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GC-MS analysis of oil rich in polyenoic fatty acid methyl esters from leaves of *Justicia secunda* Vahl growing abundantly in the lowland rain forests of the Niger Delta region of Nigeria

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Abstract
This study reports for the first time the GC-MS analyses of the chemical constituents present in the essential oil extracted by hydrodistillation from fresh leaves of the tropical plant *Justiciasecunda* Vahl. The results reveal that the oil is rich in polyunsaturated or polyenoic fatty acid methyl esters with well-known biological activities. The fatty acid esters account for 46.66% of the compounds detected with the most abundant being 9, 12, 15- Octadecatrienoic acid, methyl ester (36.56%). The other compounds of significant quantity identified are 9-Octadecenamide (7.12%), E-14-Hexadecenal (6.3%), Trifluoroacetoxy hexadecane (6.00%), 1-Heptadecene (5.68%), Hexadecanoic acid, methyl ester (Methyl Palmitate) (5.06%), 9, 12- Octadecadienoic acid, methyl ester (4.33%), Phytol acetate (3.40%).These compounds were identified by comparing their mass spectra with fragmentation patterns of standards available in the National Institute of Standards and Technology (NIST2.0) and WILEY 7 libraries.

Keywords: essential oil, GC-MS, *Justiciasecunda* Vahl, Polyenoic fatty acids methyl ester

1. Introduction
The therapeutic potential of medicinal plants have been largely attributed to the fact that they are a rich source of secondary metabolites with interesting biological activities, present either in their essential oil yields or other plant extracts [1, 2, 3]. Essential oils are composed of volatile odorous organic compounds present in the lipid soluble portions of plant fluids [4]. These oils which give plants their characteristic odour are extracted from fresh/dried parts of medicinal as well as ornamental plants by various conventional methods: hydro distillation, steam distillation, microwave assisted distillation, solvent extraction, cold pressing and super critical fluid extraction [5]. Plant essential oils are utilized as raw material either for their healing properties or for their fragrance in many consumer goods such as detergents, soaps, food flavours, spices, cosmetics, pharmaceuticals, and pesticides [6]. Hence with these diverse industrial applications, the characterization of the chemical constituents of plant essential oils by the almost exclusive hyphenated analytical method, GC-MS has gained importance and is currently receiving attention in basic and applied research.
Justiciasecunda Vahl, originally from South America is a perennial herb that grows up to 90 cm, with purplish green stem, evergreen leaves and pink flowers. It is abundant in the low land rain forests of the Niger Delta region of Nigeria and other tropical and subtropical countries. It belongs to the family, *Ancanthaceae* and is largely an uncultivated plant that is employed in folk medicinal preparations by the local people. Several communities in the Niger Delta region of Nigeria and other African countries employ the red aqueous leaf extract in providing relief to such conditions such as anaemia and hypertension [7, 8]. The phytochemical screening of this plant extract by researchers reveal the presence of the bioactive compounds: anthocyanins and other flavonoids, tannins, saponins, steroids, and alkaloids [7, 9] The fresh leaves are also observed to possess very strong fragrance suggestive of the presence of essential oil. The subsequent confirmation of the presence of essential oil will further enhance the industrial and therapeutic potential of this evergreen plant growing abundantly in the rainforests of the Niger delta region of Nigeria. Relatively little research has been published on the analysis of the volatile compounds present in the essential oil extracted from this highly resourceful medicinal plant. This paper reports for the first time the hydro distillation of fresh leaf samples of *justiciasecunda*vahl and the GC-MS analysis of the resultant compounds in its essential oil.

The individual spectra were compared with standards provided by the NIST and WILEY libraries. The biological activity of the major compounds identified, is based on the Phytochemical and Ethnobotanical Database created by Dr. Jim Duke of the Agricultural Research Service/USDA as reported in related studies [1, 10].

2. Materials and methods

2.1 Collection of samples and extraction of essential oil by hydrodistillation.

Fresh mature green leaves of the plant *JusticiaSecunda*Vahl were harvested from the uncultivated land and backyard gardens in and around the Federal University Otuoke. The plant was identified by Botanists in the Department of Biology, Federal University Otuoke and the Department of Plant Science and Biotechnology, University of Port Harcourt. A viable sample with Herbarium number UPH/V/1279 was deposited in the UPH reference Herbarium. The leaves were transported in Ziploc bags to the Natural Product Chemistry & Process Division (NPC&PD) Laboratory of the Institute of Himalayan Bioresource Technology in Palampur, Pradesh, India (CSIR-IHBT) where extraction of the essential oil was achieved by hydrodistillation using an all glass Clevenger apparatus at 40ºC for 5 hours. The resultant oil/water mixture was collected and partitioned using dichloromethane (DCM). It was then dried over anhydrous Sodium Sulphate and concentrated at 27ºC and reduced pressure using a rotorvapor (Buchi 850V) to give the pure oil. The resultant oil was stored below 4 ºC prior to GC-MS analysis in the NPC & PD lab.

