



GC-MS Analysis of the *Rauwolfia vomitoria* Ethanol Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author IIA designed the study and prepared the manuscript performed the statistical analysis. Author OATE substantively revised visualised and approved of the work and author MNIE managed literature, reviewed, edited the work and references.

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ABSTRACT

Bioactive compounds are the frontline potent agents in both nutraceuticals and pharmaceutical industries. The bioactive compounds are gaining much importance for their ability in enhancing resistance to various diseases and to improve the health of people both by traditional and modern ways of administrations. *R. vomitoria* is one of the medicinal plants used traditionally to manage hypertension, diabetes and mental disorder. This present study sought to characterize the bioactive components of *R. vomitoria* leaf and root ethanol extracts using Gas-Chromatography-Mass Spectrophotometry (GC-MS). The results of the GC-MS analysis provide different peaks indicating the presence of 22 phytochemicals in the plant leaf and 16 phytochemicals in the root. The major bioactive compounds in the leaf were squalene (18.69%), phytol (16.47%), n-hexadecanoic acid (15.68%), 7-tetradecenal, (Z) (12.90%), 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z)-, (9.56%) and others, while the roots contains; cis-vaccenic acid (32.13%), n-hexadecanoic acid (15.41%), (E)-9-octadecenoic acid ethyl ester (9.83), cyclohexanecarbonitrile 1-(-4- chlorophenyl (9.45%), 8H-azeceno[5,4-b] indol-8-one, 5-ethylidene (7.66%) and other minor compounds. Pharmacological

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activities of these compounds indicated that the compounds present in the leaf of the plant can be used as a crude drug which could be developed into a novel drug. Some of these compounds have antimicrobial, antioxidant, hepatoprotective, hypocholesterolemic as well as cancer preventive activities amongst others. The findings suggest that there is an indication that both *R. vomitoria* leaves and roots contain potent bioactive compounds that may be linked to its beneficial effects on health, with the leaf taking the lead. It is therefore recommended as a plant of phytopharmaceutical significance.

Keywords: *Rauwolfia vomitoria*; leaf; root; bioactive compounds; phytochemicals.

1. INTRODUCTION

In many countries worldwide medicinal plants remain the dominant form of medicine for the treatment and prevention of a wide range of diseases. Medicinal plants used as alternative drugs are indicative of the vital role that plants play in many developing countries, and are also sources of novel plant-derived constituents that could be leads for treatment of malaria and other diseases [1]. Plants have the capacity of synthesizing the organic compounds and are called as secondary metabolites, they have unique and complex structures. The secondary metabolites are used in the treating of chronic as well as infectious diseases [2]. One of the plants of medicinal value from the humid tropics is *Rauwolfia*, a tropical shrub with white or greenish flowers. The plant *Rauwolfia vomitoria* belongs to the family *Apocynaceae*. *Rauwolfia vomitoria* is called serpent wood, serpent snake root and swizzle stick, as well as, "asofeyeje" in Yoruba, "ira" in Igbo, "wadda" in Hausa, "akata" in Bini and "utoenyin" in Efik as vernacular names. It is mostly found in the forest of the southern part of Nigeria [3]. Research showed that herbal preparations of alkaloid extract of *R. vomitoria* have been used in traditional folk medicine in Africa as antihypertensive [4]. *Rauwolfia vomitoria* is used for treating nervous conditions [5] and can also act as antioxidant and anti-inflammatory [6], antiglycemic [7], anticonvulsant [8], Administration of ethanolic leaf and root bark extracts of *Rauwolfia vomitoria* on the 7th through 11th day of gestation may be cardiotoxic on the fetal heart of the developing rats and the extract of the root bark has more teratogenic potentials than the leaf extract [9]. The root bark extract of *R. vomitoria*, has great potential in the management of psychotic disorders [8]. Methanolic extract can be used as antimalaria [10].

