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Variation in Essential Oil Constituents of the Leaf of Anacardium Occidentale *found in Ondo, South Western-Nigeria*

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Abstract GC-MS analysis of the essential oil from the fresh leaves of *Anacardium occidentale* from Nigeria detected sixty components. The essential oil was hydro-distilled by using Clevenger-type apparatus. The main constituents in the essential oil from the leaves were beta-caryophyllene (17.36 %), alpha-copane (13.50 %), germacrene D. (10.67 %), germacrene B (9.93 %) and gamma-cadinene (8.17 %).

Keywords: *Anarcardium occidentale, essential oils, hydro-distillation and germacrene B and climate change*

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1. Introduction

Essential oils are volatile compounds from natural sources, usually plants. [1]

Essential oils of a large number of plants possess useful biological, pharmacological and therapeutical activities and are commercially important compounds. Their utilization in the various industries is influenced by the nature of their constituents.[2] Volatile oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes.[3]

The Anacardiaceae family is a rich source of these volatile oils. [4] *Anacardium occidentale* is native to the north-east coast of Brazil, and it is widely cultivated throughout the tropics. The world largest producer of cashew nut (*A. occidentale*) in 2010 was Nigeria. [5] Its various parts are widely used in traditional system of medicine for treating sores, snakes bites and rashes, the leaves of *A. occidentale* also possess antifungal, antidiarrheal and antipyretic activities. [6]

It is known that the essential oil composition pattern could be affected by the geographical and climatic conditions.[7] Burt, in year 2004 also reported that the chemical constituents of essential oils may vary based on the extraction methods, harvesting seasons and geographical sources.[8] Previous study on the essential oil extracted from the leaf of *A. occidentale* from Nigeria shows variation in the chemical composition; limonene, beta-caryophyllene, alpha-pinene, alpha-terpineol and alpha-ylangene have been observed to be the major constituents. [9] Study has also shown that beta-caryophyllene, alpha-pinene, germacrene D, p-cymene and beta-ocimene are the predominant components.[10] Another Study about the essential oils of leaves of *A. occidentale* from Minas Gerais State in Brazil described the major oil composition to be E-caryophylene, Germacrene D, alpha- copane, cadinene, Bicyclogermacrene and Germacrene B, [11] while the essential oils of *A. occidentale* Maia, *et al*. (2000) from Para State in Brazil was found to contain (E)-beta-ocimene, alpha-copaene, (E)-caryophyllene and δ-cadinene. [12]

This report seeks to investigate further on the variations in composition observed in the leave oils of *A. occidentale* plant with respect to geographical location.

2. Materials and Methods

2.1. Plant Material

The leaves of *Anacardium occidentale* were collected in April, 2013 at the premises of Wesley University of Science and Technology, Ondo state, Nigeria. The plant was authenticated by Mr. Ibhanesebhor G. of the herbarium section, Obafemi Awolowo University, Ile-Ife, Nigeria. A voucher specimen IFE 17003 was deposited at the herbarium.

2.2. Isolation of the essential oil

Fresh leaves of the plant were cut from the tree branch. The leaves were then reduced into smaller size to increase surface area and washed with distilled water; 200 g of the leaves was water-distilled by Clevenger-type apparatus for

3 h. The oil phase was trapped with 1 mL of hexane. The oil obtained was separated from the aqueous phase, the solvent was allowed to evaporate under ambient room temperature and stored under refrigeration prior to analysis.

2.3. Gas Chromatography/Mass Spectrometry Analysis

The essential oils were analyzed using Hewlett-Packard 6890 GC/MS system powered with HP ChemStation Rev. A 09.01[1206] Software. The capillary column type was HP-5MS (30.0 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier gas was hydrogen at constant flow rate of 1.0 mL/min and average velocity of 37 cm/s; the pressure was 22 psi. The initial column temperature was set at 100 °C (hold for 2 min) to the final temperature of 250 °C at the rate of 5 °C /min, Volume injected was 1.0 µL and split ratio was 20:1.

2.4. Identification of Constituents

The total chromatogram was auto-integrated by ChemStation and the constituents were identified by comparison with published mass spectral database (NIST02.L) and data from literature. Alkanes were used as reference points in the calculation of relative retention indices (RRIs). Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatograms.

