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GC–MS analysis of bioactive compounds in three extracts of *Clerodendrum volubile* P. Beauv leaves

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Abstract
This study is to investigate and characterize the chemical composition of the different crude extracts from the leaves of *Clerodendrum volubile* (*C. volubile*), an indigenous plant used in folklore medicine in the Niger delta area of Nigeria. The air-dried leaves were powdered and subjected to selective sequential extraction using solvents of increasing polarity through percolation with full column of water, ethanol and methanol to obtain three different extracts. The leaf extracts was further subjected to gas chromatography–mass spectrometry (GC-MS). GC-MS analysis of the crude extracts of *C. volubile* revealed different types of high and low molecular weight chemical entities with varying quantities present in each of the extracts. Furthermore, the leaf extracts possess unique physicochemical characteristics which may be linked to the compounds naturally present in the leaves of *C. volubile*. The presence of these biologically active compounds may form the basis for the medicinal properties exhibited by the plant.

Keywords: *Clerodendrum volubile*, leaves, Gas chromatography-mass spectrometry, crude extract

Introduction
Plants play a major role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments (WHO, 2008; Bachrach, 2012) [1-2]. They can be a source of chemical compounds of biological and pharmacological importance. History reveals that plants are sources of pharmacologically useful drugs, and will continuously be important for the screening of novel naturally occurring chemical entities (Atanasov *et al*., 2015) [3]. A crucial part in the investigation of plant is the identification of the biologically active secondary metabolites they possess leading to further biological and pharmacological studies (Guo *et al*., 2013; Momin *et al*., 2014; Farid *et al*., 2015) [4-6].
Clerodendrum volubile P. Beauv (*C. volubile*) belongs to the family of Verbenaceae. It is a climbing shrub commonly growing in deciduous forests across Africa (Burkill, 1985) [7]. It is commonly known among the Urhobo and Itsekiri tribes of the Niger-Delta of Nigeria as "Obenetete", the Yorubas in Ondo state call it as "Marugbo" (Erukainure *et al.,* 2011; Fred-Jaiyesimi and Adekoya, 2012) [8-9]. In the southern part of Nigeria, which is densely populated by the Ijaws, Urhobos and Itsekiris, it is commonly described as a delicious green leafy vegetable that is consumed as food and traditionally, the plant was found to be effective in treatment of arthritis, diabetes, rheumatism, dropsy, swellings, oedema, and gout (Fred-Jaiyesimi and Adekoya, 2012) [9]. It is also used as an anti-abortifacient and sedative (Ogunwa *et al*., 2016) [10].
Compounds present in other species of *Clerodendrum* exhibited remarkable biological activities. Plants under the same genus might also exhibit the same biological activities because of similar active principles present in them (Islam and Rahman, 2015; Chidinma *et al*., 2016) [11-12]. Identification of bioactive compounds present in *C. volubile* is still conducted in Nigeria to further enrich the information about this important, edible and endemic plant of the Niger Delta area of Nigeria for easy referencing in the future. There are no published scientific reports on the bioactive compounds present in the different extracts of *C. volubile* leaves using gas chromatography–mass spectrometry (GC–MS). Therefore, this study aimed to investigate and characterize the bioactive compounds in the different crude extracts of *C. volubile* leaves.

Materials and Methods
Plant sample
Fresh leaves of the plant *C. volubile* were purchased from Oja-Oba Main Market, Akure, Nigeria. The plant was identified by Mr. A.A. Shorungbe of Department of Biology, Federal University of Technology Akure, Nigeria and deposited in the herbarium (FUTA/BIO/0121).

Extraction of crude extracts

The leaves of the plant were air dried at room temperature for about 7days. The dried leaves were ground using an electric blender. Starting with 250 g, the dried and powdered samples were subjected to selective sequential extraction using solvents, namely: distilled water, ethanol and methanol (Casuga *et al*., 2016) [13]. Then the extract was evaporated to dryness using a rotary evaporator.

GC–MS analysis

The GC–MS analysis of bioactive compounds from the different extracts of the leaves of *C. volubile* was carried out using Model 7890A (Agilent Technologies) interfaced with a mass selector detector model 5975°C. The electron ionization was kept at 70 eV with anion source temperature of 250°C, using Helium as the carrier gas and HP-5MS (30 mm × 0.25 mm × 0.320 µm) as the stationary phase. The oven temperature was kept at 80°C held for 4 minutes and ramped to 270°C at the rate of 3.5°C/minutes holding for 6 minutes. 1 mg each of the extracts was dissolved in 1ml of acetonitrile. The mixture was vortexed and sieved through 0.4 millipore filter into a 5ml rotavapour flask and dried using rotavapour. 700 µl dichloromethane was added and transferred into screw cap tubes. 1 µl each of the prepared extracts was injected into the column at 300°C. The split mode was employed with a split ratio of 50:1. Relative quantity of the chemical compounds present in each of the extracts of *C. volubile* was expressed in percentage based on the peak area produced in the chromatogram.