Aqueous extract of *Rauwolfia vomitoria* can be used to treat typhoid, and jaundice [11] while, *Rauwolfia vomitoria* with or without vitamin E improved the immunity and enhances the

hematological indices [12]. Aqueous methanolic extract of *Rauwolfia vomitoria* leaves are used also as antisickling agents [13].

There has been an increasing interest on natural product research especially on medicinal plants which seem to have restorative properties [14]. Bioactive compounds are used in pharmaceutical industries as potents agents in treatment of many diseases. Bioactive compounds are gaining importance for the treatment of many diseases in recent days [15]. Identification and evaluation of these active compounds otherwise known as phytochemicals of uncommonly used plants could help provide information that would be useful in the development of a new drug [16] or in the production of a nutraceuticals. Phytochemicals are naturally occurring chemical compounds found in medicinal plants, leaves, vegetables and roots. They possess variety of protective properties against various diseases. The phytoconstituents from most medicinal plants for example flavonoids are considered as supplemental interventions for health substenance and disease management. The biological activity of flavonoids in neurodegenerative disorders, inflammation, cancer and cardiovascular diseases involves the regulation of cell growth and production, enzyme activity and the accent of cellular signaling cascades [17].

Over the last few decades, use of herbal drugs has been emphasized due to their easy availability, therapeutic potential, least side effects and minimum cost. At present nearly 80% of the world populations rely on plant based drugs for their health care need [18]. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds [19]. The combination of the separation technique (GC)

with the best identification technique (MS) makes GC-MS an ideal technique for qualitative and quantitative analyses for volatile and semi-volatile compounds. This study therefore, aims at utilizing a rapid method, Gas Chromatography-Mass Spectrometry (GC-MS) technique, for quantitative determination of bioactive compounds in *Rauwolfia vomitoria* Afzel leaf and root extracts.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals and reagents used were of analytical grade.

2.1.1 Plant collection and identification

The leaf and root of *R. vomitoria* were collected from Lambo Lasunwom village, Ikorodu, Lagos State, Nigeria in April, 2015. The plant was identified and authenticated by Prof J.D. Olowokudejo, Department of Botany, University of Lagos. A voucher specimen was deposited in the University herbarium with reference number LUH 6213.

2.1.2 Preparation of leaf and root extract of *R. vomitoria*

R. vomitoria leaves were washed with distilled water to free them of dust and sand. The cleaned leaves were air dried at room temperature ($28 \pm 2.0^\circ\text{C}$) until dry and ground to a powdery form. Roots were cleaned and cut into tiny pieces. The roots were left to dry and then ground to a coarse powdery form with Christy-Norris Laboratory Hammer Mill and kept in an air tight container until needed for use.

2.2 Extraction by Maceration

600g of the dried ground leaf and root were then extracted separately with 5L of 70- 95% ethanol for 24h. Upon complete extraction, the solvents were completely evaporated using a rotary evaporator and the concentrates were dried in a Plus 11 Gallenkamp oven at 45-50 °C. Extracts were re-refrigerated at 4°C until needed.

2.3 Determination of Bioactive Constituents and their Structural Composition in *R. vomitoria*

GC-MS analysis was carried out on a GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising AOC-20i auto sampler and chromatograph interfaced to a mass

spectrophotometer (GC-MS). The instrument was equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μm film thickness. The temperature employed were; column oven temperature 80°C, injection Temp 250°C at a pressure of 108.0 kPa, with total flow and column flow of 6.20 and 1.58 mL min^{-1} , respectively. The linear velocity was 46.3 cm sec^{-1} , and a purge flow of 3.0 mL min^{-1} . The GC program ion source and interface temperature were 200.00 and 250.00°C respectively with solvent cut time of 2.50 min. The MS program starting time was 3.00 min which ended at 30.00 min. with event time of 0.50 sec, scan speed of 1666 $\mu\text{L sec}^{-1}$, scan range 40 – 800 u and an injection volume of 1 μL of the plant extract (split ratio 10:1). The total running time of GC-MS was 30 min. the relative percentage of the extract was expressed as percentage with peak area normalization. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute standard and technology (NIST) having more than 62,000 patterns [20]. The spectrum of the known compounds stored in the NIST library. The name, molecular weight, and structure of components of test materials were ascertained.