3. Results

The GC Chromatogram showing the components of the essential oil is as shown below (Figure 1). Selected calibration curves along with correlation coefficients of standards are presented in Figure 2 to Figure 7.

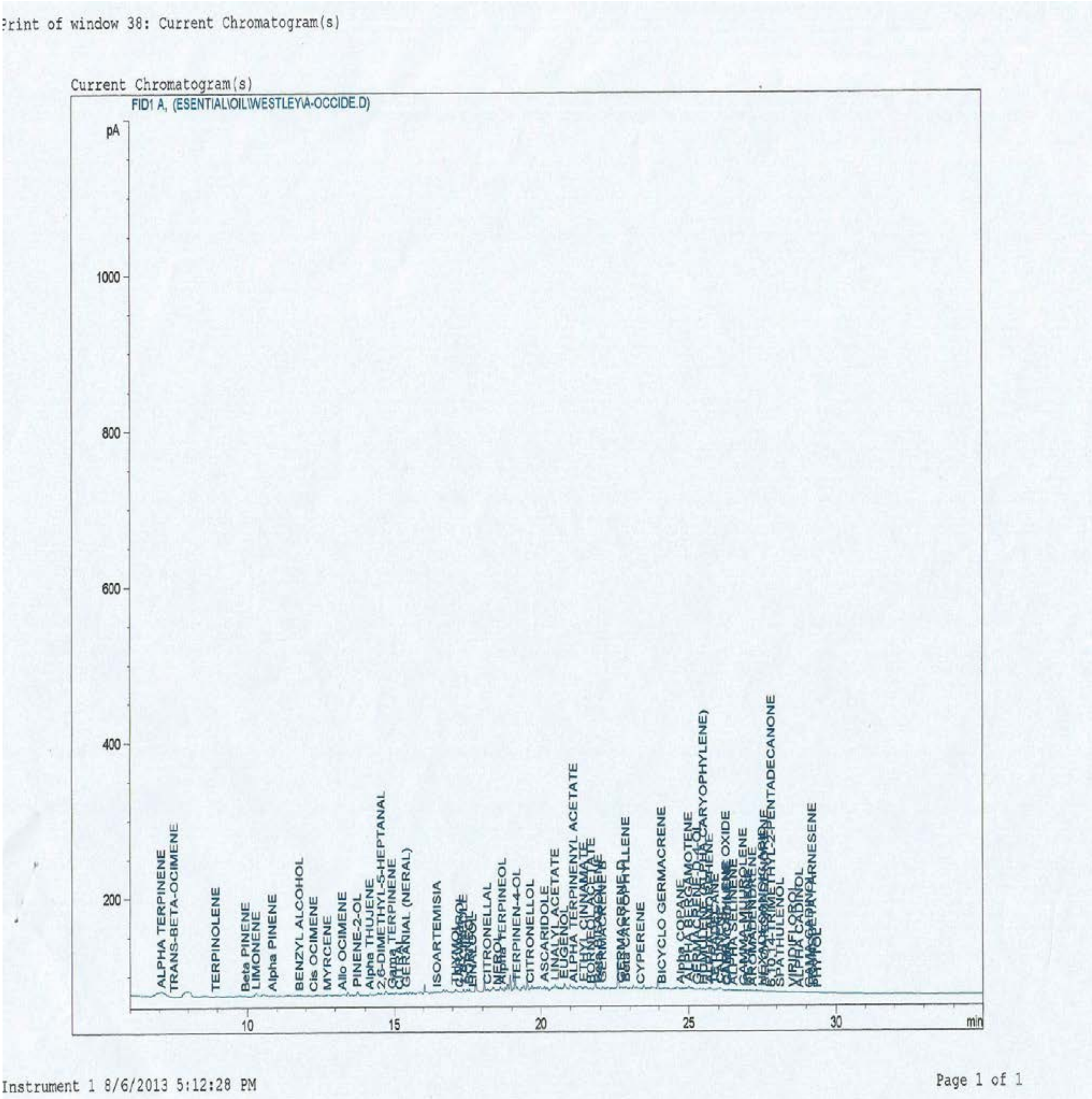

Figure 1. Chromatogram of essential oil of *Anarcardium occidentale*

Table 1. Chemical Components of the Leaf Oil of Anacardium occidentale

S/N	**NAMES**	**RRI**	**% Composition**
1	alpha terpinene	1001	0.79
2	trans-beta-ocimene	1012	0.22
3	Terpinolene	1052	0.22
4	beta-pinene	1080	0.35
5	Limonene	1092	4.79
6	alpha-pinene	1105	0.16
7	benzyl alcohol	1129	0.53
8	cis-ocimene	1141	0.06
9	Myrcene	1151	0.06
10	allo-ocimene	1163	0.22
11	pinene-2-ol	1175	0.22
12	alpha-thujene	1188	0.23
13	gama-terpinene	1205	0.45
14	Citral	1212	0.19
15	geranial (neral)	1216	0.36
16	Isoartemisia	1242	0.22
17	Thymol	1256	0.28
18	Carvacrol	1258	0.28
19	1,8- cineole	1263	0.53
20	Borneol	1265	0.44
21	Linalool	1270	0.18
22	Citronellal	1281	0.35
23	Nerol	1288	0.17
24	alpha-terpineol	1293	0.58
25	terpinen-4-ol	1305	0.21
26	Citronellol	1314	0.32
