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Research Article

Evaluation of the Chemical Composition of the Seeds of *Vigna subterranea* and Identification of their Chemical Structure (Bambara Groundnut)

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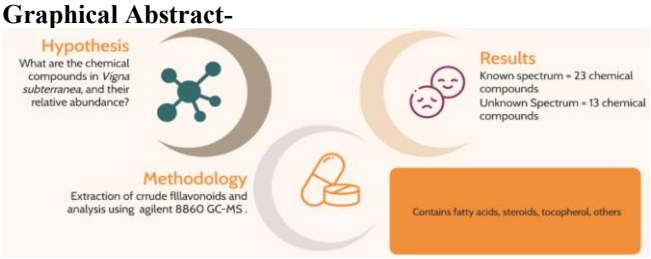
Abstract— The purpose of phase 1 of this research was to identify the chemical composition in the aqueous seeds extracts of *Vigna subterranea*, so as to determine their functions and derive their chemical structures. As part of the Chemical compounds identified, those with unknown spectrum, in which the unique mass fragmentation pattern produced by the compounds were yet to be positively identified by comparing it to existing, known mass spectral libraries or reference standards, were also discovered. A further analysis in a phase 2 study would be carried out, adopting the use of different solvents with varying polarities, and other chemical assay methods for a further deep dive into the identification of volatile compounds and others, not soluble in water or that do not exist in the GC-MC library, but could be present in other libraries.

Research Design: The aqueous seed extracts of *Vigna subterranea* (Bambara Groundnut) were evaluated to determine its chemical composition, their functions and derive their structures. The chemical components were identified through the application of Gas Chromatography - Mass Spectrometry. The objective of the study was to fully identify all chemical components, their functions and structure. If limitations exist, to continue with future research, adopting more complex methods.
Healthy shelled seeds were bought from local traders, with 10 seeds grounded into powder using a wooden mortar and pestel.. Distilled water was adopted as the solvent for extraction of the crude flavonoids. Each seed weighed between 1g to 1.5g, Crude flavonoid was extracted, by concocting and shaking vigorously in distilled water.
The chemical components were determined through the application of GC-MS, using hexane as the base for plant parts extracted by water. The chemical components, their functions and structures were determined by comparison to the NIST Mass Spectral Library.

Methodology: The methodology for extraction was the use of distilled water as solvent, by concocting the powdered seed without applying heat. Heat was not applied due to its ability to destroy proteins. Hence mechanical shaking was adopted for thoroughly mixing the powdered seeds with the solvent.GC-MS was adopted for the identification of the chemical components of the aqueous seeds extracts of *Vigna subterranea*, while desk research from existing literature was adopted to determine the chemical structures. The aggregated data resulting from the chromatography separation was used for sophisticated data analysis, with visibility into the concentration of each component.

Keywords— NIMR, Viga Subterranea, Thin Layer Chromatography, Aqueous Extract, NIST, GC-MS

Graphical Abstract-



- The figure above summarizes the research hypothesis, methodology adopted and key findings
- A GC-MS was the only method of analysis adopted
- Compounds of both known and unknown spectrums were confirmed, which would require future work using other methods of analysis

1. Introduction

Vigna subterranea also known as Bambara Groundnut is a leguminous and nutritious seed native to West Africa and found in the tropical forest of West Africa. It is used as a delicious delicacy by the Igbos in Nigeria to make a local food called Okpa. It is also boiled and cooked as porridge.

The seeds come in an oval shape and in different colours (white, red, dark-brown, black, and cream). The oval shape has a semblance to the human eye outside its socket. It has a drought resistant semi-coat, its radicule, hypocotyl, hilum and inner endosperm. Physical characteristics include a slightly bouncy nature when thrown on hard floors, with a resounding stone like sound. It is drought and pest resistant, tolerates very high temperatures and produces high yield in poor soil. It grows in pods with very hardy skins. The seeds without the pods come in different lengths between 0.7cm - 1cm. The leaves of *Vigna subterranea* are not known to be consumed, but are rather used as animal feed due to their richness in hydrogen and phosphorus.

Bambara nuts are believed by different African tribes to have varying health benefits, which range from prevention of malnutrition, to protection against anemia, to prevention against bone disease, to supply of energy and prevention of insomnia. and due to its perceived high nutrient content, Though there are yet to be verified evidence and documented research and confirmed clinical trials on the health benefits of *Vigna subterranea*. There has been several scientific analysis of its chemical components, and the seeds have been found to contain phenolic compounds, dietary fibre, amino acids, fatty acids, tocopherols, tocotrienol and phytosterols, alkaloids, flavonoids, sphingolipids, t-ferrulic acid, p-coumaric acid, and Salicylic acid.

Due to an incidental improvement in eye sight after rubbing on the eye lashes as reported by local residents, this research is set to investigate and identify the chemical and structural composition of *Vigna subterranea* seeds, using distilled water as the only solvent for extraction and further analysis in a GC-MS, after which the results would be compared against the use of other polar solvents and more complex chemical analysis methods in a phase 2 research. Chemical components with unknown spectrum would be documented and compared against all existing libraries and standards. Where no similarity is found, the substance might be a "known unknown" (known in literature but not in the specific library used) or a truly novel "unknown unknown" (a new or unexpected substance not previously documented in available databases), such as a new designer drug or an unexpected metabolite. This would require further analysis, such as combining mass spectral data with retention index information or using high-resolution mass spectrometry for empirical formula determination.

The hypothesis was formed using the relative abundance as an independent variable against the mass-to-charge of ions produced during the ionization process. In the GC-MS. This helps in identifying and quantifying the components in the aqueous seed extracts of *Vigna subterranea*. The m/z value was expressed in units of mass, divided by the charge number of the ion, and the horizontal axis in a mass spectrum. This measurement principle is fundamental to the analysis of complex mixtures. What chemical components exist in the aqueous seed extracts of *Vigna subterranea,* and what is their relative abundance.?

This paper is organized as follows, (Section 1 contains the introduction of the research, Section 2 contain the related work on the analysis of the chemical composition of *Vigna subterranea*, Section 3 contain the theory applied for the research, Section 4 contain the method and study design, section 5 describes results and discussion, Section 6 concludes the research work with future directions).

1.1 Planting *Vigna subterranea*

The required temperature for the growing of *Vigna subterranea* is 30–35°C; above 40°C, below 15°C and growth is very limited. The shoots appear within 5–21 days. Vegetation growth might continue after the reproductive stage has started Budding of flowers commences at about 30–55 days post sowing and might continue until the plant perishes. After pollination, the flower stalks grow and pods form above or below the ground. Seedcase can attain their maximum size within 30 days. Its seeds swell and attain full growth within the next 10 days, after the parenchymal layer encompassing the embryo the embryo has vanished and brown spots appear on the outside of the pod. Seeds are mature 3–6 months post germination. *Vigna subterranea* seeds are able to fix atmospheric nitrogen through relating with bacteria of the Bradyrhizobium group

2. Related Work

The assessment of the proximate and anti-nutritional composition of different variants of *Vigna subterranea* was carried out to confirm suitability as substitute sources of protein for humans and animals. The results showed richness in protein, crude fibre, carbohydrate and some anti-nutritional factors [10].

Based on the antibacterial and antifungal properties of *Vigna subterranea* in treatments during local therapy of patients in Kaduna State, Nigeria, its roots were further evaluated to identify its antimicrobial and phytochemical components using ethanol as a polar solvent. The results showed the presence of steroid, saponins, alkaloids, tannins, and flavonoids while anthraquinone was conspicuously absent. [1].