3. RESULTS AND DISCUSSION

The mass spectrum of unknown component was compared with the spectrum of the known component stored in the National Institute Standard and Technology (NIST). Interpretation of mass spectrum of GC-MS was done using database of National Institute Standard and Technology (NIST). Major components were identified with authentic standards and recorded from computerized libraries. The compound name, probability, molecular formula, molecular weight, peak area and biological activity of the test materials were ascertained. GC-MS analysis revealed the presence of 22 compounds in *R. vomitoria* leaf extract and 16 compounds in *R. vomitoria* root extract. The results of the GC-MS analysis of the leaf extract of *R. vomitoria* are listed in Fig. 1. The list of constituents is given in Table 1. The results of the GC-MS analysis of the root of *R. vomitoria* are listed in Fig. 3. The list of constituents is given in Table 2.

Five major components were identified and characterized to be seen in *R. vomitoria* leaf extract (Fig. 2). Likewise, five components were identified and characterized as the major bioactive compounds (Fig. 4). The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure

of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library.

The compounds in the leaf and root extracts of *Rauwolfia vomitoria* used in this study are, diterpene, titerpene, fatty acids, their ethyl esters, organic hydrocarbons. Others include compounds whose biological activities is yet unknown. The identified major compounds possess some important biological potential for future drug development. However, isolation and characterization of individual phytochemical constituents may proceed to discover the novel drugs and their pharmacological activities. Numerous pharmacological active compounds (tryptophan, serotonin, melatonin) have an indole nucleus. A number of compounds bearing the indole moiety have been described to own affinity toward different serotonin receptors [31] Neurotransmitters like serotonin have structure similar to indole alkaloids and this has led to the prediction of neurological activity of indoles [43]. n-Hexadecanoic acid with undecanoic acid found in *Rauwolfia vomitoria* has also being reported by [44] in a GC-MS metabolite profiling of the methanol stem bark extract of *T. pachysiphon* (Apocyanaceae) to be the most predominant metabolites with n –Hexadecanoic acid (27.49%), Oleic acid (14.60%) and Octadecanoic acid (6.38%). n-Hexadecanoic acid with undecanoic acid have been reported to be an acidifier, acidulant, increase aromatic acid decarboxylase activity, inhibitor of uric acid production and arachidonic acid [45]. Acidifiers are chemicals that reduce the pH of the body and are needed for food digestion in patients suffering from achlorhydria [45]. These phytocompounds will be beneficial since it increases gastric acid when ingested. Phytol,

one of the major compounds detected in this experiment seems to possess antimicrobial activity. Also the interaction between other major and minor components could contribute to the antimicrobial properties. Phytol is a diterpene with antimicrobial properties, significantly against many bacterial strains [46]. Phytol has been reported to have activities such as antimicrobial, anti-cancer, anti-inflammatory, anti-diuretic, immune-stimulatory and anti-diabetic activities [47]. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamin E and K1. It is used along with simple or corn syrup as a hardener in candies. It was found to possess as well as preventive and therapeutic results against arthritis [48].

Similarly, phytol and squalene also showed the various biological activities as reported for *Coldenia procumbens* Linn [2]. Research work also revealed GC-MS profiling of some other Apocyanaceae family namely *Gongronema latifolium*, *Vincetoxicum rossicum* and *Marsdenia edulis* species revealed biologically functional compounds with therapeutic properties including linoleic acid, phytol, neophytadiene, n-hexadecanoic acid, squalene, transfarnesol, 5-pentadecen-7-yne, and mercaptoacetic acid [49]. Squalene, another constituent identified in GCMS is a natural triterpene known to decrease immobility time in FST [50]. [51] identified squalene have the property of antioxidant. Squalene is a hydrocarbon and a triterpene and possesses chemopreventive activity against colon carcinogenesis. It also has the property of antioxidant [52] and possesses chemopreventive activity against colon carcinogenesis [53]. Thus, it is possible that these major compounds identified in the plant from GC-MS are responsible for the antidepressant-like, antimicrobial, antioxidant, hepatoprotective, hypocholesterolemic as well as cancer preventive activities amongst others.

