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GAS CHROMATOGRAPHY (GC) – MASS SPECTROMETRY (MS) ANALYSIS OF THE ISOLATED OIL OF *Tetracarpidium conophorum (*WALLNUT).Mull (Arg) ROOT BARK

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ABSTRACT

The root bark was collected fresh, washed with distilled water, air-dried under shade in the laboratory for four weeks and pulverized to a powdered form. Extraction was done in soxhlet extractor using hexane as a solvent. The crude extract was concentrated and partitioned with hexane and methanol (8:2).Vacuum liquid chromatography was adopted to separate the hexane fraction using solvent system of hexane; methanol (4:1). The yellow oil recovered was screened for phytochemicals and analyzed by GC-MS. Glycosides, phenolics and alkaloids were indicated in large amount; eugenols and terpenes were also present while saponin, tannins and steroid were absent. Major components detected by GC-MS were 2-Hydroxy hexadecanoic acid (Rt:17.59, 14.83%), a hydroxyl acid; 2-Hexenal (Rt:16.11, 12.36%), an unsaturated aldehyde ; Oxalic acid, allyl hexadecyl ester (Rt:17.38, 11.26%) a high molecular weight ester; Squalene (Rt:19.96, 8.97%) and alpha tocopherol (Rt:18.03, 7.14%) . The result indicated that the yellow oil constitute important phytochemcals like terpenes with the presence of squalene.

Key words: *Tetracarpidium conophorum* (Wall nut) Gas Chromatography – Mass Spectrometry, oil.

INTRODUCTION

Tetracarpidium conophorum Mull. (Arg), family Euphorbiaceae, locally called 'okhue' in 'Bini', *ukpa* (Igbo) and *awusa* or *asala* (Yoruba). It is a climbing shrub measuring 10-20ft long. The nut is used as a male fertility agent and the leaves are used for the treatment of dysentery and to improve fertility in males.Walnut comprises such families as *Juglandaceae* (English walnut), *Euphorbiaceae* (African walnut) and *Olacaceae* (African walnut). Each family has its own peculiar characteristics but they have some things in common such as the nuts. *Juglandaceae* is mostly found in the Southeast Europe, to Japan and more widely in the new world. *Tetracarpidium conophorum* (family *Euphorbiaceae*) is found in Nigeria and Cameroon while *Coula edulis* (family Olacaceae) which is also referred to as African walnut is found in Congo, The oil from the nut has found use in the formulation of wood varnish, stand oil, vulcanized oil for rubber and leather substitute. Most of the studies on the plant have been on the nutritive value of the seeds which is a snack and delicacy (Oke and

Fafunso, 1975; Adebona, 1988;Akpuaka, 2000).

This plant is cultivated principally for the nuts which are cooked and consumed as snacks (Oke, 1995). A bitter taste is usually observed upon drinking water immediately after eating the nuts. This could be attributed to the presence of chemical substances such as alkaloid. Ayodele (2003) reported the presence of oxalate, phylates and tannin in the raw *Tetracarpidium conophorum* nuts. Edem (2009) reported on the proximate composition, ascorbic acid and heavy metal contents of the nut. Oyenuga (1997) reported on the amino acid and fatty acid components of the nut and on the use of its leaf juice for the treatment of prolonged and constant hiccups.

Walnuts are considered to be herb in Traditional Chinese medicine. They are said to tonify kidneys, strengthen the back and knees and moisten the intestines. It is assumed that it can stop asthma and is prescribed to be taken between bouts of asthma, but not for acute asthma. It is used for elderly as a constipation cure. Also there are claims that the bark is used in tea as laxative and chewed for toothache and helps to prevent and control high blood pressure (Akpuaka, 2000). Though the nuts are generally eaten in Nigeria, there exist little or no reported works on the root bark of the plant. In consultation with traditional herbal practitioner, the root bark of the plant is used with hot palm kernel oil in the treatment of bone fracture (Iyekowa, 1991).Therefore; this research is aimed at determining the chemical constituents of the isolated oil from the root bark of *Tetracarpidium conophorum used* in the treatment of bone fracture in Edo State, Nigeria.