The nutritional and chemical composition of *Treculia Africana* and *Vigna subterranea* seeds collected from Kogi State, Nigeria were evaluated. Their proximate composition, amino acid profile, and mineral content, were analysed adopting standard methods. Gas chromatography-mass spectroscopy was used to determine their chemical composition [4].

A further evaluation of the chemical composition of the seeds revealed the presence of ash, carbohydrates, crude fibre, zinc, magnesium, sodium, calcium, phosphorus and magnesium [9].

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With the aim to research alternative medicines due to the growing resistance of bacteria to antibiotic treatments, the leaves and seeds of *Vigna subterranean* (Bambara Nut), was shown to inhibit the growth of some Gram-positive and Gram-negative bacteria and fungi with the leaves showing more potency. Some of the phytochemical components found were tannins, flavonoids and alkaloids [6].

In Cameroon, the leaves and seeds of twenty different species of *Vigna subterranea* were analysed to elucidate how food species can solve the problems of malnourishment. The phytochemical nutrients discovered showed a difference in the percentages across the different species [5].

Considered as a super food by some tribes in sub-Saharan African, the leaves, roots and seeds of *Vigna subterranea* was analysed for its phytochemical nutrients. The results showed the presence of saponins, tannins, steroids, flavonoids, alkaloids and vitamins [3].
Vigna subterranea was shown to treat diabetic hepatic and renal issues [2].
Processing *Vigna subterranea* seeds through roasting and boiling has effects on its nutritional and antimicrobial properties [11].
Vigna subterranea seed extracts were shown to reduce ulcer in rats with mucosal injury [8].
Vigna subterranea leaf extracts had an impact on the bacteria extracted from the swab of wound [7].

3. Theory

To answer the hypothesis for the research, which is to determine the chemical components that exist in the aqueous seed extracts of *Vigna subterranea and their relative abundance*, a gas chromatography mass-spectroscopy was used, and results compared against mass-spectral libraries.

The relative abundance of a chemical compound in a plant part is calculated from mass spectrometry (MS) data by comparing the peak area (or height) of that compound to the total area of all identified compounds. Formula for Relative Abundance

Steps for Calculation using Mass Spectrometry Data Obtain
Mass Spectrum Data: The mass spectrometer generates a mass spectrum, which plots ion intensity (which correlates with abundance) versus the mass-to-charge ratio (\(m/z\)).

Identify Relevant Peaks: Identify the specific peaks (corresponding to different \(m/z\) values) that represent the various compounds in the plant sample.

Measure Peak Areas: Determine the area under each relevant peak in the chromatogram or mass spectrum. This area is proportional to the number of ions detected for that specific compound.

Sum Total Area: Add up the areas of all identified peaks in the sample to get the total area.

Apply the Formula: Use the formula above to calculate the percentage abundance for each individual compound.

Key Considerations
Normalization: The most abundant peak in a mass spectrum is typically set as the "base peak" and assigned a relative abundance of 100%, and all other peaks are normalized relative to this base peak intensity. This is different from the percentage calculation used to determine the overall composition of a mixture.

Purpose: This method helps determine the proportional makeup of the identified chemical components in the plant extract, useful for comparative studies of plant chemical profiles.

4. Experimental Method/Procedure/Design

4.1 Study Design
The investigation was carried out to identify the phytochemical compounds of the aqueous seed extracts of *Vigna subterranea* following the below methods.

4.1.1 Sourcing of Plant Material
Healthy seeds that had been removed from their pods were bought from the local market in Lagos,

4.1.2 Extraction of Crude Flavonoids
10g of dried *Vigna subterranea* seeds were ground into powder using a wooden mortar and pestle. The ground seeds were transferred into a clean beaker and mixed in distilled water in a ratio 1:2 of seeds to water. The mixed solution was filtered using filter paper of 125mm. The resulting filtered extract was placed into heparin tubes and transferred to the Gas Chromatography technician and specialist for analysis.

4.1.3 Chromatography Separation:
The crude flavonoid extract was analyzed using an Agilent 8860 gas chromatograph coupled with a 5977B mass spectrometry detector (MSD). Separation was achieved on an Elite-5MS fused silica capillary column (5% diphenyl/95% dimethyl polysiloxane; 30 m × 0.25 µm internal diameter × 0.25 µm film thickness). GC–MS analysis was performed using an electron ionization (EI) source operating at an ionization energy of 70 eV. High-purity helium (99.999%) served as the carrier gas at a constant flow rate of 1 mL/min. A sample volume of 1 µL was injected using a split ratio of 10:1. The injector temperature was set at 300 °C, while the ion source temperature was maintained at 250 °C. The GC oven temperature was initially held at 110 °C for 1 minute, then increased at a rate of 15 °C/min to 310 °C and held for 2 minutes. Mass spectra were recorded at 70 eV with a scan interval of 0.5 seconds over a mass range of 45–450 Da. The solvent delay time was set between 0 and 3 minutes. Hexane was used as the solvent for GC–MS separation.

4.1.4 Study Location:
The study location was at the Nigeria Institute of Pharmaceutical Research (NIMR)

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4.1.5 Data Collection:
The chemical constituents were identified using an Agilent 8860 gas chromatograph coupled with a 5977B mass spectrometry detector (MSD). The acquired data were matched with entries in the NIST14 mass spectral library (D:\MassHunter\Library\NIST14.L). The resulting reports were extracted as PDF files and shared with the research team via WhatsApp.

4.4 Data Analysis
The data collected from the analysis of the chemical components of *Vigna subterranea* showed the Retention Time (RT), Area% (percentage of the total area of all peaks in the signal), Qual (qualifier ion used to identify the analyte) and other info. The number of chemical components recorded and documented.

5. Results and Discussion

Hypothesis 1: What are the chemical compounds in *Vigna subterranea* seed and their relative abundance?
Based on the outcome of analysis of the aqueous seed extracts of Viga subterranea using GC-MS, the below chemical compounds were discovered

