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Analysis of the Chemical Composition of the Essential Oil extracted from *Thevetiaperuviana* seeds Using Gas Chromatography Analysis

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Abstract:*The essential oils obtained by solvent extraction from Thevetiaperuviana seeds were analysed by GC-MCS in respect to their chemical composition. Forty-eight different peaks were identified in Thevetiaperuviana seed oil by GC-MS analysis, the active principles with their retention time (RT), molecular formula, molecular weight (MW) and area (%) are reported in this study. Results showed the efficiency and reliability of the gas chromatographic analysis in identification and qualification of chemical components in the extracted oil. The most prevailing compound was Benzene, 1, 3 dimethyl, o- xylene, p-Xylene (10.44%).*
Keywords: *Chemical components, Essential Oil, Gas Chromatography.*

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I. INTRODUCTION

Essential oils are products usually extracted from natural raw materials such as leaves, fruits, seeds and roots. Essential oils of plants and other products from secondary metabolism have had a great usage in folk medicine feel flavouring, fragrance and pharmaceutical industries [1]. The chemical compositions of essential oil extracted from different parts of plants such as leaves, roots, and stem from various plants have been studied by Thiem et al.[2], Coutinho et al.[3], and Stefanello et al.[4]. Coutinho et al.[3] observed that the chemical composition of essential oils from different reproductive and vegetative parts of a plant was similar in relation to major components; however the composition percentage of these was very different. Variations in the chemical composition at different phenological stages have been associated with the alteration of the chemical composition in antimicrobial activities, e.g., studies of the essential oils of *Salvia sahendica*[5].

Thevetiaperuviana is cultivated as an ornamental plant and planted as large flowering shrub or a tree, diffusely branched and dense crown. Leaves are dark green, glossy and linear. Flowers are funnel like with petals that are spirally twisted in small clusters at the tip of twigs. Flowers are yellow to dull orange or peach, tubular, with 5 petal lobes. The fruits are fleshy, triangular drupe, green in colour turning yellow, and then black on ripening. Each fruit contains a nut which is longitudinally and transversely divided containing one to four seeds in its kernel [6]. The sap is milky white [7].The plant starts flowering after one and a half year from plantation and there after blooms thrice a year [8].

Extracts from *Thevetiaperuviana* plant species contain glycosides, whose toxicity against snails, slugs [9], bacteria [10], insects [11] and humans [12] have been studied. *Thevetiaperuviana* plant extracts have been reported to have antifungal properties against *Cladosporiumcucumerinum*[13] and a potential source of biologically active compounds [14]. Toxicity and repellent effects of medicinal plant extracts on subterranean termites (Isoptera: Rhinotermitidae) have also been studied by Verena-Ulrike and Horst, [15]. *Thevetiaperuviana* seeds have high oil content with major glyceride of palmitric, steric, and linoleic acid [16, 17]. Due to the presence of toxins oil is non-edible.

The toxicity of the glycoside is reflected in the accidental poisonings that occur among children that feed on the seed of the plants [6, 18, 19]. Some adults have reportedly died after consuming oleander leaves in herbal teas [20]. According to Saravanapavanatha[21], the kernel of about ten fruits may be fatal to an adult while kernel of one fruit may be fatal to children. Generally, small children and livestock are at higher risk of *Thevetiaperuviana* poisoning [6].

GC-MS is one of the best techniques to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids, esters etc. [22]. Gas chromatography (GC) or gas chromatography-mass spectroscopy (GC-MS) was used in this study to identify various chemical components present in the *Thevetiaperuviana* oil with a view that component separation and identification would allow for determination of unidentified minor components which may strongly affect the overall quality of the oil.

II. METHODOLOGY

2.1. Plant Material

The seeds of *Thevetiaperuviana* were collected from Ile Ife Osun state, Nigeria. Oil extraction was performed according to methods described by Oyekunle[23]. Solvent free oil was kept inside clean sample bottles tightly sealed before they were taken for analysis.