2.2 GC-MS Analyses

The recovered essential oil was dissolved in GC grade dichloromethane (DCM) and analysed using GC-MS. The GC analysis was carried out on Gas chromatogram; an AOC – 20i auto sampler coupled, and DB-5MS capillary column (30m x 0.25mm id., film thickness 0.25µm). The initial temperature of the column was held at 70 ºC for 5mins and was programmed to 230 ºC at 4 ºC /min, then held for 15mins at 230 ºC, the sample injection volume was 1µL in GC grade dichloromethane. Nitrogen was used as the carrier gas at a flow rate of 1.1mL /min. The ion source was maintained at 230 ºC and the mass spectrum of compounds in samples was obtained by electron ionization at 70eV and the detector was operated in scan mode from 10 – 435 amu (atomic mass unit). A scan interval of 0.5 seconds and fragments from 10 to 435 Da was maintained. The total running time was 63 minutes.

2.3 Identification of Volatile components

The identification of the compounds detected was based on the retention time, molecular weight, peak areas and mass spectra fragmentation patterns. The interpreted molecular formula, molecular weight is based on the database for NIST 2.0 and WILEY 7 libraries

3. Results & Discussion

The fresh leaves of*justiciasecunda* subjected to hydrodistillation for five hours gave an oil yield of 0.35 % (w/w). The resultant essential oil was greenish- yellow with a strong fragrance. The analysis of the complete composition of the oil was performed by means of Gas chromatography coupled with Mass spectrometry. Table 3.1 presents 25 compounds detected in the chromatogram arranged in order of elution on the DB-5 capillary column. This comprises 100 % of the total oil. Figure 3.1 presents the total ion chromatogram (TIC) of *j. secunda*oil. The chromatogram shows 25 peaks and the heights of the peaks indicate the relative concentrations of the respective components present. The characteristic mass fragmentation patterns recorded on the MSn spectraled to the identification of these compounds based on specific molecular ions and fragments ions with m/z ratios which corresponds to the standards provided by the NIST 2.0 and WILEY 7 libraries.

The plant essential oil under investigation is observed to be rich in polyenoic fatty acidsand fatty acid methyl esters which account for 46.66% of the total compounds detected by GC-MS analysis. The major compounds are 9, 12, 15 – Octadecatrienoic acid, methyl ester (36.56%), 9 – Octadecenamide (7.12), E-14-Hexadecanal (6.35%), Triflouroacetoxy hexadecane (6.00%), 1-Heptadecene (5.68%), Hexadecenioc acid, methyl ester(5.06%), 1-Tricosanol (4.58%) 9, 12, - Octadecadienoic acid, (4.33%), phytol acetate (3.40%). These volatile compounds identified in *J. secunda*essential oil have been also been confirmed by related studies to be present in varying concentrations in the leaf extracts and essential oils of several well-known medicinal plants [11-18]. Some of the compounds detected in this oil are reported to possess important biological activities and proven medicinal uses. For instance, the polyenoic fatty acid 9, 12- Octadecadienoic acid has been reported to be the most abundant polyunsaturated fatty acid in human nutrition. It is also employed in treatment of hyperlipoidemia and atherosclerosis [19]. The major compound 9, 12, 15-Octadecatrienoic acid, methyl ester (36.56%) identified is reported to possess anti-infammatory, cancer preventive, hypocholesterolemic, nematicide, insectifuge, hepatoprotective, antihistaminic, antieczemic, antiacne, antiathritic, antiandrogenic and anticoronary properties [10].

Table 1: Chemical Constituents identified in the Essential oil of *Justiciasecunda* Vahl

	R / T	Name of Compound	Molecular Formula	Nature of Compound	MW	Peak Area (%)
1	29.24	1-Tetradecane	$C_{14}H_{28}$	Alkane	196	2.55
2	33.061	2,4-Di-tert-butyl phenol	$C_{14}H_{22}O$	Phenol	206	3.30
3	35.626	1-Heptadecane	$C_{17}H_{34}$	Alkane	238	5.68
4	39.288	Octadecanal	$C_{18}H_{36}O$	Aldehyde	268	2.82
5	41.473	E-14-Hexadecenal	$C_{16}H_{30}O$	Aldehyde	238	6.35
6	42.625	2-Nonenal, 2-pentyl	$C_{14}H_{26}O$	Aldehyde	210	0.19
7	42.789	6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	Ketone	268	1.04
8	43.276	1,2-bezenedicarboxylic acid bis(2-methylpropyl) Ester	$C_{16}H_{22}O_4$	Ester	278	0.70
9	44.882	7-Hexadecanoic acid, methyl ester	$C_{17}H_{32}O_2$	Fatty ester	268	0.77
10	45.005	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty ester	270	5.06
11	45.775	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O4$	Ester	376	0.43
12	46.030	9-Octadecenoic acid	$C_{18}H_{34}O_2$	Fatty acid	282	1.02