1. **n-Hexadecanoic Acid (C16H32O2):** This is a 16-carbon chain fatty acid most commonly found in animals, plants and microorganisms, also known as palmitic acid. (figure 15). Palmitic acid has been found to be an inhibitor of HIV-1 infection. Its m/z ratio was at 55.10, with a relative abundance of 98.84 % (figure 2).
2. **cis-Vaccenic acid (C18H34O2):** This is a C18 omega-7 unsaturated fatty acid having a double bond at position 11. (figure 16). It is a key mediator of energy metabolism in adipose tissues. Its m/z ratio was at 67.10, with a relative abundance of 56.83 % (figure 3).
3. **9,12-Octadecadienoic acid (Z,Z)**-: Also known as Linolenic acid ester is a polyunsaturated fatty acid that plays a crucial role in human health cell membrane structure and function. It has anti-inflammatory, hypocholesterolemic, anti-arthritic, nematicide and hepatoprotective properties. Its m/z ratio was at 69.10, with a relative abundance of 54.27 % (figure 3).
4. **Oleic Acid (C18H34O2):** This is a long chain monounsaturated omega-9 fatty acid with one double bond in its carbon chain (figure 18). . Oleic acid has been known to be beneficial for the heart's health. Its m/z ratio was at 57.10, with a relative abundance of 38.53 % (figure1).
5. **Octadecanoic Acid (C18H36O2)**: Also known as stearic acid is a 18-carbon chain saturated fatty acid (figure 19). It is used as an emulsifying and thickening agent in cosmetics and personal care, also as a stabiliser and food additive. Its m/z ratio was at 73.10, with a relative abundance of 73.13 % (figure 4).
6. **2,6,10,14,19-Eicosapentaenoic acid ,2,6,11,15,19-pentamethyl-, ethyl ester (C22H34O2):** This is an ethyl ester of the omega-3 fatty acid (figure 20). It has been known to be used in medicine to treat dyslipidemia and hypertriglyceridemia. Its m/z ratio was at 55.00, with a relative abundance of 43.20 % (figure 6).
7. **pis.,.psi.Carotene,7,7,8,8,11,11',12,12',15,15'-decahydro- (C40H66):** This is a chemical compound with a CAS number of 502-62-5 (Table 2). Also known as Lycopaoctaene, an acyclic carotene that is lycopene in which the double bonds at positions C-7, C-11, C-15, C-7', C-11' and C-15' have been reduced to single bonds (figure 21). Its m/z ratio was at 67.10, with a relative abundance of 30.67 % (figure 6).
8. **Squalene (C30H50)**:This is a triterpene, a 30-carbon isoprenoid that is used in the production of commercial cosmetics and vaccines. Its m/z ratio was at 95.10, with a relative abundance of 26.54 % (figure 6).
9. **Ergost-22-en-3-ol, (3.beta.,5.alpha.,22E,24R)-(C28H48O):** This is a spongesterol with 28 carbon atoms, 48 hydrogen atoms and 1 oxygen atom (figure 23). It is known to have an estrogen effect. Its m/z ratio was at 69.10, with a relative abundance of 94.61 % (figure 7).
10. **1H-Benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetram ethyl-, cis- (C15H26O)**: Also know as Widdrol (figure 24) is an organic compound with antimicrobial and antioxidant property. It is mainly used in the cosmetics industry in skin and hair care products. Its m/z ratio was at 151.05, with a relative abundance of 61.05 % (figure 7).
11. **1-Cyclohexene-1-acetaldehyde,2,6,6-trimethyl-(C11H18O):** Used mainly in Research and Development, to prepare certain reagents. It is known to cause irritation to the eyes and skin. Its m/z ratio was at 73.10, with a relative abundance of 73.13 % (figure 7).
12. **4-Hydroxybenzoxazolone (C7H5NO3):** This is an organic bioactive compound used in traditional medicine with anti-inflammatory and analgesic properties. Its m/z ratio was at 151.10, with a relative abundance of 73.20 % (figure 8).
13. **gamma.-Tocopherol (C28H48O2):** This is a tocopherol in which the chroman-6-ol core is substituted by methyl groups at positions 7 and 8 (figure 27). It has the ability to inhibit or retard oxidation reactions. Its m/z ratio was at 57.10, with a relative abundance of 55.33 % (figure 8).
14. **Ricinoleic acid (C18H34O3):** This is used for its laxative properties, anti-inflammatory effects, and applications in cosmetics and industrial products (figure 28). Its m/z ratio was at 67.10, with a relative abundance of 39.46 % (figure 10).
15. **6-Octadecenoic acid, (Z)- (C18H34O2):** often used as a raw material for lubricants and plays a role in lubricating and reducing friction in the manufacture of machinery, metals and plastic products. In addition, it can also be used in the preparation of cosmetics and household chemicals (figure29). Its m/z ratio was at 81.10, with a relative abundance of 32.20 % (figure 10).
16. **2-Methyl-Z,Z-3,13-octadecadienol (C19H36O2):** This is a long chain unsaturated alcohol, with a unique configuration, characterised by the two double bonds at the 3rd and 13th carbon positions (figure 30). Its m/z ratio was at 98.10, with a relative abundance of 59.38 % (figure 11).
17. **22-Stigmasten-3-one (C29H48O)**: This is one of the chemical compounds with known spectrum, discovered by

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the GC-MS (figure 31). Its m/z ratio was at 67.10, with a relative abundance of 40.51 % (figure 11).

18. **1,19-Eicosadiene (C20H38):** This is one of the chemical compounds with known spectrum, discovered by the GC-MS (figure 32). Its m/z ratio was at 69.10, with a relative abundance of 36.95 % (figure 11).
19. **Stigmastan-6,22-dien, 3,5-dedihydro- (C29H48):** This is one of the chemical compounds with known spectrum, discovered by the GC-MS (figure 31). Its m/z ratio was at 81.10, with a relative abundance of 41.35 % (figure 12).
20. **Stigmasterol (C29H48O):** This is known to inhibit the absorption of cholesterol from the diet (figure31). Its m/z ratio was at 83.10, with a relative abundance of 36.34 % (figure 12).
21. **10-Undecenehydroxamic acid (C11H21NO2):** This is structurally derived from undecanoic acid ($C_{11}H_{22}O_2$), where the hydroxyl group of the carboxylic acid is replaced by a hydroxamate (-NHOH) moiety . This modification enhances its metal-chelating capacity, particularly for iron ions, which is critical to its antimicrobial activity. Its m/z ratio was at 67.10, with a relative abundance of 56.97 % (figure 13).
22. **Bicyclo[10.1.0]tridec-1-ene (C13H22):** This is known to inhibit the absorption of cholesterol from the diet (figure 33. Its m/z ratio was at 81.10, with a relative abundance of 50.11 % (figure 13).
23. **.delta.-Tocopherol (C27H46O2):** Delta-tocopherol is a tocopherol in which the chroman-6-ol core is substituted by a methyl group at position 8. Its m/z ratio was at 137.05, with a relative abundance of 77.36 % (figure 14).

Chemical Compounds of Unknown Spectrum

About 13 chemical compounds on an unknown spectrum were also discovered. They were represented at 13.473min, 14.863mins, 15.024mins, 15.212mins, 15.745mins, 15.796mins, 15.865mins, 15.893mins, 16.437mins, 18.388mins, 18.537mins, 18.886mins, and 20.025mins the GC-MS analyzer. Figure 2 -figure 14 shows the graphical representation of all chemical compounds in the unknown spectrum.

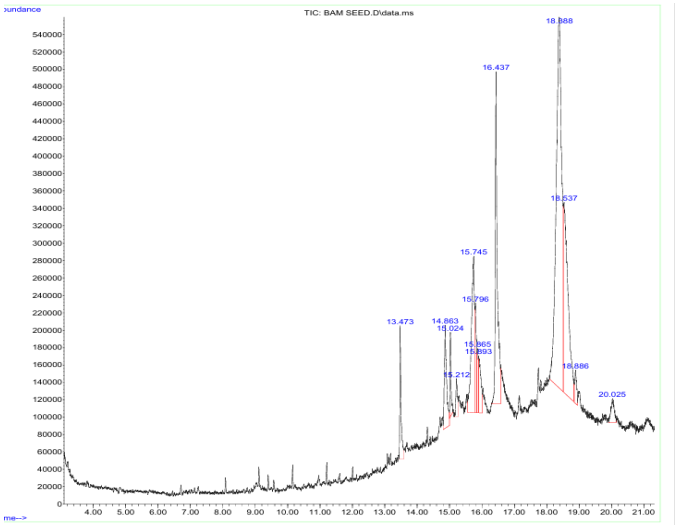

Figure 1: The relative abundance for known chemical compounds of Vigna subterranea