27	Ascaridole	1326	0.10
28	linalyl acetate	1335	0.40
29	Eugenol	1342	0.40
30	alpha-terpinenyl actate	1345	Tr
31	ethyl cinnamate	1356	0.52
32	borneol acetate	1363	0.47
33	beta-bisabolene	1367	0.59
34	**germacrene b**	1372	9.93
35	**germacrene d**	1386	10.67
36	**beta-caryophyllene**	1391	17.36
37	Cyperene	1405	0.13
38	bicyclo germacrene	1420	7.34
39	**alpha-copane**	1434	13.50
40	alpha-bergamotene	1441	0.11
41	germacrene-d-4-ol	1449	0.22
42	humulene	1454	0.47
43	alpha-amorphene	1458	2.17
44	Tetradecane	1463	1.56
45	Cadinene	1466	1.56
46	carryophlene oxide	1471	0.27
47	**gamma-cadinene**	1473	8.17
48	alpha-selinene	1478	0.66
49	gamma-muurolene	1485	0.08
50	beta-selinene	1490	0.53
51	aromadendrene	1495	0.39
52	hexadecanoic acid	1502	6.45
53	alloaromadendrene	1505	0.58
54	6,10,14-trimethyl-2-pentadecanone	1510	0.18
55	spathulenol	1518	0.27
56	viridiflorol	1528	0.27
57	alpha-cadinol	1535	0.73
58	gamma-cadinol	1543	1.14
59	trans-beta-farnesene	1545	0.18
60	phytol	1550	0.18

rri: relative retention indices calculated against n-alkanes. [13]
% calculated from tic data.
tr = trace (<0.1%).

