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Analysis of the Constituents of *Rauwolfia vomitoria* Ethanol Root Extract using GC-MS

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***Abstract*— The aim of the work was to identify the chemical compounds present in the ethanol roots extract of *Rauwolfia vomitoria* Afel (Apocynaceae) using Gas Chromatography - Mass spectroscopy (GC-MS). The GC-MS analysis revealed fifty seven compounds ranging from high and low molecular weight chemical entities at different concentrations. The major compounds present include Ethyl Oleate (10.59%), 9,12-Octadecadienoic acid, ethyl ester (8.26%), Hexadecanoic acid, ethyl ester (8.11%), Bis(2-ethylhexyl) phthalate (6.05.%), Hexanedioic acid, bis(2-ethylhexyl) ester (5.12%), 1,8(2H,5H)-Naphthalenedione, hexahydro-8a (3.89%), 2-(4-Hydroxybutyl)cyclohexanol (3.89) Benzophenone (3.07) Benzene, 1,1'-(1-methylethylidene)bis[4-(2-propenyloxy)- (2.45%), Dibutyl phthalate (2.39%), Hexadecanoic acid, methyl ester (2.88%), 10-Hydroxyhexadecan-1-yl acetate (2.25%), 1-Heptatriacotanol (2.24%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl (2.08%) The minor ones such as Azulene (0.25%), Cyclohexanol, 5-methyl-2-(1-methylethyl)- (0.20%), Naphthalene, 1,4-dimethyl- (0.18%), are also present. These chemical compounds are pharmacologically significant.**

***Index Terms*— *Rauwolfia vomitoria*, ethyl oleate, aphrodisiac agent, GC-MS,.**

I. INTRODUCTION

The use of herbal medicine for the treatment of diseases and infections is as old mankind. Many drugs which are commonly used in modern medicines have their origin either directly or indirectly from herbal sources collectively known as medicinal plants. Medicinal plants are plants with organs that contain substances that can be used for therapeutic purposes. Most of these substances are also precursors of useful synthetic drugs. A broader classification of medicinal plants would include; food plants, spices, perfumery plants, microscopic plants (like fungi, actinomycetes used for isolating drugs especially antibiotics), fibre plants like cotton, flax, jutes used for preparing surgical dressings [1], [2].

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Many individuals prefer medicinal herbs to orthodox medicines for diverse reasons, some of which may include: dissatisfactory efficacies of orthodox medicines, inconveniences of side effects, high cost, superstition and belief in efficacy of herbal medicines; furthermore there is a strong belief that herbal medicines do not have side effects [3]. In Nigeria many indigenous plants are liberally used to treat sexual dysfunction, diabetes, heart diseases, skin infection, stomach aches, cancer and a host of other conditions [4], [5]. The south-south region of Nigeria is endowed with many medicinal plants which play a very significant role in the treatment of the disease conditions peculiar to this region. *Rauwolfia vomitoria* (Afzel) is one of those medicinal plants found in abundance in this region that possesses phytochemicals that have effect against diseases and may contain the pharmacologically active compounds which can be used to treat ailing conditions. *Rauwolfia vomitoria Afzel* of Apocynaceae family is a rainforest tree of about 12 m high with trunk, whorled branches and conspicuous red globose fruit. It has oval leaves with straight venation and clusters of flowers. *Rauwolfia vomitoria* (Afzel) is recognized in natural medicine for its many therapeutic effects, among which are; treatment of diarrhea, malaria, hypertension, mental disorder, male infertility etc [6].

The root bark is a powerful purgative and has emetic effect. It is also known for its aphrodisiac, antisporic and insecticidal properties. The plant Rauwolfia vomitoria has many alkaloids and the most abundant and active is reserpine. Rauwolfia vomitoria has been extensively studied for various ailments; Amole et al., [7] documented its usefulness in lowering blood pressure. Dieudonne et al., [6] reported that treatment with aqueous extract of *Rauwolfia vomitoria* improved the number, transit and motility of sperm cells in the epididymis and vas deferens and increased production of sperm in the testes. Etim et al., [8] also reported the effectiveness of *Rauwolfia vomitoria* roots extract in improving as well as protecting the functionality of sexual organ thereby increasing the sexual desire and can be classified as aphrodisiac agent.

. This research work was undertaken to identify the oily constituents of the root of extract. The GC-MS analyzes of oil extract of the root of this plant identified a total of 57 constituents.

II. MATERIALS AND METHODS

A **Collection, Identification Preparation and Extraction of Plant Material**

The root of *Rauwolfia vomitoria* used for this work was

obtained from Itak village in Ikono Local Government Area of Akwa Ibom State and was identified by Mrs. E. G. Udoma, a taxonomist in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. Herbarium specimen with voucher number UUPH 6c was prepared and deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo for future reference. The plant material of *Rauwolfia vomitoria* collected was washed with distilled water chopped into small pieces and dried under shade for three weeks and weighed. The root plant material was pulverized to coarse powder using mortar and pestle. The powdered root sample (1.6 kg) was macerated for 72 h with 50% v/v aqueous ethanol at room temperature, after which the extract was filtered using Whatman filter paper (No. 4) and concentrated to dryness in vacuo at a temperature of 40oC using Rotavapor R-3 (CH-9230, Switzerland) to obtain the ethanol extract (97 g) then stored in a desiccator until when required for use.