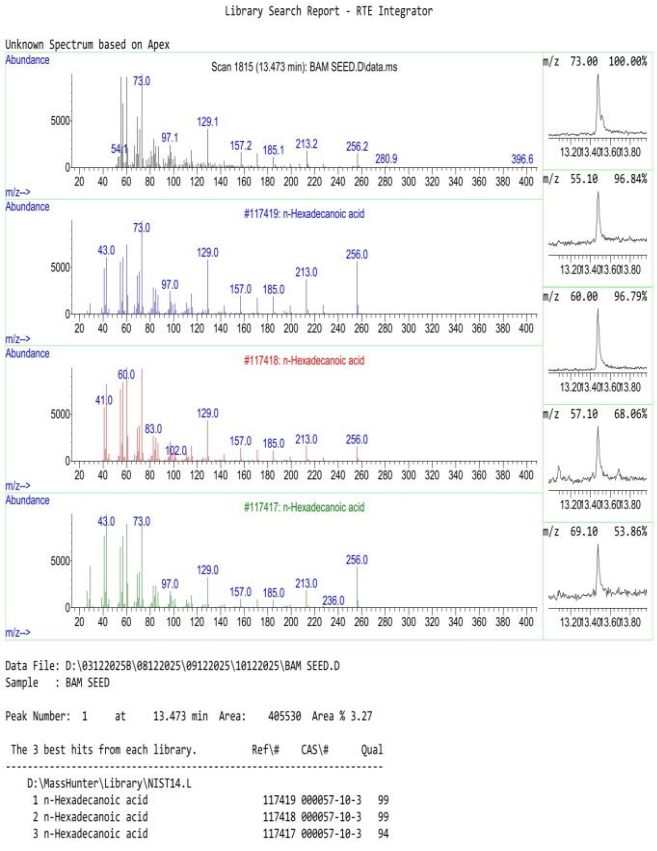

Figure 2: The relative abundance for unknown spectrum based on Apex alongside n-Hexadecanoic Acid at RT 13.473

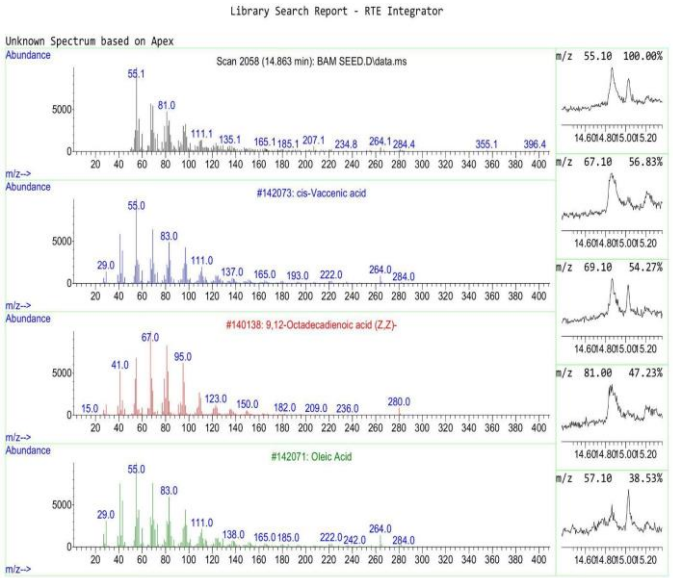

Figure 3: The relative abundance for unknown spectrum based on Apex alongside cis-Vacennic Acid, Octadecadienoic Acid, Oleic Acid at RT 14.863

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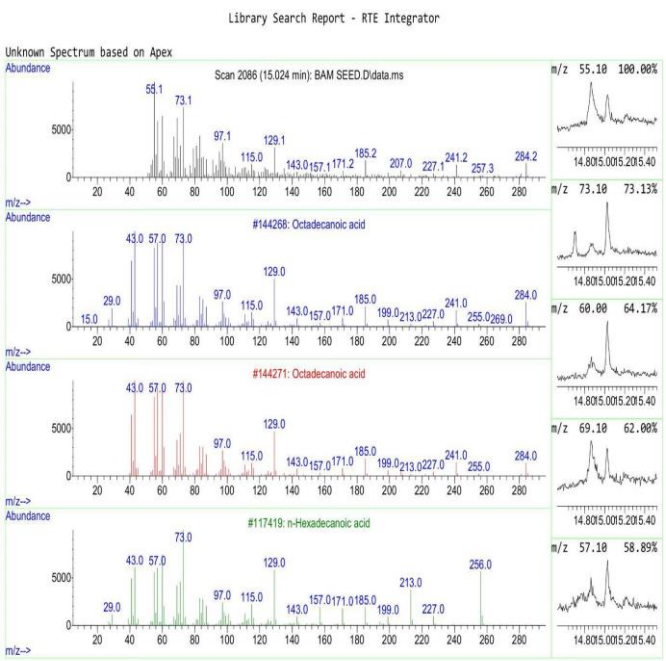

Figure 4: The relative abundance for unknown spectrum based on Apex alongside Octadecadienoic Acid, Hexadecanoic Acid at RT 15.024

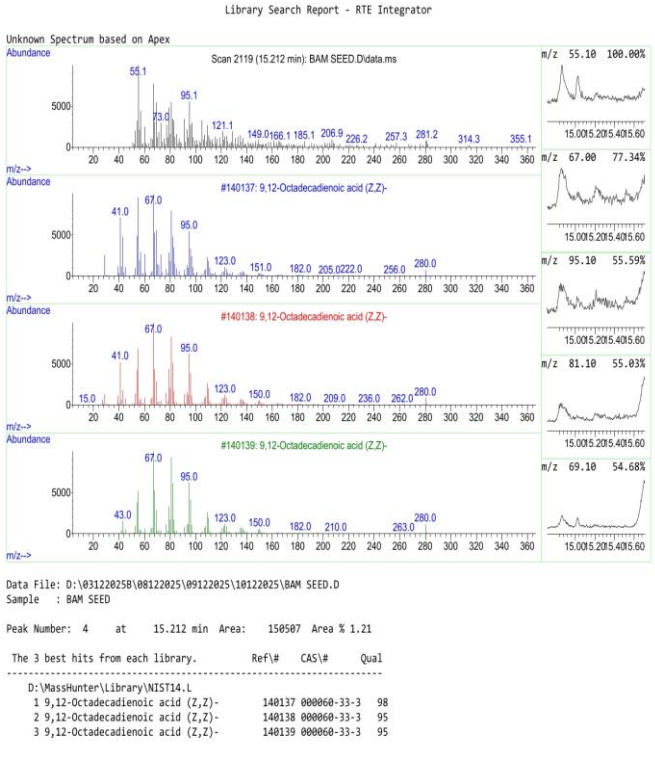

Figure 5: The relative abundance for unknown spectrum based on Apex alongside Octadecadienoic Acid at RT 15.212

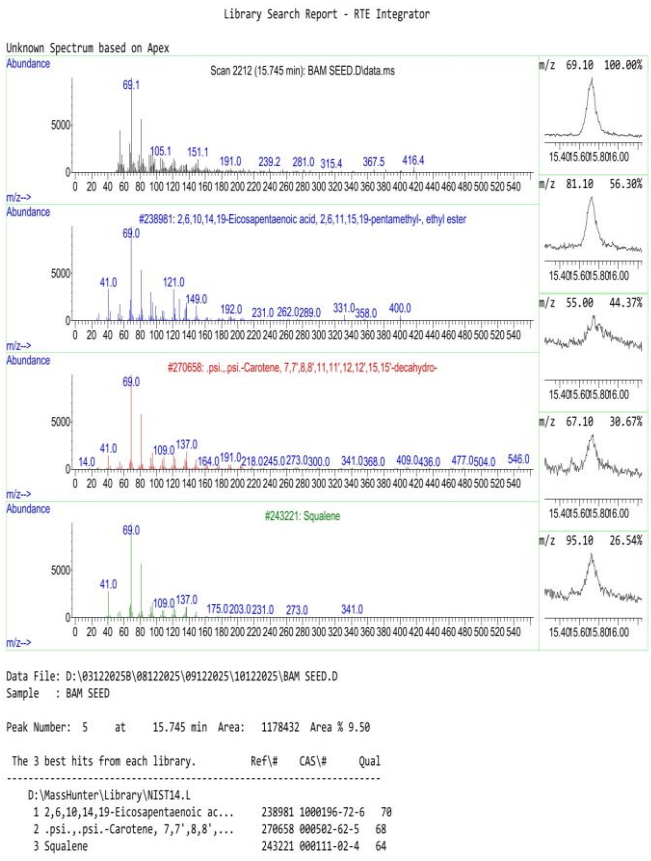

Figure 6: The relative abundance for unknown spectrum based on Apex alongside Eicosapentaenoic Acid, Carotene, decarhydro-squalene, at RT 15.745