MATERIALS AND METHOD

Sample Collection and Treatment

The root bark of *T. conophorum* was collected fresh from a bush in Ekiadolor community in Ovia North East Local Government Area of Edo State, Nigeria. The plant root bark was identified by Prof. J.F. Bamidele, a taxonomist n the Department of plant Biology and Biotechnology, University of Benin. The bark was washed with distilled water, air-dried under shade in the laboratory for four weeks and pulverized to a powdered form. 50g of the powdered sample was extracted with soxhlet extractor using hexane as a solvent The crude oil extract was dried using Na_2SO_4 and concentrated in a rotary evaporator (yield: 7.31%).

Phytochemical screening of oil fraction

Phytochemical screening was done to find the presence of the active chemical constituents such as carbohydrates, alkaloids, cardiac glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, and eugenols by using the standard procedures (Kokate *et al*. (2009) and Evans and Trease (2002).

Isolation of Oil

4.8ml of the crude oil extract was partitioned with 100ml of hexane: methanol mixture (ratio: 8: 2) and shaken vigorously in a separatory funnel. The upper hexane fraction was separated, concentrated and then subjected to vacuum liquid chromatography (VLC), using silca gel (particle size: 200-425 mesh) as the solid phase and hexane: methanol mixture (4:1) as the mobile phase. A yellow oily phase obtained was dried over Na_2SO_4 and concentrated to recover the pure oil (0.89ml by volume: yield 16%)

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GC-MS Analysis

The analysis was carried out on a GC-Mass spectrometer filled with an HP-5 MS (5% phenysiloxane) column at a temperature programme of 70^{o}C (2 minutes) increase at 10^{o}C/min to 280^{o}C and held for 7minutes. The carrier gas was nitrogen and flow rate, 1.80mL/min.

RESULTS

Phytochemical Screening

Table 1: Phytochemical Screening of hexane extract of *T. conophorum*

S/N	Phytochemical constituents	Name of the Test	Hexane Extract
1	Glycosides	General Test	++
2	Saponin	Foam Test	_
3	Flavonoid	Lead acetate Test	_
4	Phenolics	Ferric chloride	++
5	Tannin	Ferric chloride	_
6	Eugenol	KOH/HCl	+
7	Steroid	Acetic acid/H_2SO4	_
8	Terpenes	Salkowski Test	+
9	Alkaloids	Picric acid Test	++

GC-MS ANALYSIS

The GC-MS chromatogram of the isolated yellow oil given in Figure 1 showed 21 peaks indicating from the search list of the chemical abstract service twenty-one compounds. The chemical compounds identified in the oil fraction are presented in Table 2.

24/09/2015 16:31:51 GCMS-QP2010SE SHIMADZU,JAPAN

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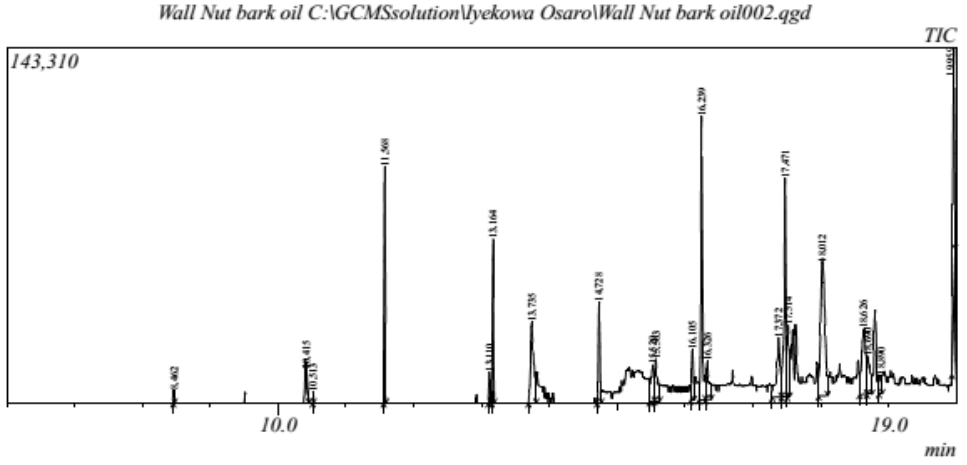
Sample Information