2.2.Gas Chromatography Mass Spectrometry (GC-MS) analysis:

GC-MS analysis was carried out on a Agilent 19091S-433HP-5MS system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30mm×250µm ×0.25 µm, composed of 5% phenyl methyl silox), operating in Electron multiplier volts 1329.412 eV; Helium (99.99%) was used as carrier gas at a constant flow of 1.5mL/min and an injection volume of 1 µl was employed (split ratio of 10:1); Injector temperature of 150 °C; Ion-source temperature of 250 °C. The oven temperature was programmed from 35 °C (isothermal for 5 min.), with an increase of 4 °C/min, to 150 °C, for 2min, then 20 °C/min to 250 °C, for 5 min. isothermal at 250 °C. Mass spectra were taken at average velocity of 44.297 cm/sec; a hold up time of 1.1287 min, pressure of 11.604 psi and frequency of 50 Hz. Total GC running time was 45 min.

2.3. Identification of Components:

Based on Hema*et al*.[24] and Udayakumar and Gopalakrishnan[22] interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area with the total area. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library.

III. RESULTS

The result pertaining to GC-MS analysis leads to the identification of the number of compounds from the GC fractions of the oil extract from *ThevetiaPeruviana* seeds and these compounds were identified through mass spectrometry attached with GC. Fig. 1 shows forty-eight different peaks identified in *ThevetiaPeruviana*seedoilby GC-MS analysis. The active components with their retention time (RT), molecular formula, molecular weight (MW) and area (%) are presented in Table 1. The most prevailing compound was (Peak 1) Benzene, 1, 3 dimethyl, o- xylene, p-Xylene (10.44%) while the least prevailing compound was peak 42, with Area of 0.33.

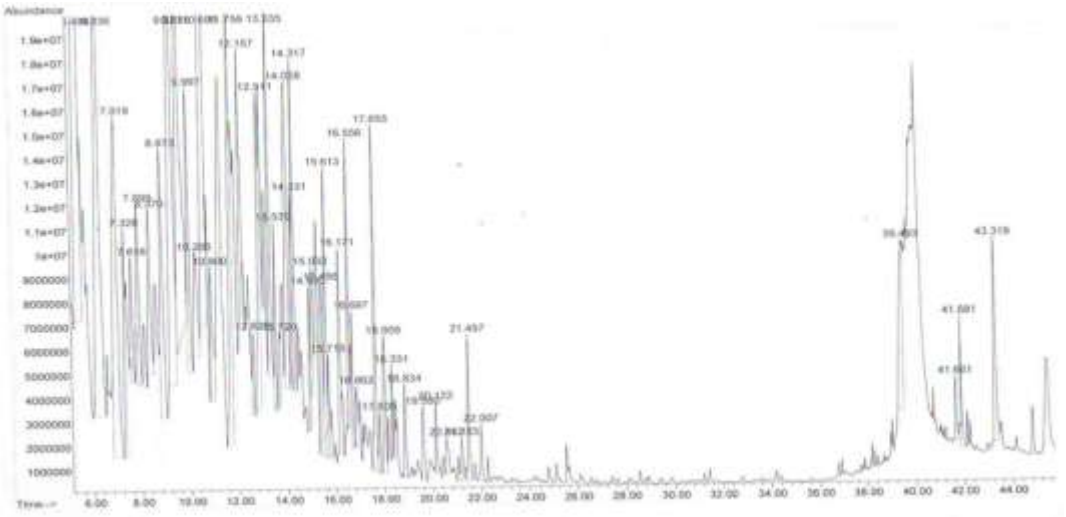

Fig. 1: GC-MS Chromatogram of oil extracted from *ThevetiaPeruviana* seeds

Table 1: Chemical composition (%) of essential oil extracted from*ThevetiaPeruviana* seeds