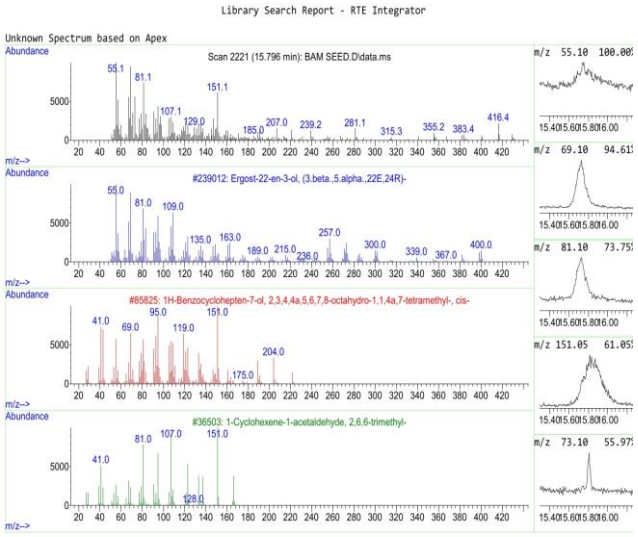

Figure 7: The relative abundance for unknown spectrum based on Apex alongside other known compounds with known spectrum at RT 15.796

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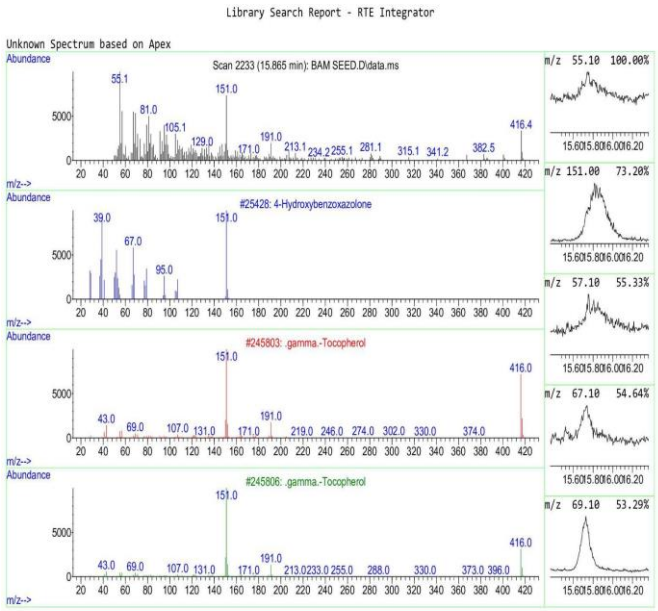

Figure 8: The relative abundance for unknown spectrum based on Apex alongside hydroxybenzoxazolone and .gamma.-Tocopherol at RT 15.865

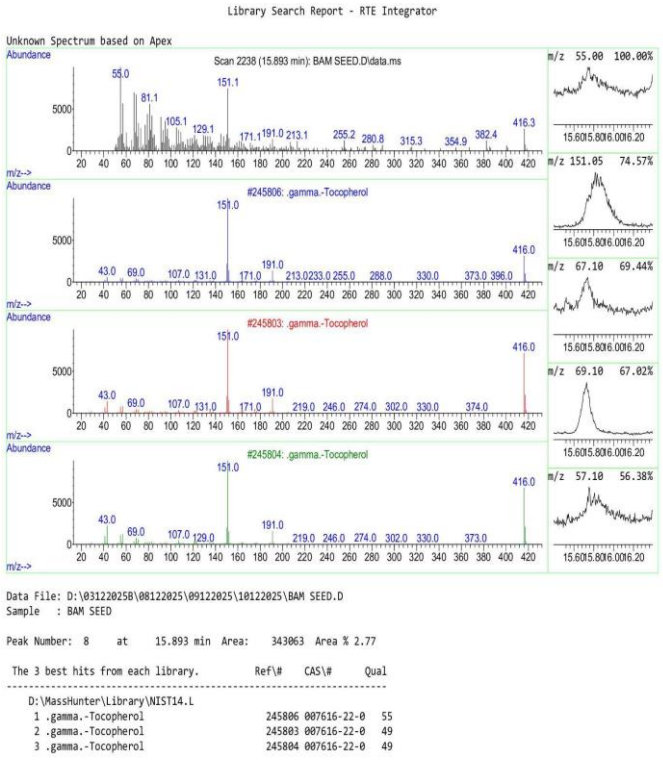

Figure 9: The relative abundance for unknown spectrum based on Apex alongside .gamma.-Tocopherol at RT 15.893

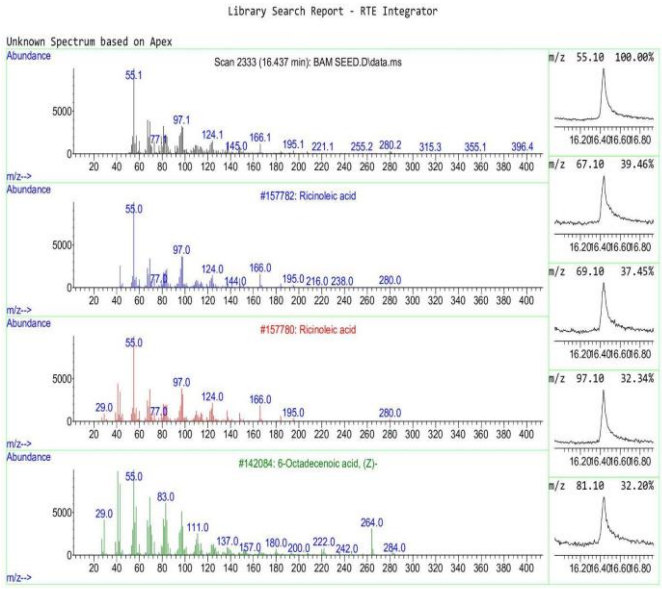

Figure 10: The relative abundance for unknown spectrum based on Apex alongside Ricinoleic Acid and 6-Ocatadecenoic Acidt RT 16.437

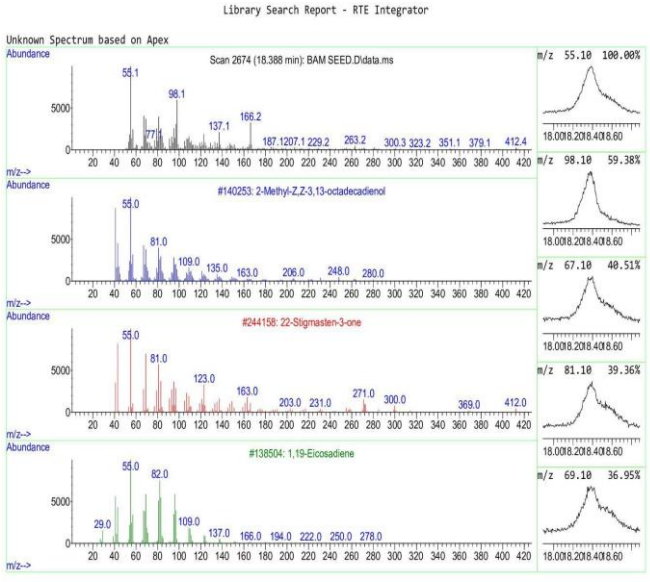

Figure 11: The relative abundance for unknown spectrum based on Apex alongside an Octadecadienol acid, a Stigmasten and an Eicosadiene at RT 18.388