Analyzed by	: SBen OS
Analyzed	: 24/09/2015 11:51:17
Sample Type	: Unknown
Level #	: 1
Sample Name	: Iyekowa
Sample ID	: Wall Nut bark
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 2
Injection Volume	: 1.00
Data File	: C:\GCMSsolution\Iyekowa Osaro\Wall Nut bark oil002.qgd
Org Data File	: C:\GCMSsolution\Iyekowa Osaro\Wall Nut bark oil002.qgd
Method File	: C:\GCMSsolution\Iyekowa Osaro\F.qgm
Org Method File	: C:\GCMSsolution\Iyekowa Osaro\F.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\Ronald_Tuning_20_08_2014.qgt
Modified by	: Admin
Modified	: 24/09/2015 13:34:20

Wall Nut bark oil C:\GCMSsolution\Iyekowa Osaro\Wall Nut bark oil002.qgd



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.462	8.450	8.475	4510	0.28	5205	0.67	0.87		Butane, 2,2-dimethyl-
2	10.415	10.410	10.450	-7097	-0.44	-668	-0.09	10.62	MI	Phenol, 2-amino-4-(1H-1,2,3,4-tetrazol-1-y
3	10.513	10.505	10.525	3352	0.21	4629	0.59	0.72		2,2-Dimethyl-propyl 2,2-dimethyl-propanes
4	11.568	11.550	11.590	109385	6.71	95452	12.24	1.15		Pentadecanal-
5	13.110	13.095	13.125	10776	0.66	12547	1.61	0.86		5,10-Pentadecadien-1-ol, (Z,Z)-
6	13.164	13.145	13.185	64912	3.98	66285	8.50	0.98		13-Oxabicyclo[10.1.0]tridecane
7	13.735	13.690	13.805	110813	6.80	32672	4.19	3.39		n-Decanoic acid
8	14.728	14.700	14.760	60283	3.70	40892	5.24	1.47		1,2-Cyclopentanediol, 3-methyl-
9	15.520	15.470	15.545	48271	2.96	14796	1.90	3.26	V	4-Dodecanol
10	15.563	15.545	15.625	45069	2.77	16775	2.15	2.69	V	Sulfurous acid, 2-ethylhexyl isohexyl ester
11	16.105	16.080	16.130	36371	2.23	20212	2.59	1.80	V	4-Nonene, 5-nitro-
12	16.239	16.200	16.300	201387	12.36	114331	14.66	1.74	V	Octadecanoic acid, 2-hydroxy-1,3-propaned
13	16.326	16.300	16.385	45664	2.80	15395	1.97	2.97	V	Acetic acid, trifluoro-, 2,2-dimethylpropyl e
14	17.372	17.280	17.415	87241	5.35	24105	3.09	3.62	V	Oxalic acid, allyl pentadecyl ester
15	17.471	17.415	17.500	183507	11.26	88306	11.32	2.08	V	1-Octadecyne
16	17.514	17.500	17.540	54025	3.32	28847	3.70	1.87	V	1-Octadecyne
17	18.012	17.965	18.100	241645	14.83	53671	6.88	4.50	V	Nitric acid, nonyl ester
18	18.626	18.565	18.675	116432	7.14	26316	3.37	4.42	V	Oxalic acid, allyl nonyl ester
19	18.690	18.675	18.750	48039	2.95	15192	1.95	3.16	V	Oxalic acid, allyl nonyl ester
20	18.890	18.855	18.900	18837	1.16	7477	0.96	2.52	V	1-Hexyl-2-nitrocyclohexane
21	19.959	19.925	19.975	146165	8.97	97511	12.50	1.50		Squalene
				1629587	100.00	779948	100.00			

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Figure 1: GC-MS Analysis of Wall nut root bark oil.

