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.

**Article Information**

DOI: 10.9734/EJMP/2021/v32i630398

Editor(s):
(1) Dr. N. Karmegam, Government Arts College, India.
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Complete Peer review History: https://www.sdiarticle4.com/review-history/68096

Received 14 March 2021
Accepted 19 May 2021
Published 07 August 2021

**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
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, hypcholesterolemic 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], antglycemic [7], anticonvulsant [8]. Administration of ethanolic leaf and root bark extracts of Rauwolfia vomitoria on the 7th through 14th 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 ± 2.0°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 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 μL 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* (Apocyanacae) to be the most predominant metabolites with *n*-Hexadecanoic acid (27.49%), Oleic acid (14.60%) and Octadecanoic acid (6.38%). *n*-Hexadecaneoic 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
Spectra of compounds identified by GC-MS

**Squalene**

**Phytol**

**n-Hexadecanoic acid**

**7-Tetradecanal, (Z)-**

**9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z)-**

*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.

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Component</th>
<th>Retention time</th>
<th>MW</th>
<th>Area %</th>
<th>Nature of Compound</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Undecanoic acid</td>
<td>26.332</td>
<td>186</td>
<td>1.02</td>
<td>Fatty acid</td>
<td>Acidifier, urinary acidulant</td>
</tr>
<tr>
<td>2</td>
<td>3-O-Methyl-d-glucose</td>
<td>26.718</td>
<td>194</td>
<td>1.68</td>
<td>Sugar moiety</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1-Acetyl-2,2,6,6-tetramethyl-4-acetyloxypiperidine</td>
<td>26.945</td>
<td>241</td>
<td>1.76</td>
<td>Antimicrobial [21].</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hexadecan</td>
<td>27.520</td>
<td>240</td>
<td>1.45</td>
<td>Alkanal</td>
<td>Antimicrobial, Antioxidant [22]</td>
</tr>
<tr>
<td>5</td>
<td>2-Pentadecanone, 6,10,14-trimethyl</td>
<td>27.624</td>
<td>268</td>
<td>0.22</td>
<td>Diterpenoids</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>6</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>27.935</td>
<td>296</td>
<td>0.25</td>
<td>Terpene alcohol</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>7</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>28.241</td>
<td>296</td>
<td>0.47</td>
<td>Terpene alcohol</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>8</td>
<td>n-Hexadecanoic acid</td>
<td>28.641</td>
<td>256</td>
<td>1.85</td>
<td>Fatty acid</td>
<td>Antibacterial and antifungal [23]</td>
</tr>
<tr>
<td>9</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>29.083</td>
<td>284</td>
<td>0.49</td>
<td>Fatty acid ester</td>
<td>Anti-inflammatory [24]</td>
</tr>
<tr>
<td>10</td>
<td>n-Hexadecanoic acid</td>
<td>29.859</td>
<td>256</td>
<td>15.68</td>
<td>See above</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>30.077</td>
<td>284</td>
<td>3.98</td>
<td>Fatty acid</td>
<td>Antimicrobial, Antiandrogenic, Flavor, Hemolytic</td>
</tr>
<tr>
<td>13</td>
<td>Phytol</td>
<td>31.992</td>
<td>296</td>
<td>16.47</td>
<td>Diterpene</td>
<td>Anticancer Antioxidant, Antiinflammatory, Diuretic. See above</td>
</tr>
<tr>
<td>14</td>
<td>7-Tetradecenal, (Z)</td>
<td>32.531</td>
<td>210</td>
<td>12.90</td>
<td>Alkanal</td>
<td>Larvicidal and repellent activity [26]</td>
</tr>
<tr>
<td>15</td>
<td>9,12,15- Octadecatrienoic acid, ethyl ester, (Z,Z)-</td>
<td>32.760</td>
<td>306</td>
<td>9.56</td>
<td>Linolenic acid ester</td>
<td>Anticancer, Antimicrobial, Antioxidant and Hypocholesteralemic [27]</td>
</tr>
<tr>
<td>16</td>
<td>Octadecanoic acid, ethyl ester</td>
<td>33.119</td>
<td>312</td>
<td>3.38</td>
<td>Fatty acid ester</td>
<td>Antifungal, Antimicrobial [28] Anti-cancer [29]</td>
</tr>
<tr>
<td>17</td>
<td>Hexacosane</td>
<td>34.596</td>
<td>366</td>
<td>0.48</td>
<td>See above</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>9-Octadecanamide, (Z)-</td>
<td>35.946</td>
<td>281</td>
<td>1.64</td>
<td>Amide</td>
<td>See above</td>
</tr>
</tbody>
</table>
Table 2. List of compounds identified at various retention times from root of *Rauwolfia vomitoria* by GC-MS