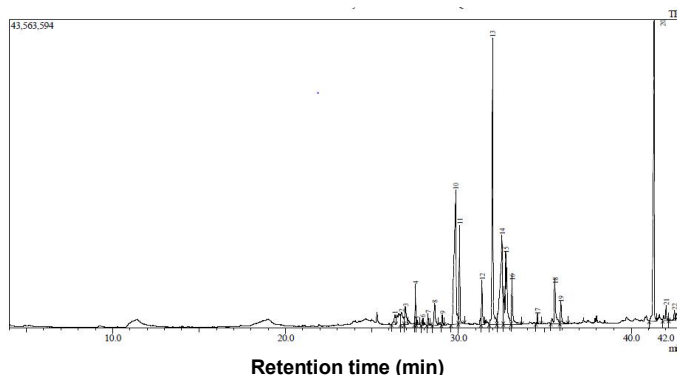


Fig. 1. GC-MS chromatogram of the bioactive constituents in *Rauwolfia vomitoria* Leaves

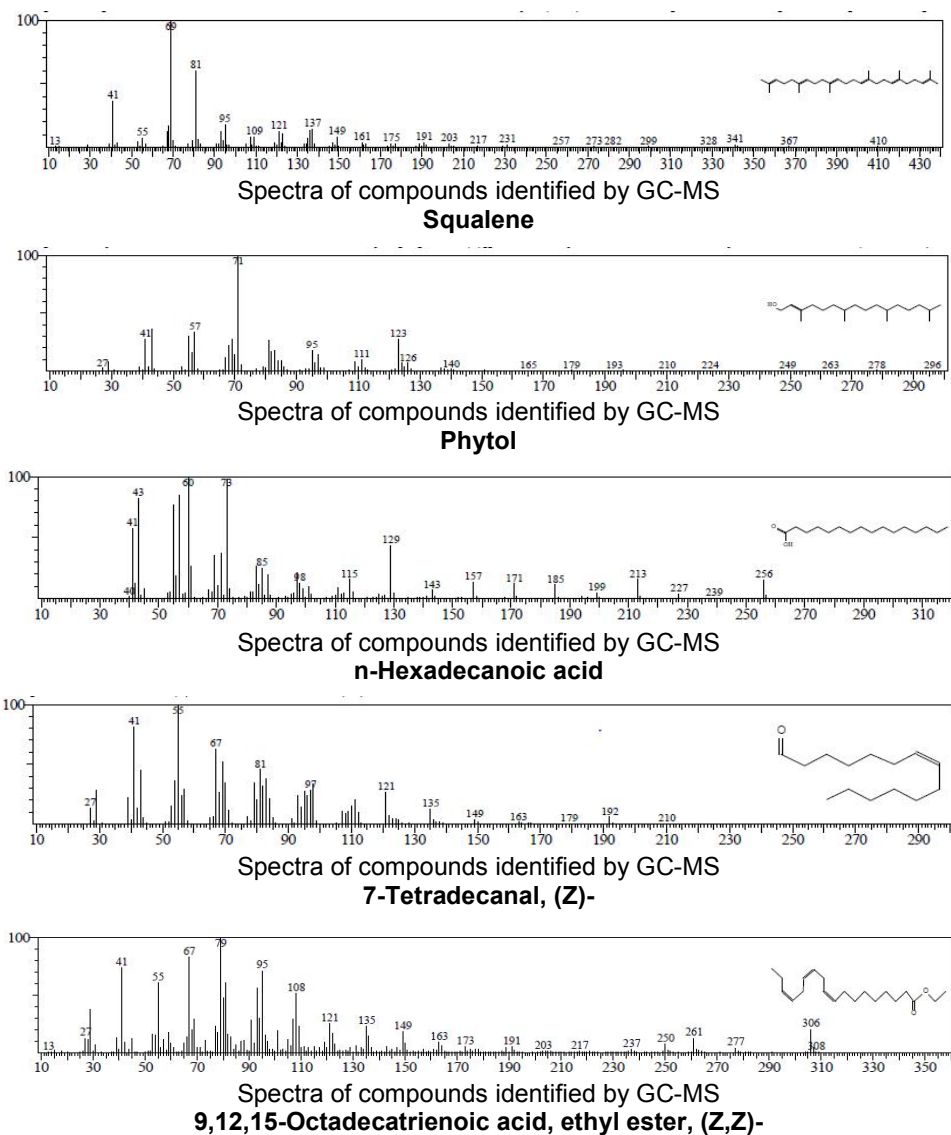


Fig. 2. Mass spectrum of major compounds of *Rauwolfia vomitoria* root extract

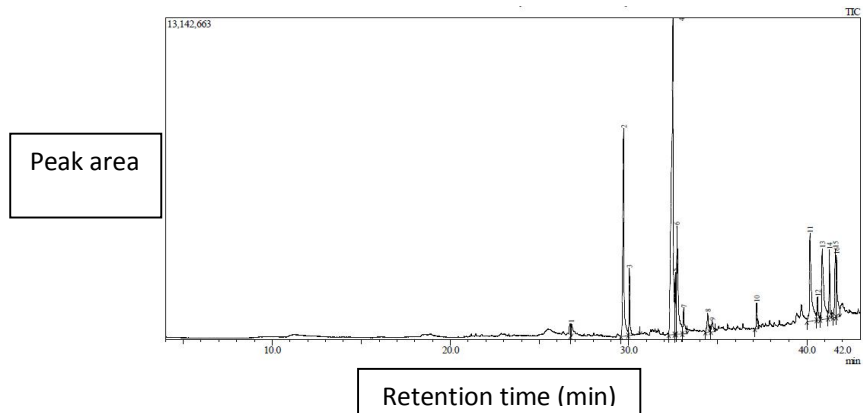


Fig. 3. GC-MS chromatogram of the bioactive constituents in *Rauwolfia vomitoria* Root

Table 1. List of compounds identified at various retention times from leaves of *Rauwolfia vomitoria* by GC-MS.

Peak No	Component	Retention time	MW	Area %	Nature of Compound	Activity
1	Undecanoic acid	26.332	186	1.02	Fatty acid	Acidifier, urinary acidulant
2	3-O-Methyl-d-glucose	26.718	194	1.68	Sugar moiety	
3	1-Acetyl-2,2,6,6-tetramethyl-4-acetyloxypiperidine	26.945	241	1.76		Antimicrobial [21].
4	Hexadecanal	27.520	240	1.45	Alkanal	Antimicrobial, Antioxidant [22]
5	2-Pentadecanone, 6,10,14-trimethyl	27.624	268	0.22	Diterpenoids	Antimicrobial
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	27.935	296	0.25	Terpene alcohol	Antimicrobial
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	28.241	296	0.47	Terpene alcohol	Antimicrobial