Identification of chemical constituents

The compounds present in the extracts of *C. volubile* were tentatively identified by matching the relative retention times generated for unknowns on HP- 5MS column with those of standard substances in NIST14 library analysed under similar conditions (Akilan *et al*., 2014) [14].

Results

Bioactive compounds present in the extracts

The bioactive compounds present in aqueous, methanol and ethanol extracts obtained from *C. volubile* leaves are shown in Tables 1–3. Their identification and characterization were based on their elution order in a HP-5MS column. The elution time, molecular formula and the amount of these bioactive compounds were also presented. Based on abundance, the top three major compounds present in the aqueous extract were 13-docosenamide (77.09%), cyclopropanecarboxylic acid, tridecyl ester (10.91%), and n-hexadecanoic acid (7.05%). The methanol crude extract contained methanesulfenyl chloride (35.56%) followed by trichloro hexadecane (20.75%) and 2,4-di-tert-butylphenol (12.47). The ethanol crude extract showed had oxirane (36.20% and 14.31%), methyl 2-octylcyclopropene-1-heptanoate (12.41%) and Hexadecanoic acid, methyl ester (9.96%) as the top three major compounds. The GC chromatograms of the three extracts presented in Figures 1–3 show the retention time in the column and the detected peaks which correspond to the bioactive compounds present in the extract.

Table 1: Chemical composition of *C. volubile* aqueous leaf extract

Pk#	RT (min)	Area%	Library/ID(C:\Database\NIST14.L)	Formula	Mol. Wt (g/mol)
1	18.777	4.95	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
2	19.217	7.05	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4
3	21.918	10.91	Cyclopropanecarboxylicacid, tridecyl ester	$C_{17}H_{32}O_2$	344.5
4	25.992	77.09	13-Docosenamide	$C_{22}H_{43}NO$	337.6

Keys: Retention Time (Rt)

Table 2: Chemical composition of *C. volubile* methanol leaf extract

P k#	RT (min)	Area%	Library/ID(C:\Database\NIST14.L)	Formula	Mol. Wt (g/mol)
1	8.763	2.40	Octacosane, 2-methyl-	$C_{29}H_{60}$	408.79
2	10.634	7.63	Eicosane	$C_{20}H_{42}$	282.55
3	11.206	35.56	Methanesulfenyl chloride, trichloro	CCl_4S	185.89
4	13.873	10.86	Neophytadiene	$C_{20}H_{38}$	278.52
5	15.692	20.75	Hexadecane	$C_{16}H_{34}$	226.44
6	16.270	12.47	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32
7	16.768	9.16	Tridecane, 1-iodo-	$C_{13}H_{27}I$	310.26
8	19.377	1.17	3-Oxatricyclo [3.2.1.0(2,4)] octane, (1α,2β,4β,5α)-	$C_7H_{10}O$	110.15

Keys: Retention Time (RT)

Table 3. Chemical composition of *C. volubile* ethanol leaf extract

P k#	RT (min)	Area%	Library/ID(C:\Database\NIST14.L)	Formula	Mol. Wt (g/mol)
1	8.855	0.63	Oxirane, 2,2-dimethyl-3-(3, 7, 12, 16, 20- pentamethyl-3, 7, 11, 15, 19-heneicosapentaenyl	$C_{30}H_{50}O$	426.72
2	8.883	0.35	8-Hexadecenal, 14-methyl-,	$C_{17}H_{32}O$	252.44
3	11.470	12.41	Methyl 2-octylcyclopropene-1-heptanoate	$C_{19}H_{34}O_2$	294.47
4	11.504	7.15	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans	$C_{20}H_{30}$	270.45
5	11.561	3.33	5-Hydroxyimino-4-phenylhydrazono-4,5,6,7- tetrahydrobenzofurazan		
6	11.595	9.96	Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.43
7	12.620	0.51	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.45
8	16.305	1.21	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34
9	16.968	1.57	Diethyl phthalate	$C_{12}H_{14}O_4$	222.24
10	16.986	0.70	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43
11	18.056	1.82	4,4,5,6,7-tetrahydrobenzofurazan	$C_6H_7N_3O_2$	153.13
12	18.593	8.54	Phenanthrene,	$C_{14}H_{10}$	178.23
13	19.103	0.70	Methyl 2-octylcyclopropene-1-heptanoate	$C_{19}H_{34}O_2$	294.47
14	19.126	0.62	8-Hexadecenal	$C_{16}H_{30}O$	238.40
15	21.197	36.20	Oxirane	C_2H_4O	44.05
16	21.225	14.31	Oxirane	C_2H_4O	44.05

