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Phytochemical analysis of N-Hexane nut extract of *Tetracarpidium conophorum* (Euphorbiaceae) using Ultraviolet-Visible, fourier transform infrared and gas chromatography mass spectrometry techniques

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Abstract

The use of African walnut, *Tetracarpidium conophorum* had been in existence for time immemorial among the West African coastal region and sub-saharan Africa. Ethno-medicinal uses of the above nut include remedy for stomach ache, sperm boost as well as treatment of bacterial infection. The aim of the study was to chemo-metrically profile the bio active principle present in n-hexane nut extracts of *T. conophorum* which will be useful in the justification of the ethno-medicinal uses and claims. Chemometric profile of n-hexane nut extract of *T. conophorum* was performed by using ultraviolet-visible spectroscopy (UV-VIS), (UV-2500pc Spec), fourier transform infrared spectroscopy (FTIR) model-8400S spec.) and GC-MS (model-QP 2010 plus Spec). The compound detection employed the NIST Ver.2.0 – year 2005 library. The biological activity are based on Dr. Duke's phytochemical and ethnobotanical databases. Peaks corresponding to carotenoids and its derivatives were revealed by the UV-VIS spectroscopy. The FTIR spectrum confirmed the presence of alcohol, alkenes, alkanes, esters, and Carboxylic acid functional groups. The chemo-metric profile of the n-hexane nut extract of *T. conophorum* revealed eleven phyto-chemotypes with different pharmacological activities, overall, methyl (8E, 11E, 14E)- 8,11, 14 –icosatrienoate (31.61%) and Oleic acid (57.27%) was the prevailing compounds. Their presence provides important scientific evidence for their ethno-medicinal use and thus recommended as a plant of phyto-pharmaceutical importance.

Keywords: *Tetracarpidium conophorum*, carotenoids, chemo-metric, alkane, phyto-chemometric

1. Introduction

Tetracarpidium conophorum (family Euphorbiaceae) commonly called the African walnut, is a perennial shrub 10-20 feet long that is found growing wild in forest zones of sub-saharan Africa [1]. Studies have shown that the African walnut possesses some wound healing properties [2], antibacterial [3], antioxidant [4] and immune-stimulating activities [5]. Hence the present research is aimed at characterizing the bioactive principle present in the n-hexane nut extract using Ultraviolet-visible spectrophotometry (UV-VIS), fourier transform infrared spectroscopy (FTIR) and Gas- Chromatography-Mass spectrometry (GC-MS) and comparing with already established databases.

2. Materials and methods

2.1 Chemicals

Methanol, n-hexane, distilled water, were of analytical grade and purchased from Sigma Aldrich (St. Lous, MO, USA).

2.2 Plant material and extraction

The cooked African walnut (*T. conophorum*) was purchased from a local market in Port Harcourt, Nigeria. The nuts were identified and authenticated by Mr. Osuala of the department of pharmacognosy, University of Port Harcourt, Nigeria. A voucher specimen (UPC 108505) of the sample was deposited in the herbarium of the department.

2.3 Preparation of extract

Cooked *T. conophorum* nuts were cut into pieces and air-dried. The dried nuts were ground into powder using a Willey mill (Thomas Willy Mills, Swedesboro, NJ, USA). Five hundred grams of the pulverised powder was extracted with n-hexane (800 ml) for 48 h. The resultant oily extract was collected and stored in a refrigerator (-14 °C) till further use.

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2.4 UV-VIS and FTIR spectroscopic analysis.

The n-hexane extract was centrifuged at 3000 rpm for 10 min and thereafter filtered through 0.45 μ m synthered glass funnel. A 1:10 dilution of the centrifuged solution was made with the solvent. The extract obtained was scanned in the wavelength ranging from 200-900 nm using UV-2500 PC Series Ver.2.30 spectrophotometer and the characteristic peaks detected. The diluted extract above was used in carrying out the FTIR analysis using FTIR-8400s spectrophotometer system. Through the various characteristic peaks obtained, the various functional groups present were deduced. The peak values of the UV-VIS and FTIR were recorded. The above analysis were carried out in duplicate.