8	n-Hexadecanoic acid	28.641	256	1.85	Fatty acid	Antibacterial and antifungal [23]
9	Hexadecanoic acid, ethyl ester	29.083	284	0.49	Fatty acid ester	Anti-inflammatory [24]
10	n-Hexadecanoic acid	29.859	256	15.68		See above
11	Hexadecanoic acid, ethyl ester	30.077	284	3.98	Fatty acid	Antimicrobial, Antiandrogenic, Flavor, Hemolytic
12	Phytol	31.377	296	2.44	Diterpene	Antimicrobial, Anti-inflammatory, Anticancer [25]
13	Phytol	31.992	296	16.47	Diterpene	Anticancer Antioxidant, Antiinflammatory, Diuretic. See above
14	7-Tetradecenal, (Z)	32.531	210	12.90	Alkanal	Larvicidal and repellent activity [26]
15	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z)-,	32.760	306	9.56	Linolenic acid ester	Anticancer, Antimicrobial, Antioxidant and Hypocholesteralemic [27]
16	Octadecanoic acid, ethyl ester	33.119	312	3.38	Fatty acid ester	Antifungal, Antimicrobial [28] Anti-cancer [29]
17	Hexacosane	34.596	366	0.48		
18	9-Octadecenamide, (Z)-	35.605	281	3.88	Amide	Anti-inflammatory activity, antibacterial activity and antioxidant [30].
19	9-Octadecenamide, (Z)-	35.946	281	1.64	Amide	See above