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Table 2: GC-MS Analysis of isolated yellow oil of *T. conophorum*

Peak No	Retention Time (Rt)	Name of compound	Area percent (%)	Mol. Formula	Mol. weight
1	8.46	2,2 dimethyl butane	0.28	C_6H_{14}	86
2	10.42	4-(1H-1,2,3,4-tetrazol) 2- amino phenol	0.44	$C_7H_7N_5O$	177
3	10.51	Nicotinicacid,2-tetrahydrofuryl methyl ester	0.21	$C_{11}H_{13}NO_3$	207
4	10.52	2,2 dimethyl butane	6.71	C_6H_{14}	86
5	11.57	Pentadecanal	0.66	$C_{15}H_{30}O$	226
6	13.11	5, 10 pentadecadien-1-ol	3.98	$C_{15}H_{28}O$	224
7	13.17	14-Methyl-8-hexadecen-1-ol	6.80	$C_{17}H_{34}O$	254
8	13.74	n-Decanoic (Capric) acid	3.70	$C_{10}H_{20}O_2$	172
9	14.73	2-Ethyl,2-methyl Eicosanoic acid (Arachidic acid isomer)	2.96	$C_{24}H_{48}O_2$	368
10	15.52	4-duodecanol	2.77	$C_{12}H_{26}O$	186
11	15.57	2, 4, 6 Trimethyl nonene	2.23	$C_{12}H_{24}$	168
12	16.11	2-Hexenal	12.36	$C_6H_{10}O$	98
13	16.24	15-Hydroxylpentadecanoic acid	2.80	$C_{15}H_{30}O_3$	258
14	16.33	2-Octylthio thiophene	5.35	$C_{12}H_{20}S_2$	228
15	17.38	Oxalic acid, allyl hexadecyl ester	11.26	$C_{21}H_{38}O_4$	354
16	17.47	1-Octadecyne	3.32	$C_{18}H_{34}$	250
17	17.59	2-Hydroxy hexadecanoic acid	14.83	$C_{17}H_{34}O_3$	286
18	18.03	Alpha tocopherol (vitamin E)	7.14	$C_{29}H_{50}O_2$	430
19	18.63	Oxalic acid, allyl nonyl ester	2.95	$C_{14}H_{24}O_4$	256
20	18.80	Cyclohexane	1.16	C_6H_{12}	84
21	19.96	Squalene (Terpene)	8.97	$C_{30}H_{50}$	410
		Total	**100.00**		

DISCUSSION

In Table 1, glycosides, phenolics and alkaloids were indicated in large amount; eugenols and terpenes were also present while saponin, tannins and steroid were absent. Phenolic and alkaloids are useful bioactive agents that have physiological effect in man (Sofowora, 1982)

From Table 2, major components detected from the isolated yellow oil of *T. conophorum* are 2-Hydroxy hexadecanoic acid (Rt:17.59, 14.83%), a hydroxyl acid; 2-Hexenal (Rt:16.11, 12.36%), an unsaturated aldehyde ; Oxalic acid, allyl hexadecyl ester (Rt:17.38, 11.26%) a high molecular weight ester; Squalene (Rt:19.96, 8.97%) and alpha tocopherol (Rt:18.03, 7.14%) while minor components among others were n-decanoic (caproic) acid(Rt:13.74, 3.7%), a saturated fatty acid; 4-duodecanol (Rt:15.52, 2.77%), a high molecular weight alcohol and 5, 10 pentadecadien-1-ol (Rt:13.11,3.98%),a large molecular weight unsaturated alcohol. The result indicated that the yellow oil constitute important phytochemcals like terpenes with the presence of squalene which is the direct precursor to cholesterol; alpha tocopherol (vitamin E) which has antioxidant properties and required for the proper function of many organs in the human body (Edem *et al*., 2009). High molecular weight alcohols were also detected from the oil.

The isolated oil of the *T. conophorum* root bark contains components like squalene, a terpene which corroborates the phytochemicals detected in the hexane extract of *T. conophorum.* Squalene being a precursor to cholesterol is an important bioactive chemical constituent which among others (hydroxyl acid, unsaturated alcohol and alpha tocopherol) are present in the oil of *T. conophorum*. This suggests that the oil should have activity when subjected to antimicrobial, wound healing activity, pharmacological studies and bone ossification as claimed by traditional medicine.

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