2.5 GC-MS analysis

Preparation of extracts for GC- MS analysis:

The concentrated extract was re-dissolved in n-hexane, vortexed and filtered through 0.45 μ m synthered glass funnel. A 1 μ l aliquot of the sample solution was injected into the GC-MS equipment.

2.6 Instrumentation and chromatographic conditions

GC-MS analysis was conducted on a Model QP 2010 Plus Shimadzu, Tokyo, Japan. Comprising a AOC-20i auto-sampler and gas-chromatograph interphased to a mass spectrometer (GC-MS) instrument equipped with a VF 5 ms Fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μ m film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The carrier gas, Helium (99.99%) was used at a constant flow rate of 1.58 ml/min, injector and mass transfer line temperature were set at 250 and 200 $^{\circ}$ C respectively, and an injection volume of 1 μ l was employed (split ratio 10:1), the oven temperature was programmed from 80 $^{\circ}$ C (isothermal for 2min) with an increase of 9 $^{\circ}$ C/min to 200 $^{\circ}$ C for 4 min, 10 $^{\circ}$ C /min to 280 $^{\circ}$ C, ending with a 5min isothermal at 280 $^{\circ}$ C.

The MS operation parameters were as follows; ionization energy, 70eV ; ion source temperature, 200 $^{\circ}$ C, solvent cut

time, 2.5 min, relative detector gain mode scan speed 1666 μ /sec; scan range 40-800 μ , the interface temperature is 250 $^{\circ}$ C. The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

2.7 Identification of components

Calculations were made for the relative percentage amount of each phyto-components by comparing their average peak area to the total area. The detection employed the NIST Ver. 2.0-year 2005 library. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Searvce/USDA [6]. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown phyto-components was compared with the spectrum of the known components stored in the NIST library. The name and molecular weight of the phyto-components of the test materials were established.

Table 1: UV- VIS values of n-hexane nut extract of *Tetracarpidium conophorum*

Wavelength (nm)	Absorbance
482.80	2.159

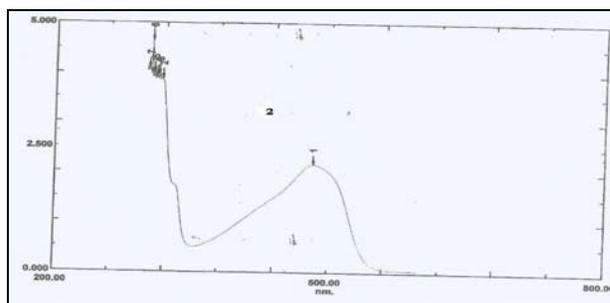


Fig 1: UV-VIS spectrum of n-hexane nut extract of *T. conophorum*.

Table 2: FTIR peak values and functional groups of different phyto-components identified in the n-Hexane extract of the nuts of *Tetracarpidium conophorum*.

Peak values(nm)	3444.02	2942.51	1638.58	1402.30	1245.01	1050.28
Functional group	O-H, Stretch H-bonded	C-H, Stretch, Saturated	C=C Stretch, Conjugated	C-H Stretch, Bonded	C-O Stretch, Weak.	C-O Stretch, Weak.

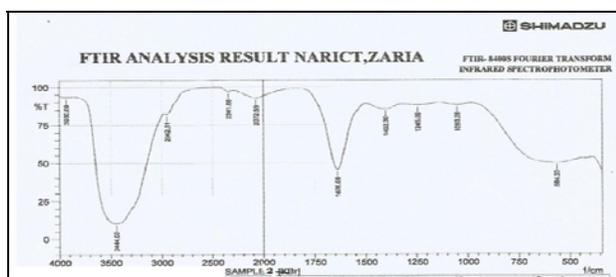


Fig 2: FTIR spectrum of n-Hexane nut extract of *Tetracarpidium conophorum*.

