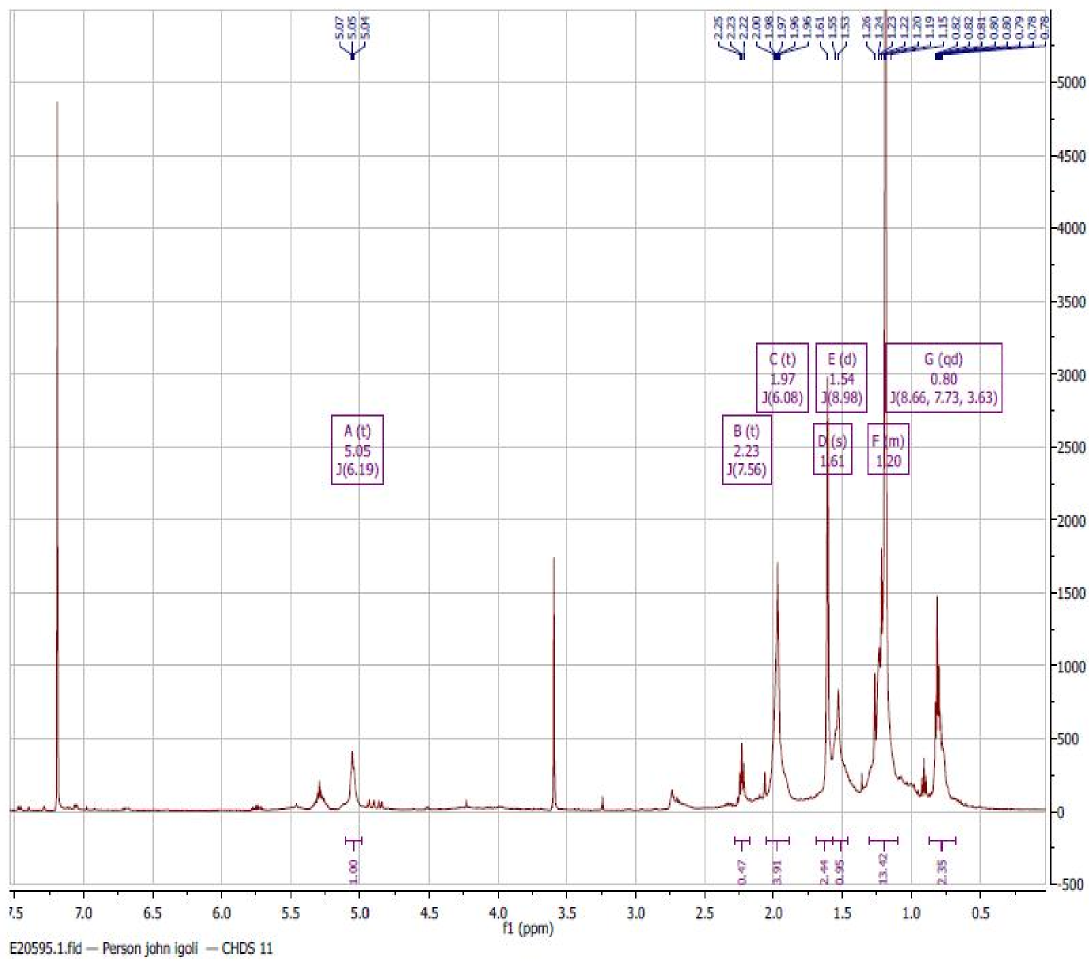
Table 4. Experimental chemical shifts and multiplicity of CHDS11 compared to literature

Proton	Experimental chemical shift δ and multiplicity	SDBS literature	Anatoli et al Literature
CH – 9,10,12,13	5.05 (t)	5.58-5.12	5.35 (m)
CH – 8,14	2.23 (t)	2.764	2.77 (t)
CH_2 – 2	1.97 (t)	2.33	2.33
CH_2 – 3	1.54 (d)	1.63	1.61
CH –11	1.61 (s)	2.02	2.07 (q)
CH – 15	1.20 (m)	-	1.30 (m)
CH – 16	1.20 (m)	–	1.30 (m)
CH – 17	1.20 (m)	1.10–1.53	1.31(m)
CH_2 – 4,5,6,7	1.20 (m)	1.10 – 1.53	1.33(m)
CH_3 – 18	0.80 (qd)	0.9	0.9 (t)