20	Squalene	41.333	410	18.69	Titerpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, chemo preventive, Lipoxgenase-inhibitor, Pesticide [31], [2]
21	2-methylhexacosane	42.030	380	1.13	Branched alkane	Antibacterial
22	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentame	42.517	426	0.55		

Table 2. List of compounds identified at various retention times from root of *Rauwolfia vomitoria* by GC-MS

Peak No	Component	Retention time	MW	Area %	Nature of Compound	Activity
1	Benzoic acid, 3,4,5-trimethoxy	26.775	212	0.58	Aromatic compound	Food preservative, antifungal [32]
2	n-Hexadecanoic acid	29.728	256	15.41	Fatty acid	Antioxidant, Anti-inflammatory, Hypo-cholesterolemic
3	Hexadecanoic acid, ethyl ester	30.051	284	3.09	Palmitic acid ester	Anti-inflammatory, Anticancer, Hepato-protective, Anti-arthritic, Anti-coronary [33]
4	cis-Vaccenic acid	32.488	282	32.13	Omega-7 fatty acid	Anti-cancer [34]
5	9,12-Octadecadienoic acid (Z,Z)-6 32.717	32.631	280	2.84	Polyunsaturated fatty acid	Anti-oxidant [34]
6	(E)-9-Octadecanoic acid ethyl ester	32.717	310	9.83	Fatty acid ester	Neurotransmitter regulator [35]
7	Octadecanoic acid, ethyl ester	33.098	312	2.13	Fatty acid ester	Antineuroinflammation [36]
8	9-Tricosene	34.451	322	1.50	Pheromone	Pesticide [37]
9	8,11,14-Eicosatrienic acid	34.702	306	1.02	Unsaturated fatty acid	NF
10	1-Heneicosanol	37.185	312	1.55	Fatty alcohol	Anti-tuberculosis [37]
11	Cyclohexanecarbonitrile 1-(4-chlorophenyl)	40.189	307	9.45	Cyclic Hydrocarbon	
12	Spiro[androst-5-ene-17, 1' Cyclobutan] 2' one	40.611	350	1.48	Ketone compound	Anti-bacterial [38]
13	8H-Azeceno[5,4-b]indol-8-one, 5-ethylidene	40.876	326	7.66	Aromatic heterocyclic organic	Antidepressant properties [39]
14	Squalene	41.282	410	2.90	Triterpene	Neurotransmission [40] Anti-tumor [40,41]
15	Hepta-fluorobutyric acid, n-tetradecyl ester	41.611	289	4.01	Organofluorine	NF
16	Ethyl-iso-allocholate	41.677	334	4.43	Steroid	Anti-microbial [42]