Peak	RT	Area (%)	Name of the compound	Molecular formula	Molecular weight (g/mol)
1	5.488	10.44	Benzene, 1 ,3 dimethyl	C_8H_{10}	106.165
			o- xylene	C_8H_{10}	106.165
			p-Xylene	C_8H_{10}	106.165
2	6.366	8.39	p-Xylene	C_8H_{10}	106.165
			Benzene, 1 ,3 dimethyl	C_8H_{10}	106.165
			Benzene, 1 ,3 dimethyl	C_8H_{10}	106.165
3	7.019	5.37	Dodecane	$C_{12}H_{26}$	170.3348
4	7.326	1.55	Bicyclo [4.1.0] heptane	C_7H_{12}	96.1702
			Cyclopentane, 1, 3-dimethyl-2- (1-methylethenyl)-, (1.alpha.,2.alpha.,3.beta.)-	$C_{10}H_{18}$	138.2499
			3, 3-Tetramethyleneglutaric anhydride	$C_9H_{12}O_3$	168.1898
5	7.616	0.85	Benzene, 1 , 2, 3 trimethyl-	C_9H_{12}	120.1916
			Benzene, (1-methylethyl)	C_9H_{12}	120.1916
			Benzene, (1-methylethyl)	C_9H_{12}	120.1916
6	7.899	1.51	Cyclopentane, (1-methylethyl)-	C_8H_{16}	112.2126
			Ethanone, 1-(1-methylcyclopentyl)-	$C_8H_{14}O$	126.1961
			Cyclohexane, propyl	C_9H_{18}	126.2392
7	8.37	1.55	Nonane, 3-methyl-	$C_{10}H_{22}$	142.2817
			Octane, 3,6-dimethyl-	$C_{10}H_{22}$	142.2817
			Sulfurous acid, 2-ethylhexyl hexadecyl ester	$C_{24}H_{50}O_3S$	418.717
8	8.873	2.58	Benzene Propyl-	C_9H_{12}	120.1916
			Benzene Propyl-	C_9H_{12}	120.1916
			N-Benzyl-2-phenethylemine	$C_{15}H_{17}N$	211.3021
9	9.321	7.62	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	120.1916
			Benzene, 1-ethyl-2-methyl-	C_9H_{12}	120.1916
			Mesitylene	C_9H_{12}	120.1916
10	9.635	6.9	Benzene, 1, 2, 3-trimethyl-	C_9H_{12}	120.1916
			Mesitylene	C_9H_{12}	120.1916
11	9.997	2.11	Benzene, 1-ethyl-2-methyl-	C_9H_{12}	120.1916
			Benzene, 1-ethyl-3-methyl-	C_9H_{12}	120.1916
			Benzene, 1, 2, 3-trimethyl-	C_9H_{12}	120.1916
12	10.256	1.45	Cyclohexane, 1-methyl-2-propyl	$C_{10}H_{20}$	140.2658
			Ethanone, 1- (1-methylcyclohexyl)-	$C_9H_{16}O$	140.2227
			Cyclohexane, 1-methyl-3-propyl	$C_{10}H_{20}$	140.2658
13	10.969	7.5	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	120.1916
			Mesitylene	C_9H_{12}	120.1916