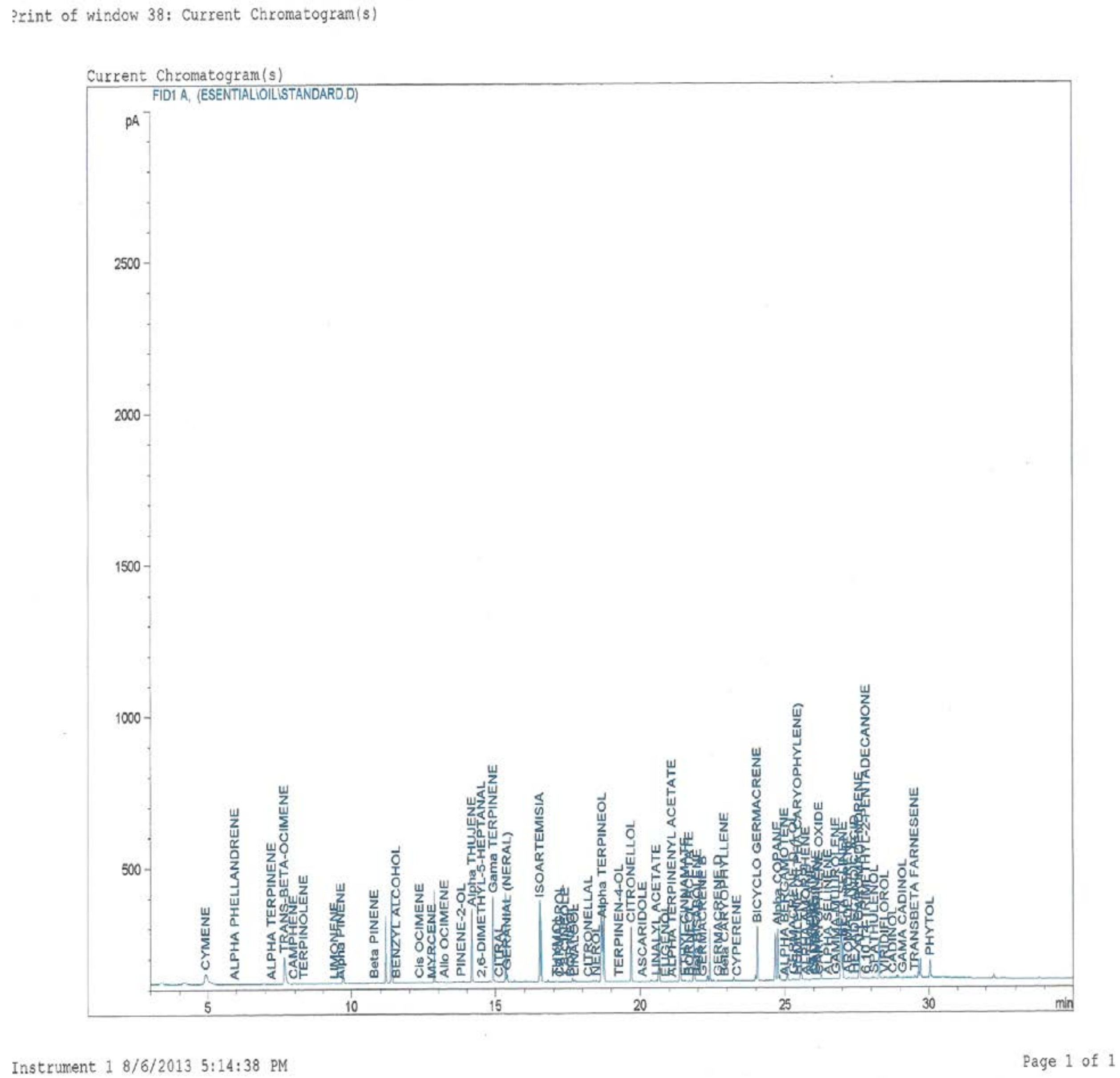

Figure 2. Chromatogram of standards (mixture)