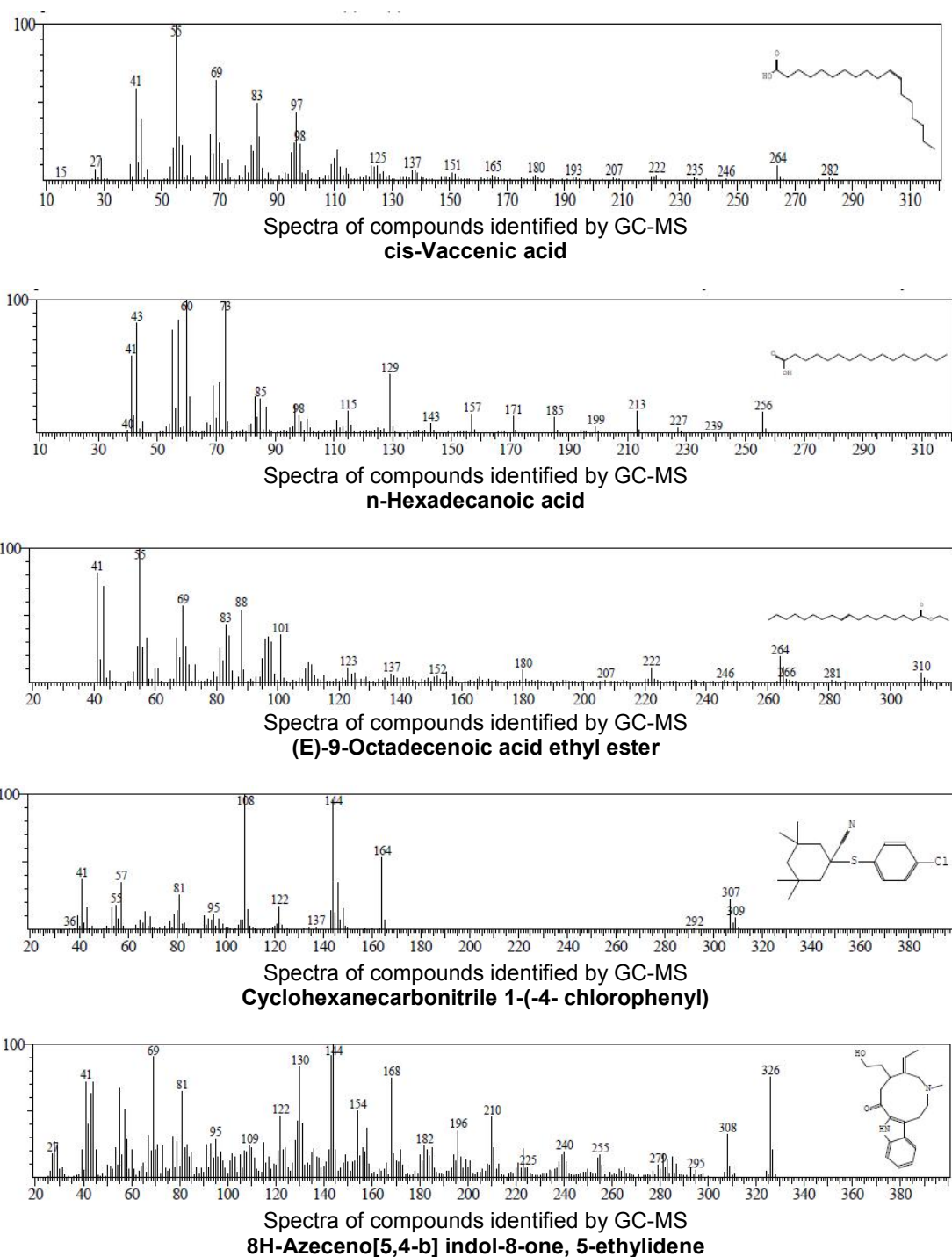


Fig. 4. Mass spectrum of major compounds of *Rauwolfia vomitoria* root extract

4. CONCLUSION

This study clearly shows that GC-MS is a powerful technique enabling fast separation and characterization of bioactive metabolites. The

high sensitivity of this technique helps in characterization of active compounds in *R. vomitoria* that can be used as drugs. The findings suggest that there is an indication that *R. vomitoria* leaves and roots contain an array of

bioactive compounds that may be linked to its beneficial effects on health. Therefore it is recommended as a plant of phytopharmaceutical importance.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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