Keys: Retention Time (RT)

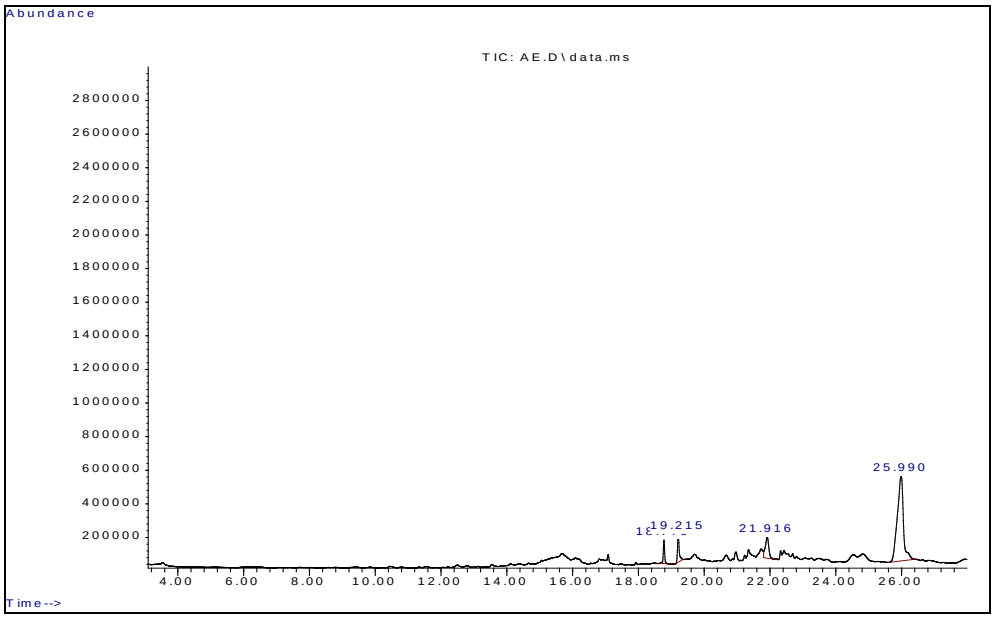

Fig. 1: GC-MS Chromatograph of aqueous *C. volubile* leaf extract

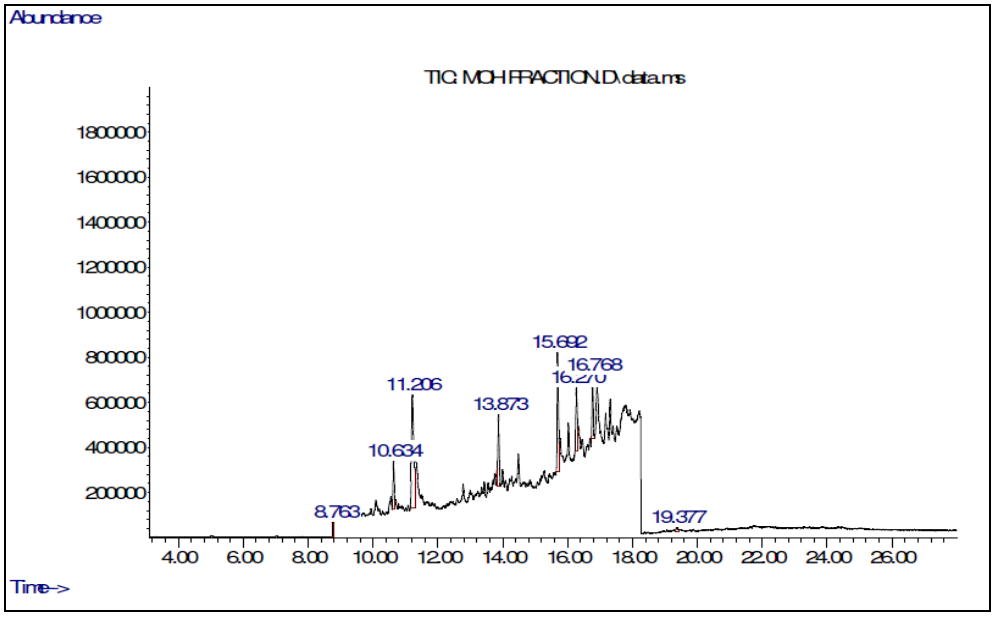

Fig. 2: GC-MS Chromatograph of methanolic extract of *C.volubile* leaf

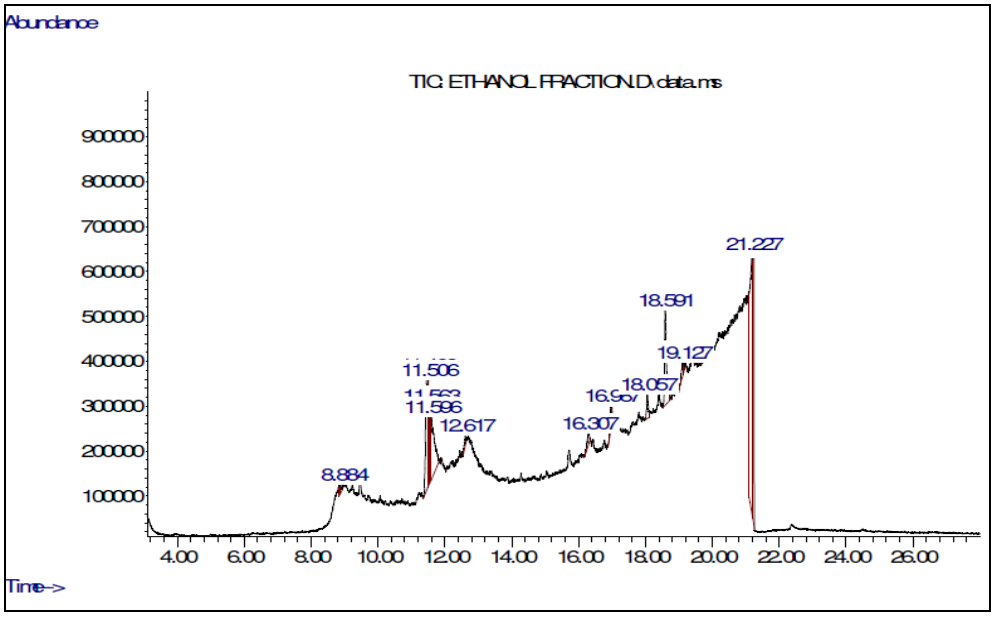

Fig 3: GC-MS Chromatograph of ethanolic extract of *C. volubile* lea

Discussion

In the present study, three different crude extracts were obtained from the leaves of *C. volubile* through selective sequential extraction with different solvents, namely; aqueous, methanol and ethanol. GC–MS analysis of aqueous, methanol and ethanol extracts revealed the presence of various bioactive compounds. Hexadecanoic acid, methyl ester is present both in aqueous (4.95%) and ethanol extracts (9.96%) but in different quantities. In addition, hexadecanoic acid is present in both aqueous and ethanol extracts but also in small quantities. The three crude extracts did not seem to contain any major common compound. Based on studies, some of the constituents revealed by GC–MS are biologically active compounds. They have been shown to possess pharmacologic activities which may be linked to the healing potential of the plant. Hexadecanoic acid, methyl ester was proven to exhibit antimicrobial effects (Ibibia *et al*., 2016) [15]. n-Hexadecanoic acid was confirmed to be present in other plants exhibiting anti-inflammatory (Sermakkani and Thangapandian, 2012) [16] antioxidant, nematicide, pesticide, anti-androgenic, hemolytic, 5-alpha reductase inhibiting (Jayamathi *et al*., 2012) [17], mosquito larvicidal effects (Thomas *et al*., 2013) [18].