B. **Gas Chromatography – Mass Spectroscopy (GC-MS) Analysis of the extract**

The extract (1.00 g) was dissolved in chloroform (2.5 mL), centrifuged at 10,000 rpm for 20 minutes and the oily supernatant was decanted and subjected to GC-MS analysis.

GC-MS Model: QP2010SE Shimadzu, (Tokyo, Japan) comprising a AOC-20i auto-sampler and gas-chromatograph inter-phased to a mass spectrometer (GC-MS) instrument equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness was used. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The carrier gas was Helium (99.99%) at a constant flow rate of 1.58 ml/min, injector and mass transfer line temperature were set at 250˚C and 200oC respectively, and an injection volume of 1 µl was employed (split ratio 10:1), the oven temperature was programmed from 80˚C (isothermal for 2 min), with an increase of 9˚C/min to 200˚C for 4 min, 10˚C/min to 280˚C, ending with a 5 min isothermal at 280˚C. The MS operating parameters were as follow: ionization energy, 70 eV; ion source temperature, 200˚C, solvent cut time, 2.5 min, relative detector gain mode, scan speed 1666 µ/sec; scan range 40-800 u, the interface temperature is 250˚C. The total running time of GC-MS was 30 min. The relative percentage of the sample was expressed as percentage with peak area normalization.

C. Identification of the constituents

The components peaks were identified by comparing their retention times with those of the standards and NIST05 mass spectral library (NIST, [9]. The concentrations of the components compounds were calculated as a percentage of the peak area to the total area of all the components in the sample

III. RESULTS

Figure 1 represents the chromatogram of the Gas chromatography (G. C.) of the ethanol extract of root of *Rauwolfia vomitoria* and Table 1 shows the analysis of the GC-MS result of the sample

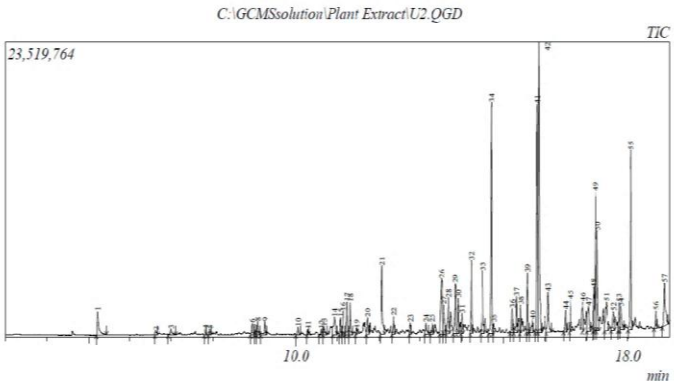

Figure 1: GC-MS Chromatogram of root plant oil extract of *Rauwolfia vomitoria*

Table 1: GC-MS analysis of the root oil extract of Rauwolfia vomitoria showing retention time molecular formula, molecular weight and percentage content of the constituents