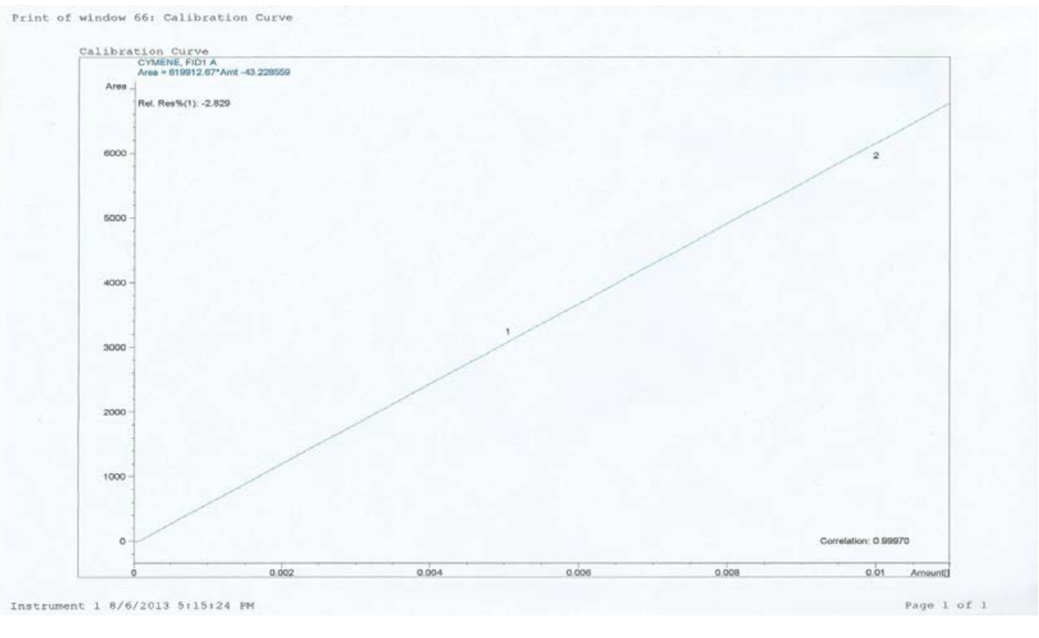

Figure 3. calibration curve of cymene

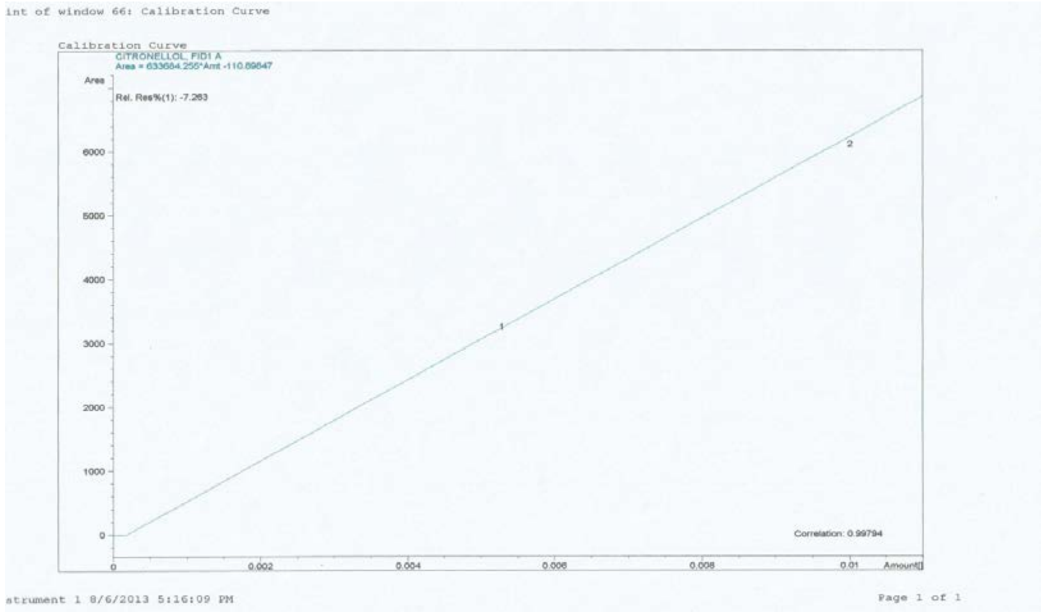

Figure 4. Calibration curve of myrcene

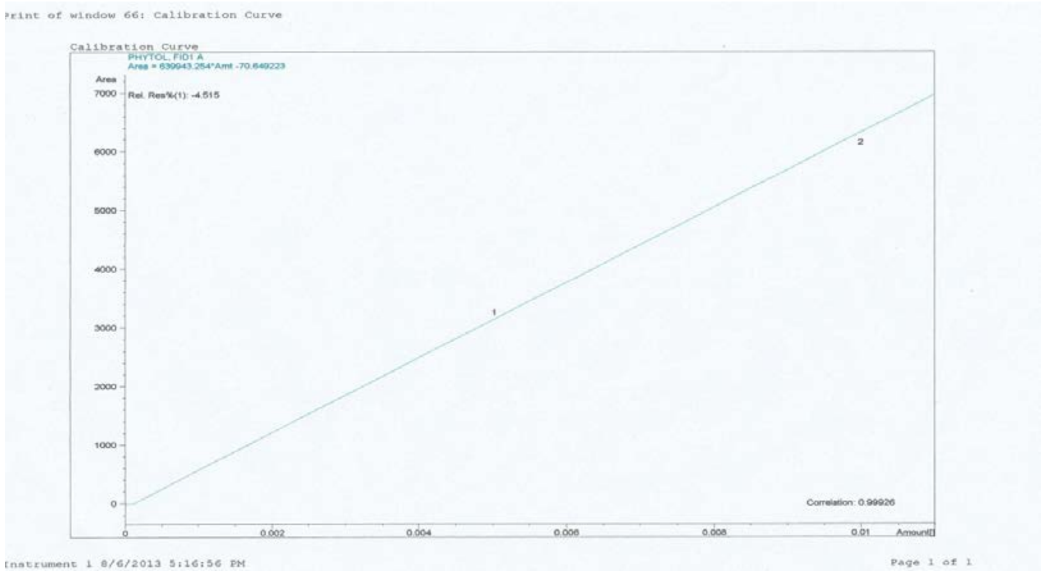

Figure 5. Calibration curve of phytol

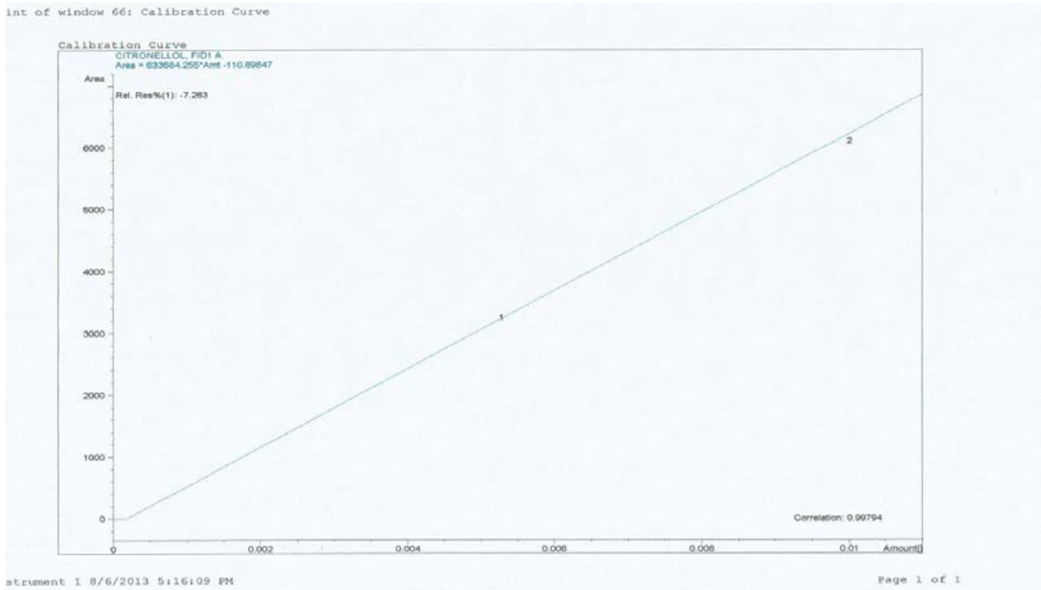

Figure 6. Calibration curve of citronellol

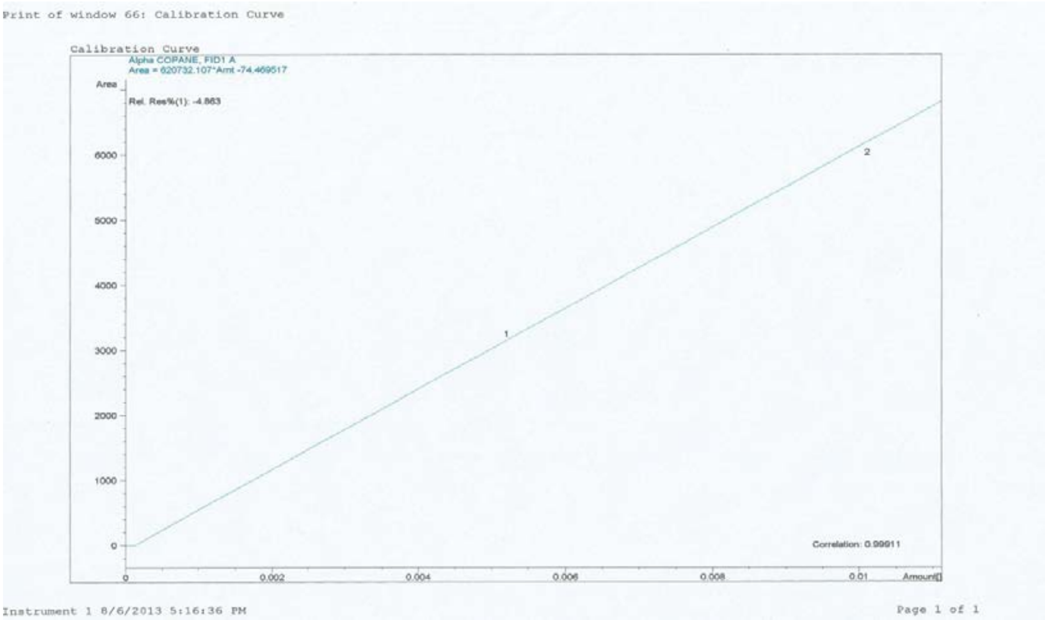

Figure 7. Calibration curve of alpha copane

4. Discussion

Sixty volatile oils were identified from the leaf of A. occidentale found in Ondo, Ondo State, Nigeria and these are listed in Table 1.

This study has shown that the essential oils of A. occidentale *found in South-western-Nigeria is rich in* beta-caryophylene (17.36 %), alpha-copane (13.50 %), germacrene D (10.67 %), germecrene B (9.93 %) and gamma-cadinene (8.17 %), with higher percentage compositions as compared to earlier reported essential oils analyses of A. occidentale done in Nigeria {see [9] and [10]}. The present result is at disparity with these {[9] and [10]} earlier reports from Nigeria in both compositions and yields.