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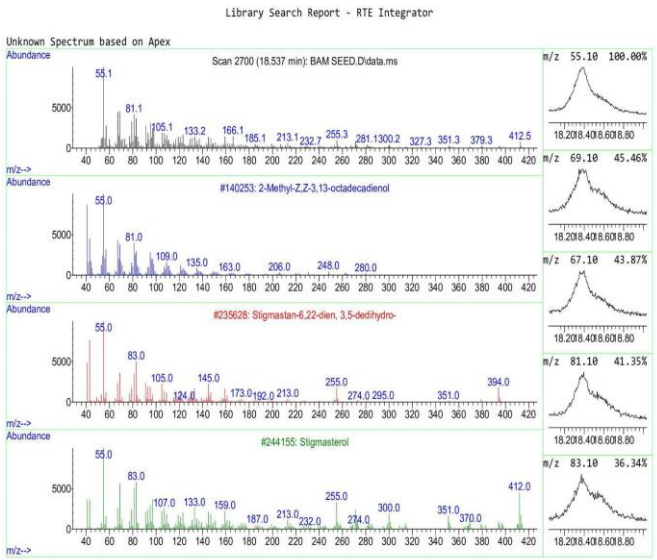

Figure 12: The relative abundance for unknown spectrum based on Apex alongside and Octadecadieno, a stigmastan, and a stigmasterol at RT 18.537

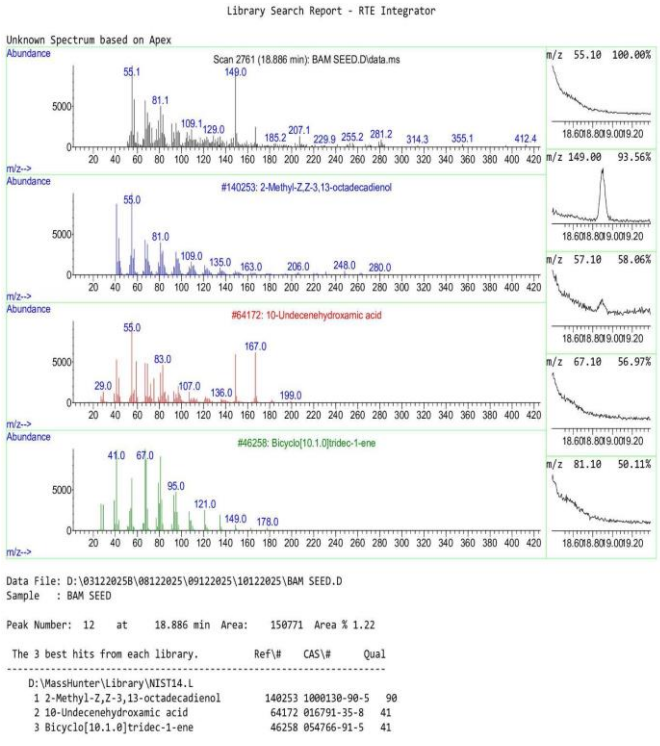

Figure 13: The relative abundance for unknown spectrum based on Apex alongside an Octadecadienol, an Undecenehydroxamic acid, and a tride-1-ene at RT 18.886

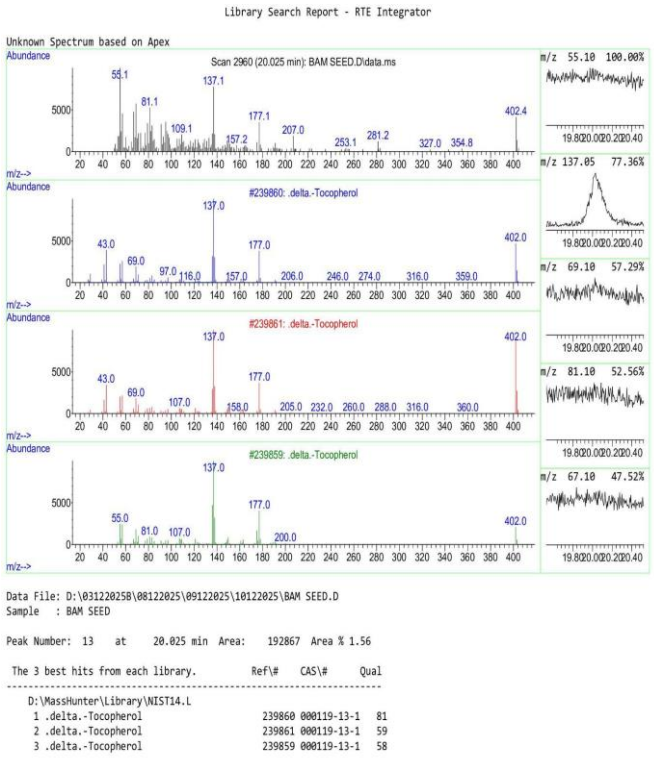

Figure 14: The relative abundance for unknown spectrum based on Apex alongside .delta..-Tocopherol at RT 20.025

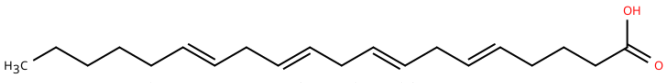

Figure 15: n-Hexadecanoic Acid ($C_{16}H_{32}O_2$)

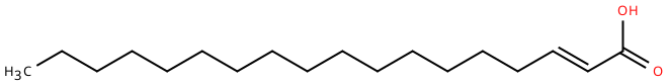

Figure 16: cis-Vaccenic Acid ($C_{18}H_{34}O_2$)

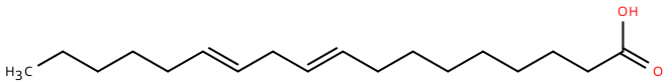

Figure 17: 9,12-Octadecadienoic acid (Z,Z) ($C_{18}H_{32}O_2$)

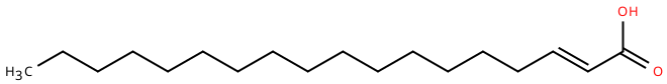

Figure 18: Oleic Acid ($C_{18}H_{34}O_2$)

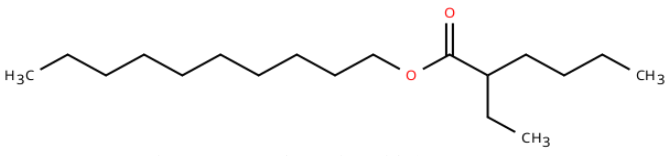

Figure 19: Octadecanoic Acid ($C_{18}H_{36}O_2$)

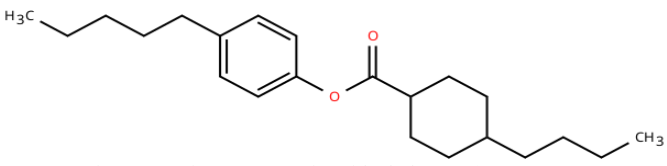

Figure 20: Eicosapentaenoic acid,ethyl ester ($C_{22}H_{34}O_2$)

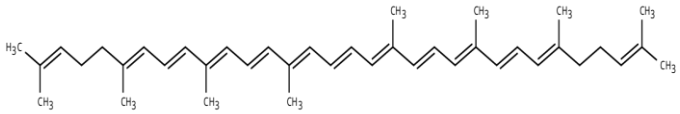

Figure 21: psi,psi-Carotene, 7,7,8,8,11,11,12,12,15,15-decahydro ($C_{40}H_{66}$)