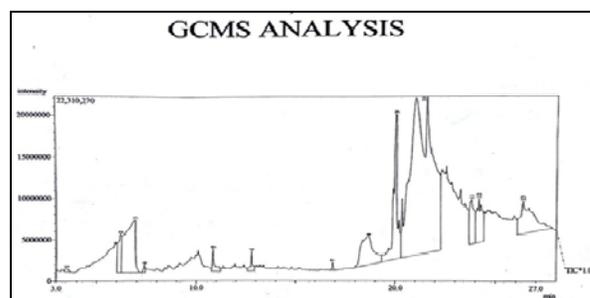


Fig 3: Gas chromatogram of the n-Hexane nut extract of *Tetracarpidium conophorum*.

Table 3: Phyto-components identified in the n-hexane nut extract of *Tetracarpidium conophorum* by GC-MS.

S/N	Retention time (mins)	Name of compound	Molecular formular	Molecular weight (gmol ⁻¹)	Peak Area (%)
1	3.496	2-methyl cyclopentanon	C ₆ H ₁₀ O	98	0.16
2	6.171	Methyl nicotinate	C ₇ H ₇ NO ₂	137	2.19
3	6.884	Glycerin	C ₃ H ₈ O ₃	92	8.85
4	7.379	5-Oxotetrahydrofuran-2 -carboxylic acid methyl ester	C ₆ H ₈ O ₄	144	0.18
5	10.883	2-cyclohexene-1-one,3-(hydroxyl methyl)-6-(1-methylethyl)	C ₁₀ H ₁₆ O ₂	168	0.83
6	12.765	5-octadecanoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	0.60
7	16.870	Tridecanoic acid methyl ester	C ₁₄ H ₂₈ O ₂	228	0.16
8	18.677	nHexadecanoic acid methyl ester	C ₁₆ H ₃₂ O ₂	256	6.08
9	20.057	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C ₁₉ H ₃₂ O ₂	296	9.57
10	21.050	Oleic acid	C ₁₈ H ₃₄ O ₂	282	57.27
11	23.835	Oleic anhydride	C ₃₆ H ₆₆ O ₂	546	3.75
12	24.191	Furan-2-carboxamide,5-benzoyl-N-(2-dimethylaminoethyl)	C ₁₆ H ₁₈ N ₂ O ₃	286	3.84
13	26.395	2-monoole	C ₂₁ H ₄₀ O ₄	356	6.52