However, the GCMS profiling of the phytochemical constituents the three Traditional African Herbal Extracts (TAHEs) prepared form the leaves of *C. volubile* also revealed the presence of some interesting volatile constituents. Based on their chemical properties, the range of identified compounds includes ethers, esters, alcohols, alkanoic acids, aldehydes and amides. As rich and promising this plant may be, few things need be addressed regarding the most abundant volatile constituents found in the three extract preparations, vis: 13-docosenamide is an amide of docosenoic acid, otherwise called erucylamide which was reported to be capable of sleep-like induction (Cravatt *et al*., 1995) [19]. Trichloromethanesulfenyl chloride, otherwise called perchloromethanethiol, which can be synthesized from carbon disulfide and chlorine, is capable of generating methanethiol and cause liver toxicity. And the presence of oxirane (ethylene oxide), a cyclic ether called epoxide seems to confer fungicidal, bactericidal and sporicidal properties on the plant leaf, and as such make the leaf extract an excellent disinfectant. Therefore ingestion of preparations of this vegetable should be done with caution by ensuring good food processing and preparation practices (GFPPP).

The presence of the identified bioactive components present in *C. volubile* leaf extract could be responsible for the medicinal properties of the plants leaves. The study revealed major bioactive compounds present in all of the three extracts. Identification of these compounds in the plant serves as the basis in determining the possible health benefits of the plant leading to further biological and pharmacological studies.

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