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Component</th>
<th>Retention time</th>
<th>MW</th>
<th>Area %</th>
<th>Nature of Compound</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzoic acid, 3,4,5-trimethoxy</td>
<td>26.775</td>
<td>212</td>
<td>0.58</td>
<td>Aromatic compound</td>
<td>Food preservative, antifungal [32]</td>
</tr>
<tr>
<td>2</td>
<td>n-Hexadecanoic acid</td>
<td>29.728</td>
<td>256</td>
<td>15.41</td>
<td>Fatty acid</td>
<td>Antioxidant, Anti-inflammatory, Hypo-cholesterolemic</td>
</tr>
<tr>
<td>3</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>30.051</td>
<td>284</td>
<td>3.09</td>
<td>Palmitic acid ester</td>
<td>Anti-inflammatory, Anticancer, Hepato-protective, Anti-arthritic, Anti-coronary [33]</td>
</tr>
<tr>
<td>4</td>
<td>cis-Vaccenic acid</td>
<td>32.488</td>
<td>282</td>
<td>32.13</td>
<td>Omega-7 fatty acid</td>
<td>Anti-cancer [34]</td>
</tr>
<tr>
<td>5</td>
<td>9,12-Octadecadienoic acid (Z,Z)-6</td>
<td>32.631</td>
<td>280</td>
<td>2.84</td>
<td>Polyunsaturated fatty acid</td>
<td>Anti-oxidant [34]</td>
</tr>
<tr>
<td>6</td>
<td>(E)-9-Octadecanoic acid ethyl ester</td>
<td>32.717</td>
<td>310</td>
<td>9.83</td>
<td>Fatty acid ester</td>
<td>Neurotransmitter regulator [35]</td>
</tr>
<tr>
<td>7</td>
<td>Octadecanoic acid, ethyl ester</td>
<td>33.098</td>
<td>312</td>
<td>2.13</td>
<td>Fatty acid ester</td>
<td>Antineuroinflammation [36]</td>
</tr>
<tr>
<td>8</td>
<td>9-Tricosene</td>
<td>34.451</td>
<td>322</td>
<td>1.50</td>
<td>Pheromone</td>
<td>Pesticide [37]</td>
</tr>
<tr>
<td>9</td>
<td>8,11,14-Eicosatrienic acid</td>
<td>34.702</td>
<td>306</td>
<td>1.02</td>
<td>Unsaturated fatty acid</td>
<td>NF</td>
</tr>
<tr>
<td>10</td>
<td>1-Heneicosanol</td>
<td>37.185</td>
<td>312</td>
<td>1.55</td>
<td>Fatty alcohol</td>
<td>Anti-tuberculosis [37]</td>
</tr>
<tr>
<td>11</td>
<td>Cyclohexanecarbonitrile 1-(4-chlorophenyl)</td>
<td>40.189</td>
<td>307</td>
<td>9.45</td>
<td>Cyclic Hydrocarbon</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Spiro[androst-5-ene-17, 1’ Cyclobutan] 2’ one</td>
<td>40.611</td>
<td>350</td>
<td>1.48</td>
<td>Ketone compound</td>
<td>Anti-bacterial [38]</td>
</tr>
<tr>
<td>13</td>
<td>8H-Azeceno[5,4-b] indol-8-one, 5-ethylidene</td>
<td>40.876</td>
<td>326</td>
<td>7.66</td>
<td>Aromatic heterocyclic organic</td>
<td>Antidepressant properties [39]</td>
</tr>
<tr>
<td>14</td>
<td>Squalene</td>
<td>41.282</td>
<td>410</td>
<td>2.90</td>
<td>Triterpene</td>
<td>Neurotransmission [40] Anti-tumor [40,41]</td>
</tr>
<tr>
<td>15</td>
<td>Hepta-fluorobutryc acid, n-tetradecyl ester</td>
<td>41.611</td>
<td>289</td>
<td>4.01</td>
<td>Organofluorine</td>
<td>NF</td>
</tr>
<tr>
<td>16</td>
<td>Ethyl-iso-allocholate</td>
<td>41.677</td>
<td>334</td>
<td>4.43</td>
<td>Steroid</td>
<td>Anti-microbial [42]</td>
</tr>
</tbody>
</table>
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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Peer-review history:
The peer review history for this paper can be accessed here: https://www.sdiarticle4.com/review-history/68096