13	46.779	Triflouroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	Alkane	338	6.00
14	49.202	9, 12-Octadecadienoic acid	$C_{18}H_{32}O_2$	Linolenic acid	280	4.33
15	49.360	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	Polyenoic fatty acid ester	292	36.56
16	49.710	3,7,11,15-Tetramethyl -2-Hexadecen-1-ol	$C_{20}H_{40}O$	Terpene alcohol	296	1.98
17	50.075	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	Ester	326	0.38
18	50.690	Eicosene	$C_{20}H_{40}$	Alkene	280	0.29
19	51.587	Tetradecanamide	$C_{14}H_{20}NO$	Amide	227	1.16
20	52.046	1-Tricosanol	$C_{23}H_{48}O$	Alcohol	340	4.58
21	52.207	9,10-Dibromopentacosane	$C_{25}H_{50}Br_2$	Alkane	510	0.39
22	52.622	Phythol Acetate	$C_{22}H_{42}O_2$	Diterpene	338	3.40
23	58.399	9-Octadecanamide	$C_{18}H_{35}NO$	Amide	281	7.12
24	59.709	Unknown	Unknown	-	-	1.68
25	60.263	Triflouroacetoxy hexadecane	$C_{18}H_{31}F_3O_2$	Alkane	338	2.33
		Total				100.00

Sample Name: BSAH1
Sample ID : Dr Hamilton-Amachree
Data File : E:\GCMS Data-2016\Oct2016\261 0 16 \BSAH1.qgd
Method File : E:\GCMS Method Files\EI-Apg-MS40B-64.0.qgm

Tuning File : E:\TUNE\03-08-2016 after pm.qgt
Acquisition Date : 27-Oct-16:
Acquisition Time : 3:32:56 PM
Analyst : Shiv Kumar

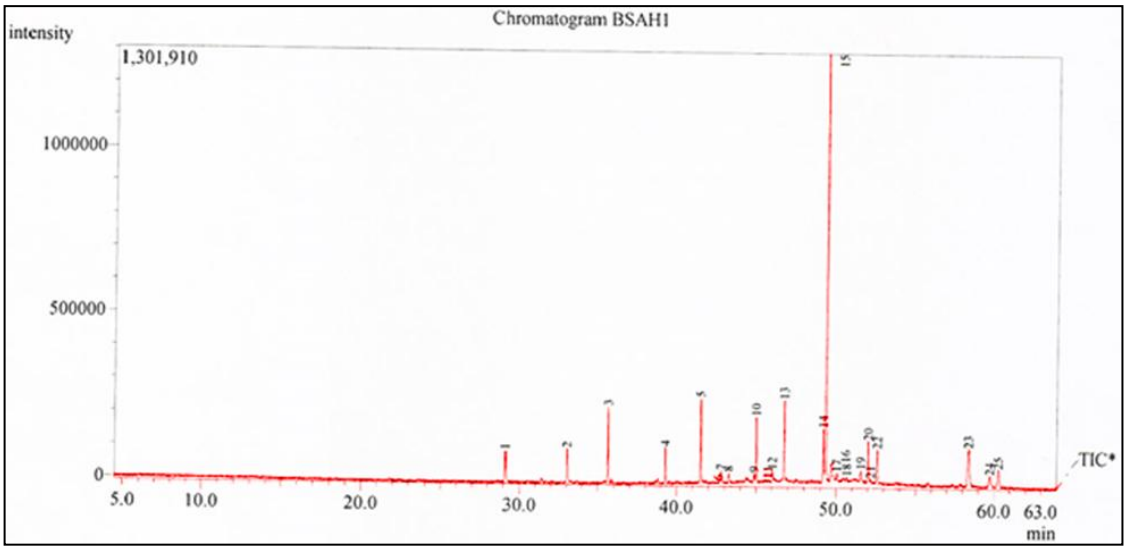

Fig 1: GC-MS Chromatogram of Essential Oil of *Justiciasecunda* Vahl

4. Conclusion

The hyphenated GC-MS analytical technique employed in the analysis of the essential oil extracted from the leaves of the plant under investigation detected 25 compounds. The identity of twenty- four of these compounds were confirmed by comparing their mass spectra with standards available in the NIST and WILEY libraries. This essential oil is observed to be rich in polyenoic fatty acid methyl esters. The most abundant compound is 9, 12, 15- Octadecatrienoic acid methyl ester (36.56%). The other compounds of significant quantity identified in this oil are 9-Octadecenamide (7.12%), E-14-Hexadecenal (6.3%), Trifluoroacetoxy hexadecane (6.00%), 1-Heptadecene (5.68%), Hexadecanoic acid, methyl ester (Methyl Palmitate) (5.06%), 9, 12- Octadecadienoic acid, methyl ester (4.33%), Phytol acetate (3.40%).The presence of these compounds with well- established biological activities and proven medicinal applications in the essential oil of *justicia secunda vahl* makes it a potential source of raw material for the pharmaceutical and related industries; and further justifies its use in traditional herbal preparations.

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6. Conflict Of Interest Statement

We declare that there is no conflict of interest.

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