			Benzene, 1, 2, 3-trimethyl-	C_9H_{12}	120.1916
14	10.9	1.21	trans-2-Methylcyclohexanol, pentafluoropropionate	$C_7H_{14}O$	114.1854
			Bicyclo [3..3.1] nonane	$C_9H_{16}O_2$	156.222
			Cyclohexane, 1-methyl-	C_7H_{12}	96.17
15	11.756	4.33	Benzene, 1, 2, 3-trimethyl-	C_9H_{12}	120.1916
			Mesitylene	C_9H_{12}	120.1916
			Benzene, 1, 2, 4-trimethyl-	C_9H_{12}	120.1916
16	12.157	1.9	Indane	C_9H_{10}	118.176
			Indane	C_9H_{10}	118.176
			Benzene, cyclopropyl-	C_9H_{10}	118.1757
17	12.658	0.45	Decane	$C_{10}H_{22}$	142.2817
			Decane	$C_{10}H_{22}$	142.2817
			Hexadecane	$C_{16}H_{34}$	226.4412
18	12.911	2.86	Naphthalene, decahydro-, trans-	$C_{10}H_{18}$	138.2499
			Naphthalene, decahydro-, trans-	$C_{10}H_{18}$	138.2499
			Naphthalene, decahydro-, trans-	$C_{10}H_{18}$	138.2499
19	13.355	2.49	Benzene, 2-ethyl-1, 4-dimethyl	$C_{10}H_{14}$	134.2182
			Benzene, 1-ethyl-3, 5-dimethyl	$C_{10}H_{14}$	134.2182
			Benzene, 2-ethyl-1, 4-dimethyl	$C_{10}H_{14}$	134.2182
20	13.539	0.93	Benzene, 1-methyl- 4-propyl	$C_{10}H_{14}$	134.2182
			Benzene, 1-methyl- 4-propyl	$C_{10}H_{14}$	134.2182
			Benzene, 1-methyl- 4-propyl	$C_{10}H_{14}$	134.2182
21	13.72	0.49	2, 5-Furandione, dihydro-3- (2-methyl-2-propenyl)-	$C_8H_{10}O_3$	154.1630
			Cyclopentane, 2-ethylidene-1, 1-dimethyl-	C_9H_{16}	124.2233
22	14.058	3.029	Benzene, 1-ethyl-2-, 4-dimethyl-	$C_{10}H_{14}$	134.2182
			o-Cymene	$C_{10}H_{14}$	134.2182
			Benzene, 1-ethyl-2, 3-dimethyl-	$C_{10}H_{14}$	134.2182
23	14.231	0.99	Indan, 1-methyl-	$C_{10}H_{12}$	132.2023
			Benzene, 1-ethenyl-3-ethyl-	$C_{10}H_{12}$	132.2023
			Benzene, 1-methyl-2- (2-propenyl)-	$C_{10}H_{12}$	132.2023
24	14.317	1.83	Benzene, 4-ethyl-1, 2-dimethyl-	$C_{10}H_{14}$	134.2182
			Benzene, 1-ethyl-2, 4-dimethyl-	$C_{10}H_{14}$	134.2182
			Benzene, 2-ethyl-1, 3-dimethyl-	$C_{10}H_{14}$	134.2182
25	14.93	0.75	IH-Indene, 2,3-dihydro-1, 1-dimethyl-	$C_{11}H_{14}$	146.2289