This study reports for the first time, the presence of germacrene B in the leaf of A. occidental *from Nigeria*.

The percentage yield (9.93 %) of germacrene B obtained in the present report was found to be higher when compared to what was obtained from A. occidentale in Brazil (7.3 %)in year 2012. [11]

All the major constituents reported in this study were found in similar or slightly higher percentage yield and composition when compared to Ricardo *et al*. (E-caryophylene (15.4 %), Germacrene D. (11.5 %), alpha-copane (10.3 %), cadinene (9.3 %), Bicyclogermacrene (8.2 %) and Germacrene B (7.3 %)) and Maia, *et al*. ((E)-beta-ocimene (28.8 %), alpha-copaene (13.6 %), (E)-caryophyllene (7.6 %), and δ-cadinene (9.1 %) {see [11]}.

The report is in aggreement with published report of Dzamic *et al*,. based on the presence of beta-phellandrene (42.7 %), (E)-caryophylene (4.4 %), alpha-pinene (4.3 %), germacrene D (4.0 %), p-cymene (3.2 %) and (E)-beta-ocimene (3.1 %)) and Morokola *et al*. based on the presence of limonene (85.9 %), beta-caryophelene (1.7 %), alpha-pinene (1.5 %), alpha-terpineol (1.1 %) and alpha-ylangene (1.0 %) in good yield, respectively, {see [9] and [10]}.

However, beta-phellandrene and alpha-ylangene which were reported as the predominant components in the earlier report of Dzamic *et al*. and Morokola *et al* are conspicuously absent in our present study and this could be attributed to varying geographical location and climate change,{see [9] and [10]}.

It is interesting to observe that beta-caryophelene which was present in small proportion of 4.4 % and 1.7 % in Dzamic *et al*. and Morokola *et al* respectively is detected in large amount (17.36 %) as a major constituent in this present study. These can be as a result of variation in geographical area or locality of the plant and genetic variability. [14]

In conclusion, the chemical compositions of the essential oils of the leaf of *A. occidentale* are affected by environmental factors such as the current climate change due to climate forcings. However, some similarities could be found in their composition regardless of the geographical and climatic conditions as revealed by this study.

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