S/N	RT	CONSTITUENT	MF	MW	%
1	5.217	3-Ethyl Pyridine	C_7H_7N	107	1.38
2	6.620	3-Ethyl-4-methyl Pyridine	$C_8H_{11}N$	121	0.28
3	6.989	6-Methylheptyl vinyl Ether	$C_{10}H_{20}O$	156	0.51
4	7.812	Cyclohexanol, 5-methyl-2-(1-methylethyl)-,	$C_{10}H_{20}O$	156	0.20
5	7.912	Azulene	$C_{10}H_8$	128	0.25
6	8.942	1-(3,3-Dimethylbutyn-1-yl)-2,2-dimethylcycl	$C_{11}H_{16}$	148	0.66
7	9.027	6-Heptenoic acid, 4-methylene-5-methyl-, methyl	$C_{10}H_{16}O_2$	168	0.34
8	9.80	Naphthalene, 2-methyl-	$C_{11}H_{10}$	142	0.62
9	9.247	Naphthalene, 1-methyl-	$C_{11}H_{10}$	142	0.68
10	10.053	Tridecane	$C_{13}H_{28}$	184	0.35
11	10.288	Naphthalene, 1,4-dimethyl-	$C_{12}H_{12}$	156	0.18
12	10.619	Dinonylamine	$C_{18}H_{39}N$	269	0.28
13	10.675	2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane	$C_{11}H_{18}$	150	0.39
14	10.934	Methoprene	$C_{19}H_{34}O_3$	310	1.27
15	11.070	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.55
16	11.131	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186	0.81
17	11.231	Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6	$C_{12}H_{20}$	164	1.18
18	11.306	4(1H)-Azulenone, octahydro-1-methylene-,	$C_{11}H_{16}O$	164	1.23
19	11.459	Bicyclo[3,3,1]non-2-ene, 7-oxa-2,8,9-trimethyl	$C_{14}H_{22}O_3$	238	0.28
20	11.721	Phthalic acid, ethyl pentadecyl ester	$C_{25}H_{40}O_4$	404	0.79
21	12.074	Benzophenone	$C_{13}H_{10}O$	182	3.07
22	12.354	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	592	1.05
23	12.760	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242	0.39
24	13.136	Benzyl Benzoate	$C_{14}H_{12}O_2$	212	0.52
25	13.283	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.57
26	13.506	1,8(2H,5H)-Naphthalenedione, hexahydro-8a	$C_{11}H_{16}O_2$	180	3.57
27	13.563	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280	1.65
28	13.678	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro	$C_{15}H_{22}O$	218	1.68
29	13.837	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl	$C_{16}H_{22}O_4$	278	2.08
30	13.920	cis-Z-.alpha.-Bisabolene epoxide	$C_{15}H_{24}O$	220	1.39
31	14.005	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.84
32	14.237	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.28
33	14.498	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	2.39
34	14.712	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	8.11
35	14.771	Tetradecane, 1-chloro-	$C_{14}H_{29}Cl$	232	0.40
36	15.216	Ethyl Oleate	$C_{20}H_{38}O_2$	310	0.95
37	15.314	1-Hexadecanol	$C_{16}H_{34}O$	242	1.43
38	15.420	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.90
39	15.704	Methyl stearate	$C_{19}H_{38}O_2$	298	1.79
40	15.704	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.80
41	15.820	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	8.26
42	15.861	Ethyl Oleate	$C_{20}H_{38}O_2$	310	10.59
43	16.082	10-Hydroxyhexadecan-1-yl acetate	$C_{18}H_{36}O_3$	300	2.25
44	16.501	Andrographolide	$C_{20}H_{30}O_5$	350	0.86
45	16.620	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-	$C_{35}H_{68}O_5$	560	1.13
46	16.915	Z,Z-8,10-Hexadecadien-1-ol	$C_{16}H_{30}O$	238	1.29
47	17.054	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	1.74
48	17.178	1-Heptatriacotanol	$C_{37}H_{76}O$	536	1.70
49	17.222	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370	5.12
50	17.264	2-(4-Hydroxybutyl)cyclohexanol	$C_{10}H_{20}O2$	172	3.89
51	17.490	1-Heptatriacotanol	$C_{37}H_{76}O$	536	2.24
52	17.658	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	1.05
53	17.788	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{18}H_{34}O$	568	1.09
54	17.837	Tridecanedial	$C_{35}H_{68}O_5$	266	1.48
55	18.069	Bis(2-ethylhexyl) phthalate	$C_{13}H_{24}O_2$	390	6.05
56	18.675	Cyclohexanol, 3-(aminomethyl)-3,5,5-trimethyl	$C_{24}H_{38}O_4$	171	0.72
57	18.887	Benzene, 1,1'-(1-methylethylidene)bis[4-(2-propenyloxy)-	$C_{21}H_{24}O_2$	308	2.45

IV. DISCUSSION

The extract by GC-MS analysis is shown to contain many fatty acids in their methyl ester forms such as Palmitic acid C16:0 (CH3 (CH2)14COOH, also known as hexadecanoic acid (a saturated fatty acid, Stearic Acid (C18:0) (C18H36O2) or octadecanoic acid with high concentrations. Stearic acid is used in many different ways as household products. It is used as a lubricant, a hardener, and an emulsifier (a chemical that allows oils and water to mix). Ethyl oleate was noted have the highest percentage of the fatty acid ester. Ethyl oleate formed by the condensation of oleic acid and ethanol is produced by the body during ethanol intoxication [10]. Ethyl oleate is used as a solvent for pharmaceutical drug preparations involving lipophilic substances such as steroids [11]. Ethyl oleate is also used by compounding pharmacies as a vehicle for intramuscular drug delivery, in some cases to prepare the daily doses of progesterone in support of pregnancy. Ethyl oleate is a major ingredient in Testosterone Cypionate injection used in treating sexual dysfunction [12], [13]. Therefore, it may be established that its presence in this plant contribute to synergizing the activity of aphrodisiac constituents of the plant.

V. CONCLUSION

In conclusion this study identifies over fifty constituent compounds of the oily extract of the root of *Rauwolfia vomitoria* some of which are very useful in the treatment of some common diseases in this locality. This work can also serve as reference point for further work on the plant.

. **Competing interests**: There are no competing interests.

Authors' contribution:

ECJ: Designed the experiment, sourced available literature and proof read the manuscript.
AUU: Interpreted the chromatogram
USB: Performed the extraction and wrote the manuscript.

VI. ACKNOWLEDGMENT

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