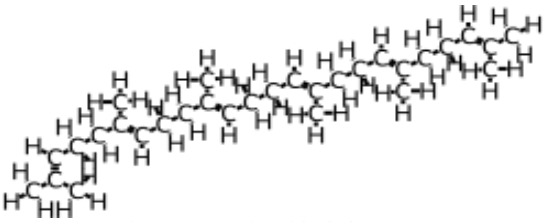

Figure 22: Eicosapentaenoic acid,ethyl ester ($C_{22}H_{34}O_2$)

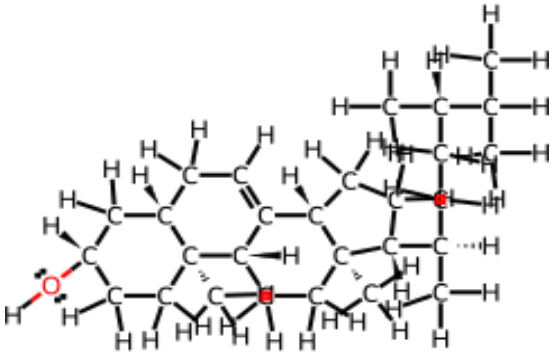

Figure 23: Ergost-22-en-3-ol, (3beta,5alpha,22E,24R)- ($C_{28}H_{48}O$)

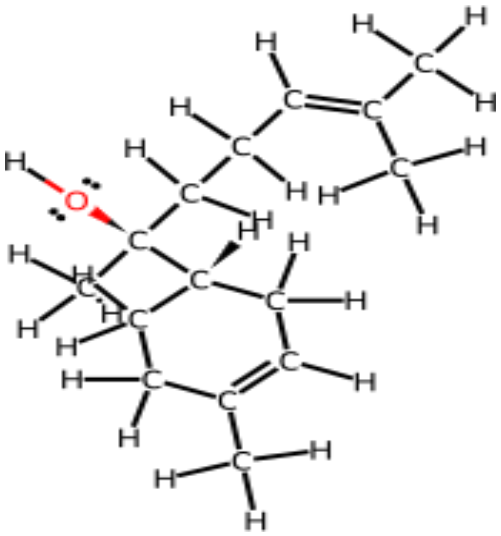

Figure 24: Widdrol ($C_{15}H_{26}O$)

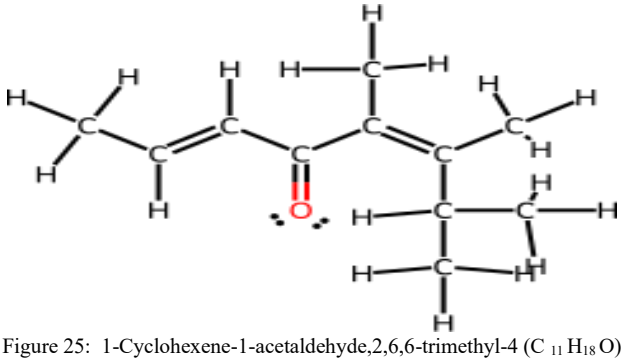

Figure 25: 1-Cyclohexene-1-acetaldehyde,2,6,6-trimethyl-4 ($C_{11}H_{18}O$)

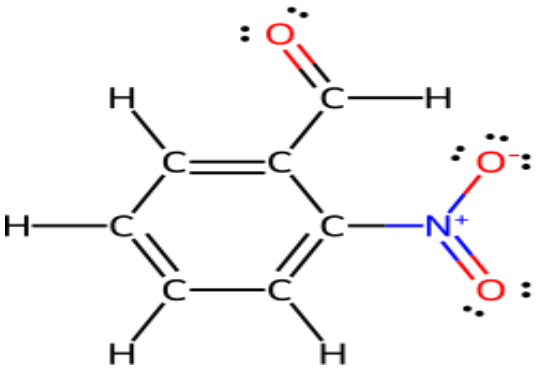

Figure 26: 4-Hydroxybenzoxazolone ($C_7H_5NO_3$)

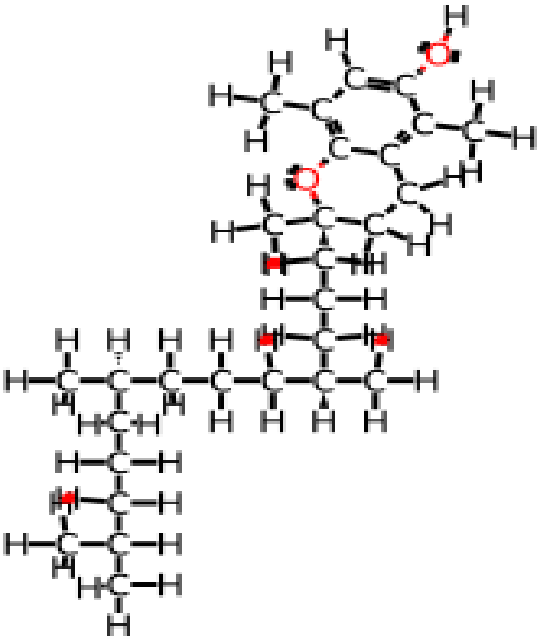

Figure 27: .gamma.-Tocopherol ($C_{28}H_{48}O_2$)

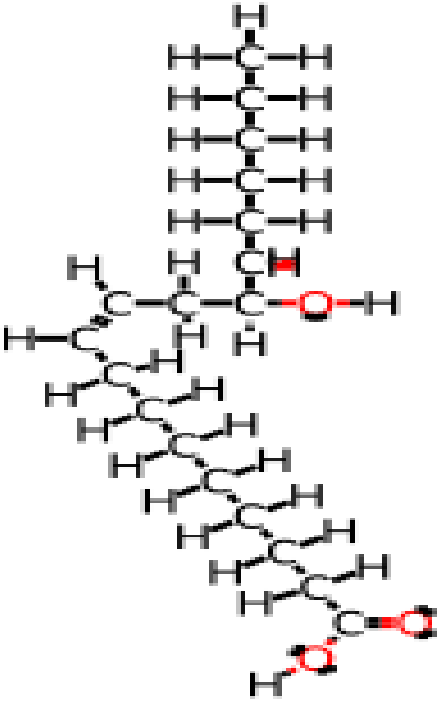

Figure 28: Ricinoleic acid ($C_{18}H_{34}O_3$)

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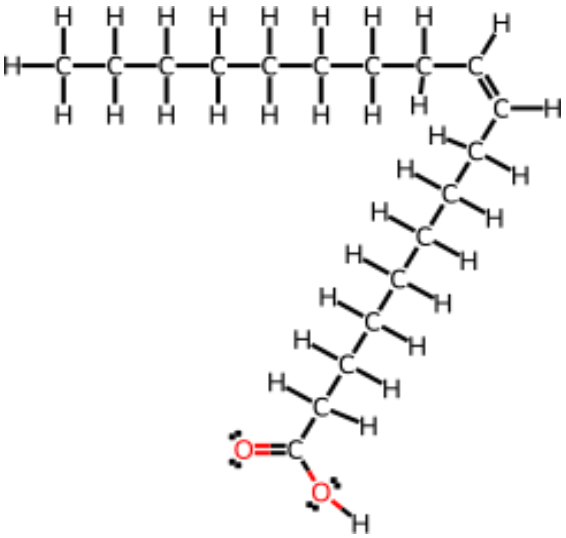

Figure 29: 6-Octadecenoic acid, (Z)- ($C_{18}H_{34}O_2$)

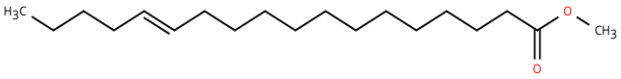

Figure 30: 2-Methyl-Z,Z-3,13-octadecadienol ($C_{19}H_{36}O_2$)