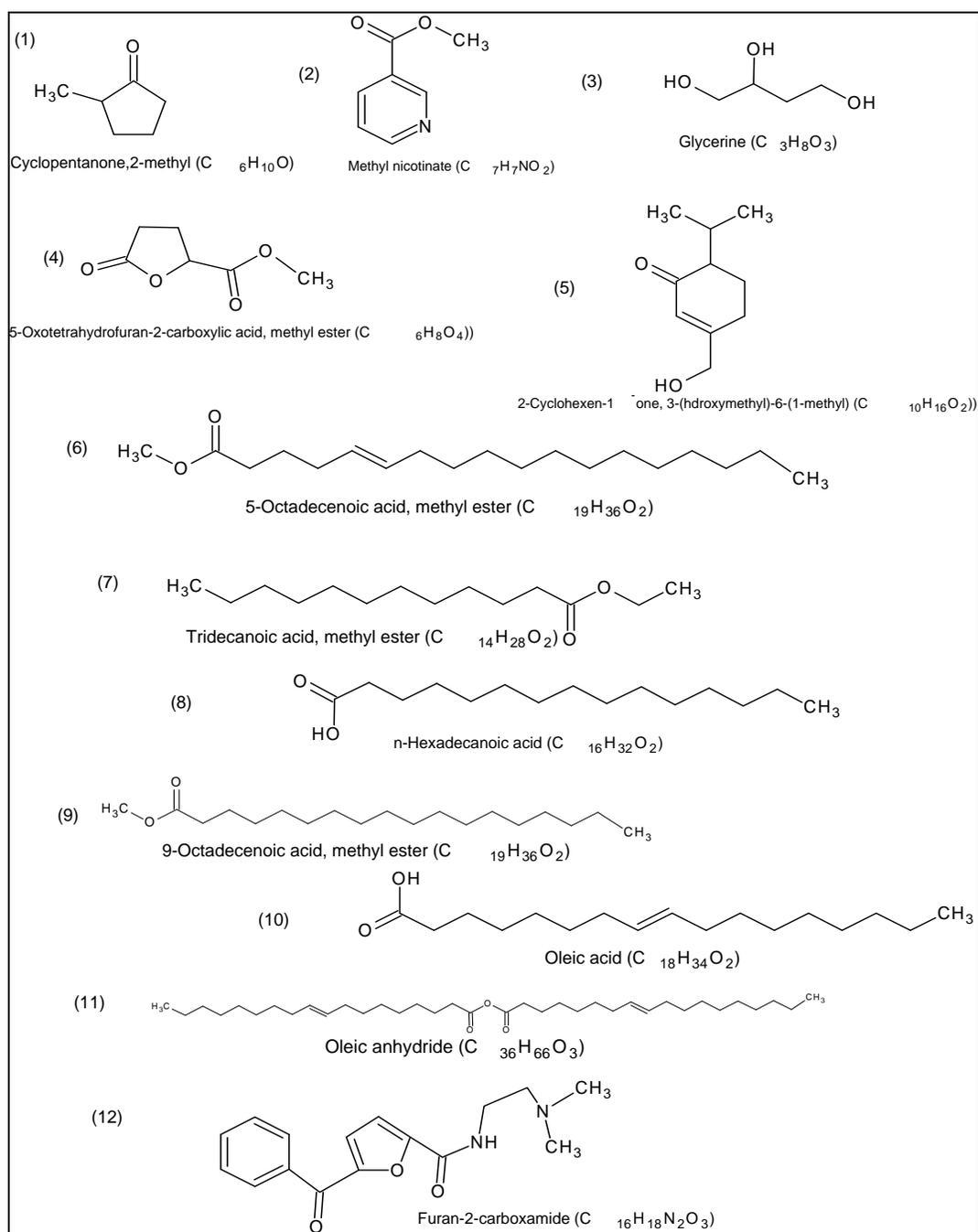
**Fig 3:** Structures of the identified phyto-components in the n-Hexane nut extract of *T. Conophorum*.

Table 4: Biological Activities of Some Active Principles Present in the Extracts of *Tetracarpidium conophorum* Nut.

Phyto-Components.	Nature of Compounds	Biological Activities ***
9, 12- Octadecadienoic acid (Z, Z)-Cis, cis-Linoleic Acid. (Linoleic acid).	Unsaturated fatty acid	5-Alpha reductase inhibitor, antiandrogenic, Antiarteriosclerotic, Anticoronary, Antifibrinolytic, Antihistaminic, Antiinflammatory, antileukotrienic, anti prostatic, hepatoprotective, carcinogenic, immunomodulator, antieczemic, hypocholesteramine.
1-H Indole	Indole	Aldose-Reductase-Inhibitor, Anti-acne, Anti-bacterial, Anti-bacterial, Anti-cariogenic, Anti-salmonella, Anti-septic, Anti-streptococcic, Cancer Preventive, Carcinogenic Insectiphile, Nematicide, Perfumery, Fragrance, Pesticide, Tumorigenic.
Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant, Lipoxygenase-Inhibitor, Perfumery, Pesticide, Sunscreen.
9- Octadecanoic acid (Z)- (Oleic acid).	Unsaturated fatty acid	5-Alpha-Reductase-Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antiallopecic, Antiandrogenic, Antiinflammatory, Antileukotriene, Cancer-Preventive, Choleric, Dermatitigenic FLavor, Hypocholesterolemic. Insectifuge, Irritant, Percutaneostimulant, Perfumery Propagic;
n-Hexadecanoic acid. (Palmitic acid).	Saturated fatty acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
Methyl (8E,11E,14E)-8,11,14-Eicosatrienoate	Unsaturated fatty acid ester	Anti-arthritis, Anti-coronary, Anti-inflammatory
Tridecanoic acid methyl ester	Unsaturated fatty acid esters	Antioxidant, Perfumery.
Glycerol	Fatty alcohol	Anti-cataract Anti-ear-wax, Anti-ketotic, Anti-neuralgic, Arrhythmigenic, Emollient, Hyperglycemic,
Methyl nicotinate	Nicotinic acid methyl ester	Anti-Crohn's, Antidote, (pesticides), Anti-schizophrenic, Anti-thyrotoxic, Choleric, Hypocholesterolemic, Hypoglycemic, Insulinase-Inhibitor, Insulinotonic, Lipolytic