Experimental NMR data showing the protons and chemical shifts in ppm

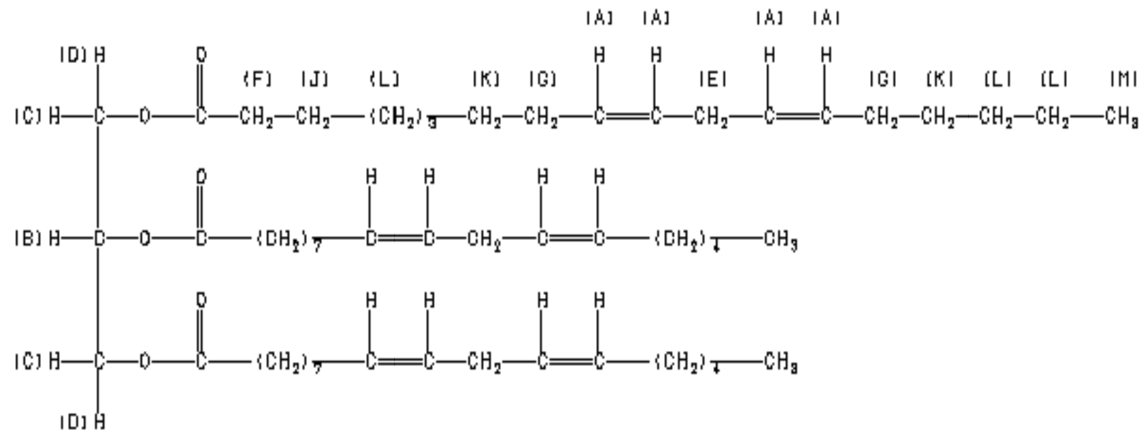


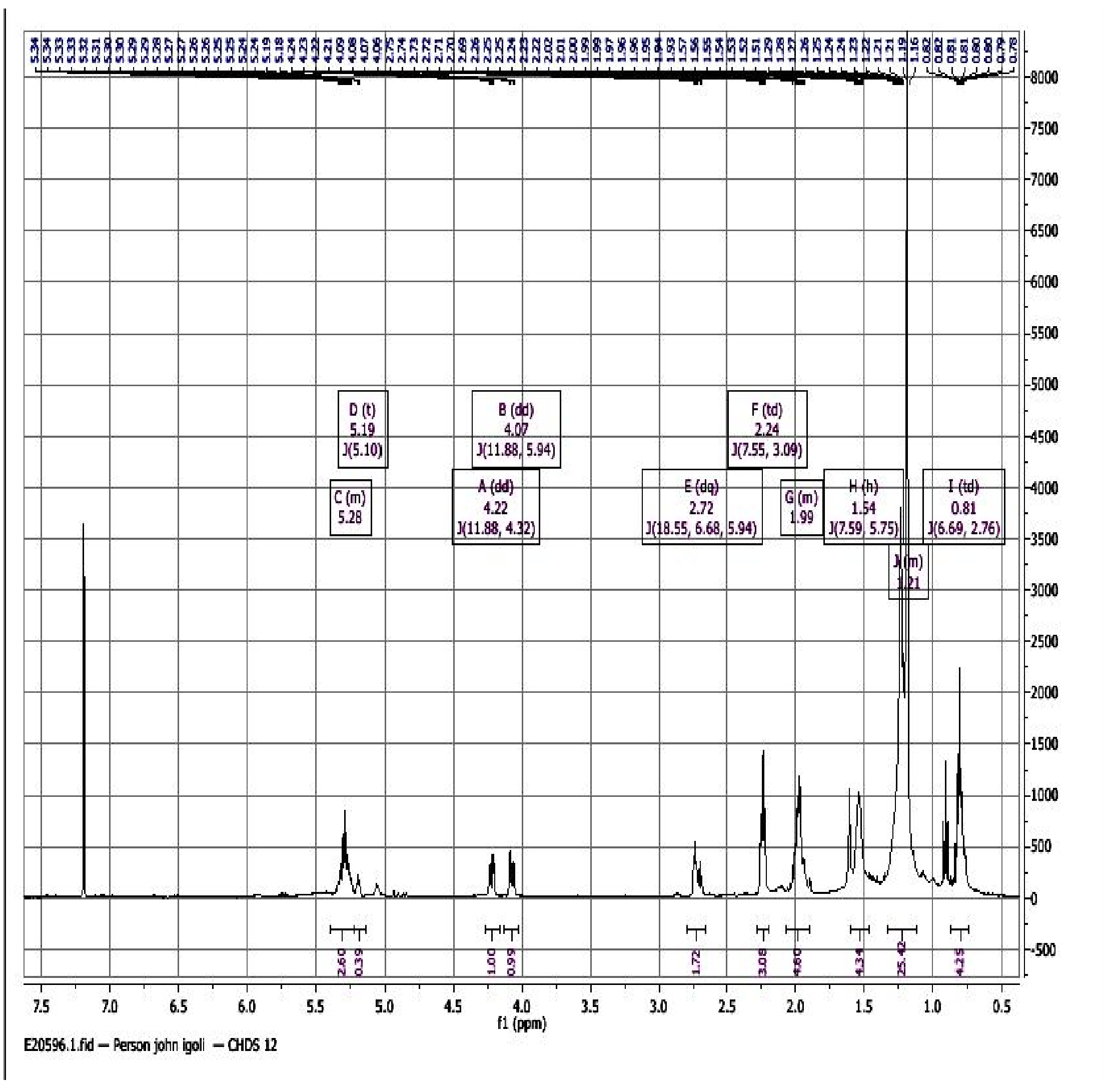
[1]H NMR spectrum of cis,cis-9,12-octadecadienoic acid