			IH-Indene, 2,3-dihydro-1, 3-dimethyl-	$C_{11}H_{14}$	146.2289
			IH-Indene, 2,3-dihydro-1, 1-dimethyl-	$C_{11}H_{14}$	146.2289
26	15.032	0.85	p-Cymene	$C_{10}H_{14}$	134.2182
			Benzene, 1, 2,4, 5-tetramethyl-	$C_{10}H_{14}$	134.2182
			o-Cymene	$C_{10}H_{14}$	134.2182
27	15.456	1.45	Benzene, 1, 2,4, 5-tetramethyl-	$C_{10}H_{14}$	134.2182
			Benzene, 1, 2,4, 5-tetramethyl-	$C_{10}H_{14}$	134.2182
			Benzene, 1, 2, 3, 5-tetramethyl-	$C_{10}H_{14}$	134.2182
28	15.613	1.85	Benzene, 1-ethyl-3, 5-dimethyl-	$C_{10}H_{14}$	134.2182
			Benzene, 2-ethyl-1, 3-dimethyl-	$C_{10}H_{14}$	134.2182
			p-Cymene	$C_{10}H_{14}$	134.2182
29	15.715	0.54	Naphthalene, decahydro-2-methyl-	$C_{11}H_{20}$	152.2765
			cis-Decalin, 2-syn-methyl-	$C_{11}H_{20}$	152.2765
			trans-Decalin, 2-methyl-	$C_{11}H_{20}$	152.2765
30	16.171	1.52	1H-Indene, 2, 3-dihydro-4-methyl-	$C_{10}H_{12}$	132.2023
			1H-Indene, 2, 3-dihydro-5-methyl-	$C_{10}H_{12}$	132.2023
			3a, 6-Methano-3aH-indene, 2, 3, 6, 7-tetrahydro-	$C_{10}H_{12}$	132.202
31	16.556	1.53	Benzene, 2-ethenyl-1, 4-dimethyl-	$C_{10}H_{12}$	132.2023
			Benzene, 1-ethenyl-4-ethyl-	$C_{10}H_{12}$	132.2023
			Benzene, 1-ethenyl-4-ethyl-	$C_{10}H_{12}$	132.2023
32	16.697	0.58	Benzene, 1-ethyl-2, 3-dimethyl-	$C_{10}H_{14}$	134.2182
			Benzene, 2-ethyl-1, 4-dimethyl-	$C_{10}H_{14}$	134.2182
			Benzene, 1, 2, 3, 5-teteramethyl-	$C_{10}H_{14}$	134.2182
33	16.892	0.39	Benzene, 1-methyl-4-(2-methylpropyl)-	$C_{11}H_{16}$	148.2447
			2-(p-Tolyl)ethylamine	$C_9H_{13}N$	135.2062
			4-Methylphenyl acetone	$C_{10}H_{12}O$	148.2017
34	17.655	2.26	Naphthalene	$C_{10}H_8$	128.1705
			Naphthalene	$C_{10}H_8$	128.1705
			Naphthalene	$C_{10}H_8$	128.1705
35	17.805	0.34	Benzene, (1, 2-dimethyl-1-propenyl)	$C_{11}H_{14}$	146.2289
			Benzene, (1-methyl-1-butenyl)-	$C_{11}H_{14}$	146.2289
			1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
36	18.009	1.10	1H-Indene, 2, 3-dihydro-1, 6-dimethyl-	$C_{11}H_{14}$	146.2289
			Benzene, (1, 2-dimethyl-1-propenyl)	$C_{11}H_{14}$	146.2289
37	18.331	0.40	1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
			1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
			1H-Indene, 2, 3-dihydro-1, 6-dimethyl-	$C_{11}H_{14}$	146.2289
38	18.834	0.54	Dodecane	$C_{10}H_{22}$	142.2817
39	19.58	0.54	2-Ethyl-2, 3-dihydro-1H-indene	$C_{11}H_{14}$	146.2289
			Benzene, (1-ethyl-1-propenyl)-	$C_{11}H_{14}$	146.2289
			Benzene, (1-ethyl-1-propenyl)-	$C_{11}H_{14}$	146.2289
40	20.122	0.39	2,2 -Dimethylindene, 2, 3-dihydro-		
			1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
			1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
41	20.562	0.36	1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
			Benzene, (1-methyl-1-butenyl)-	$C_{11}H_{14}$	146.2289
			1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
42	21.183	0.33	1H-Indene, 2, 3-dihydro-4, 7-dimethyl-	$C_{11}H_{14}$	146.2289
			Benzene, (2-methyl-1-butenyl)-	$C_{11}H_{14}$	146.2289
			Naphthalene, 1, 2, 3 ,4-tetrahydro-1-methyl-	$C_{11}H_{14}$	146.2289
43	21.497	0.88	Naphthalene, 2-methyl-	$C_{11}H_{10}$	142.1971
			Naphthalene, 1-methyl-	$C_{11}H_{10}$	142.1971
			Naphthalene, 2-methyl-	$C_{11}H_{10}$	142.1971
44	22.007	0.35	Naphthalene, 2-methyl-	$C_{11}H_{10}$	142.1971
			Naphthalene, 1-methyl-	$C_{11}H_{10}$	142.1971
			Benzocycloheptatriene	$C_{11}H_{10}$	142.1971
45	39.493	2.12	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772
			Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772
			Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772
46	41.661	0.37	9-Octadecenal, (z)-	$C_{18}H_{34}O$	266.462
			2-Methyl-z,Z-3, 13-octadecadienol	$C_{19}H_{36}O$	
			Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240.3816
47	41.881	0.61	Hexadecanoic acid, 2-hydroxy-1-		
			(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5026
			Dimethyl tetradecanedioate	$C_{16}H_{30}O_4$	286.407
			1, 15-Pentadecanedioic acid	$C_{15}H_{28}O_4$	272.3804
48	43.319	1.58	9-Octadecanoic acid (z)-, 2-hydroxy-1-		
			(hydroxymethyl) ethyl ester	$C_{21}H_{40}O_4$	356.5399
			9-Octadecanoic acid (z)-, 2, 3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.5399
			Propyleneglycolmonoleate	$C_{21}H_{40}O_3$	340.54

IV. CONCLUSION

GC-MS analysis performed in this study shows a reliable quantitative and qualitative analysis of complex essential oils. It also provides us with more information on retention indices and area of each component present in the oil. All the components present in the oil is essential and they strongly contribute to the overall qualities of the essential oil.

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