** Source Dr. Duke's Phytochemical and Ethnobotanical Databases [Online database]. ***.

3. Results and Discussion

The UV-VIS spectrum profile of *T. conophorum* was analysed at a wavelength from 200-900 nm due to the concise and clear integrity of the peaks and baseline. The n-hexane extract profile of *T. conophorum* showed peak at 482.8 nm with the absorption value of 2.159 (Table 1). The peak value of 406-485 nm indicates the presence of carotenoids and its derivatives. The precise position and relative intensities of these maxima give valuable information on the nature of the carotenoids. Other derivatives which could be responsible for the observed absorption in this region include some steroids and fatty acids. The FTIR spectrum was used to identify the functional group of the phyto-components based on the peak value in the region of infrared radiation [7]. The results of FTIR peak values and functional groups are presented in Table 2. The FTIR confirmed the presence of alkanes, alkynes, aromatics, alcohols, ketones, ether, aldehydes and esters. The gas chromatograms of n-hexane extract of *T. Conophorum* is shown in fig 3, this shows the relative concentrations of various compounds getting eluted as a function of retention time (RT). The height of the peak indicates the relative concentration of the components present in *T. Conophorum* extract. The result of GC-MS analysis of the phyto-components present in n-hexane extract of *T. conophorum* is shown in table 3. This reveals the fragmentation pattern, molecular formula, molecular weight and structure of the phyto-components identified. Thirteen phyto-components were identified and quantified from the nut extract of *T. conophorum* and these active phyto-components with their respective retention time (RT), molecular formula, molecular weight and relative percentages (Peak area %) were presented also in table 3. The n-hexane nut extract of *T. conophorum* contains: Oleic acid (57.27%), E,E,Z-1,3,12-Nonadecatriene-5,14-diol. (9.57%), Glycerin (8.85%), 2-monole (6.52%), n-Hexadecanoic acid methyl ester (6.08%), Furan-2-carboxamide-5-benzoyl-N-(2-dimethylaminoethyl) (3.75%), Methyl nicotinate (2.19%), 2-Methyl cyclopentanone (0.16%). The biological activities of some of

the phyto-components based on Dr. Duke's phytochemical and ethno-botanical databases are tabulated in table 4. The abundance of Oleic acid and other essential fatty acid in the nut extract is reported to have cholesterol lowering activities as well as anti-inflammatory effects, while methyl cyclopentanone is a congener in the biosynthesis of prostaglandins of pharmacological importance.

4. Conclusion

The revealing report on the characterization and profiling of *T. conophorum* n-hexane nut extract gives an insight on the nature of active principle in this medicinal plant. Thirteen phyto-components were identified from the nut extract of the plant by GC-MS analysis, this justifies the wide claims and ethno-medicinal uses of the nuts by the traditional West African dwellers for a wide range of ailments ranging from stomach ache, diarrhoea, constipation, infertility, wound healing and antiseptic agent.

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