[1]H NMR spectrum of CHDS12 as 1,2,3-propanetriyltris(cis,cis-9,12-octadecadienoate) in $CDCl_3$ compared to literature

Fig. 1. CIS, CIS-9,12-Octadecadienoic acid, $C_{18}H_{32}O_2$ (linoleic acid)(CHDS11)





[1]HNMR SPECTRUM OF1,2,3-propanetriyltris(cis,cis-9,12-octadecadienoate)

Fig. 2. 1,2,3-propanetriyltris(cis,cis-9,12-octadecadienoate)

Table 5. Experimental chemical shift of CHDS12 compared to literature

Proton	Experimental chemical shift δ and multiplicity	SDBS literature
CH=CH9,10,12,13	5.28 (m)	5.35
H-C-O-2	5.19 (t)	5.27
CH_2O-1^1	4.22 (dd)	4.295
CH_2O-1^2	4.07 (dd)	4.147
CH_2-11	2.72 (dq)	2.769
CH_2-2	2.24 (td)	2.315
CH_2-8,14	1.99 (m)	2.049
CH_2-3	1.54 (d)	1.61
CH_2--7,15	1.21(m)	1.36
CH_2-4,5,6,16,17	1.21 (m)	1.30
CH_3-18	0.81 (td)	0.889

Experimental NMR data showing the protons and chemical shifts in ppm

4. CONCLUSIONS

The phytochemical analysis showed the presence of steroids, glycosides, alkaloids, saponins, in n-hexane leaves crude extract, steroids, and saponins in the crude ethyl acetate extract and steroids, saponins, alkaloids. The n-hexane crude extract was fractionated, and two pure samples were obtained and were labelled as CHDS11, CHDS12.

The ^{1}H NMR spectra revealed that the sample labelled as CHDS11 contained cis,cis,-9,12-octadecadienoic acid, whereas, CHDS12 contained 1,2,3-propanetryltris(cis,cis-9,12-octadecadienoate). The presence of these compounds has shown that the traditional use of the plant could be the correct alternative.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. Primary production of the biosphere: Integrating terestrial and oceanic components. 1998;237-238.
2. Okoli RI, Turay AA, Mensah JK, Aigbe AO. Phytochemical and antimicrobial properties of four herbs from Edo State, Nigeria. Report and Opinion. 2009;1(5):67–73.
3. Suman RK, Venkateshwer G, Samuel G, Gangadhar S. Phytochemical screening of some compounds from leaf plant extracts of *Holoptelea integrifolia* and *Celestus emarginata* used by Gondu tribes at Adilabad District, Andhrapradesh, India. 2013;2:65-70.
4. Essiette UA, Bala DN, Agbakalu JA. Pharmacognistic studies of the leaves and stem of Diodia Scandens Sw in Nigeria. Scholar research library, Archives of Applied Science Research. 2010;2(5):184–198.
5. Okafor JC. tropical plants in health care delivery in Nigeria. Bodija Ibadan. 2013;7–58.
6. Akpuaka MU. Essentials of natural products chemistry. Mason, Enugu. 2009; 34–64.
7. Sofowora A. Medicinal plants and traditional medicine in Africa, 2nd Edn, Ibadan, Nigeria. 1993;150–153.
8. Yadva RNS, Munin A. Phytochemical analysis of some medicinal plants. J. of Phyt. 2011;3(12):10-14.
9. Charles Staples, Ricardo Mattos, Scott Boken, Lynn Sollenberger, William Thatcher, Tom Jenkins. Feeding fatty acids for fertility. Proceedings of the 13th Annual Florida Ruminant Nutrition Symposium. 2014;71-85.
10. Barzo P, Marmarou A, Fatouros P, Corwin F, Dunbar J. Magnetic resonance imaging-monitored acute blood-brain barrier changes in experimental traumatic brain injury. J. Neurosurg. 1996;85(21):1113–1121.
11. Teresa C. Hill. The importance of triglyceride/ HDL ratio. Advocate. 2012; 970:522-537.

12. Assmann G, Schulte E. The importance of triglyceride. Euro. J. of Epidemiology. 1992;8(1):99.
13. Anatoli PS, Elvino B, Raffaella G, Anna LS. Metabolic profile of lettuce leaves by high-field NMR spectra, MRC. 2005;625-638.
14. Finar IL. Stereochemistry and the chemistry of natural products, Dorling Kindersley, New Delhi, India. 2013;78-80.
15. Harbourne JB. Phytochemical methods, chapman and hall Ltd. London. 1997;1–29.
16. Spectral data base for organic compounds; 2016.
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