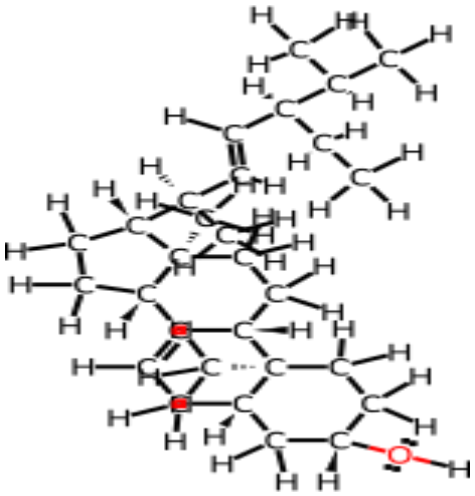

Figure 31: 22-Stigmasten-3-one , and Stigmasterol ($C_{29}H_{48}O$)

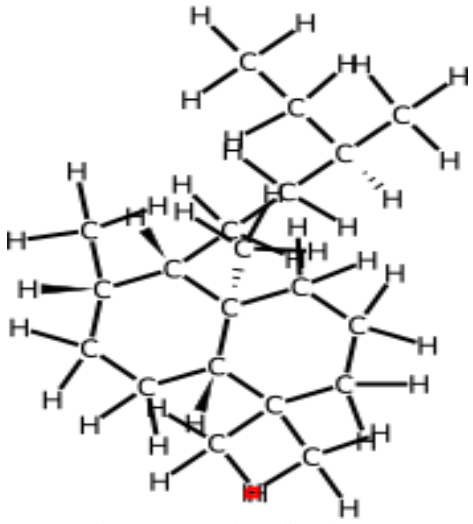

Figure 32: 1,19-Eicosadiene($C_{20}H_{38}$)

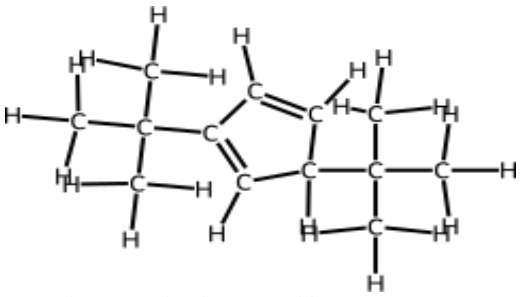

Figure 33: Bicyclo[10.1.0]tridec-1-ene ($C_{13}H_{22}$)

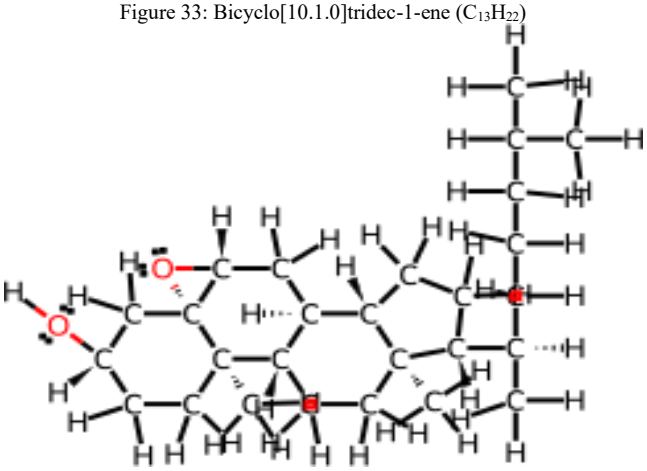

Figure 34: .delta.-Tocopherol ($C_{27}H_{46}O_2$)

Table 1. Showing results of GC-MS analysis as compared against Mass Hunter Library

pk #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	13.473	1810	1815	1835	rBV2	1533 82	4055 30	8.14%	3.270%
2	14.863	2047	2058	2081	rVV7	1177 43	5648 26	11.33 %	4.554%
3	15.024	2081	2086	2099	rVV4	9305 7	2058 52	4.13%	1.660%
4	15.212	2108	2119	2128	rBV4	4357 9	1505 07	3.02%	1.214%
5	15.745	2180	2212	2218	rVV5	1794 65	1178 432	23.65 %	9.502%
6	15.796	2218	2221	2230	rVV6	1259 60	4137 29	8.30%	3.336%
7	15.865	2230	2233	2237	rVV6	7370 2	1655 72	3.32%	1.335%
8	15.893	2237	2238	2259	rVV6	6540 0	3430 63	6.88%	2.766%
9	16.437	2309	2333	2358	rBV2	3815 61	1664 172	33.39 %	13.418%
10	18.388	2623	2674	2695	rBV8	4206 07	4983 334	100.0 0%	40.181%
11	18.537	2698	2700	2751	rVV8	2231 96	1983 674	39.81 %	15.994%
12	18.886	2755	2761	2773	rVV5	3998 7	1507 71	3.03%	1.216%
13	20.025	2940	2960	2981	rBV5	2740 3	1928 67	3.87%	1.555%

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Table 2. Showing results of GC-MS analysis as compared against Mass Hunter Library2

PK#	RT	Area%	Library /ID	Ref#	CAS#	QUAL
1	13.473	3.27	n-Hexadecanoic acid	117419	1957-10-3	99
			n-Hexadecanoic acid	117418	1957-10-3	99
			n-Hexadecanoic acid	117417	1957-10-3	94
2	14.863	4.55	cis-Vaccenic acid	142073	000506-17-2	97
			9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3 96	96
			Oleic Acid	142071	000112-80-1	96
3	15.024	1.66	Octadecanoic acid	144268	1957-11-4	96
			Octadecanoic acid	144271	1957-11-4	95
			n-Hexadecanoic acid	117419	1957-10-3	59
4	15.212	1.21	9,12-Octadecadienoic acid (Z,Z)-	140137	000060-33-3	98
			9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3	95
			9,12-Octadecadienoic acid (Z,Z)-	140139	000060-33-3	95
5	15.745	9.5	2,6,10,14,19-Eicosapentaenoic acid,	238981	1000196-72-6	70
			2,6,11,15,19-pentamethyl-, ethyl ester,pis.,.psi.Carotene,77"8,"°,11,11',12,12',15,15'-decahydro-Squalene	270658	000502-62-5	68
				243221	0111-02-04	64
6	15.796	3.34	Ergost-22-en-3-ol, (3.beta., 5.alpha.,22E,24R)-1H-Benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-1-Cyclohexene-1-acetaldehyde,2,6,6-trimethyl-	239012	036422-25-0	52
				85825	006892-80-4	25
				36503	000472-66-2	25
7	15.865	1.34	4-Hydroxybenzoxazolone	25428	028955-70-6	52
			gamma.-Tocopherol	245803	007616-22-0	42
			gamma.-Tocopherol	245806	007616-22-0	38
8	15.893	2.77	gamma.-Tocopherol	245806	007616-22-0	55
			gamma.-Tocopherol	245803	007616-22-0	49
			gamma.-Tocopherol	245804	007616-22-0	49
9	16.437	13.42	Ricinoleic acid	157782	000141-22-0	74
			Ricinoleic acid	157780	000141-22-0	64
			6-Octadecenoic acid, (Z)-	142084	000593-39-5	62
10	18.388	40.18	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90-5	56
			22-Stigmasten-3-one	244158	004736-95-2	52
			1,19-Eicosadiene	138504	014811-95-1	45
11	18.537	15.990	2-Methyl-Z,Z-3,13-octadeca	140253	1000130-90-5	92

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			dienol			
			Stigmastan-6,22-dien, 3,5-dedihydr o-Stigmasterol	235628244155	107304-12-1 000083-48-7	90. 78
12	18.886	1.220	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90-5	90
			10-Undecenehydroxamic acid	64172	016791-35-8	41
			Bicyclo[10.1.0]tridec-1-ene	46258	054766-91-5	41
13	20.025	1.560				
			.delta.-Tocopherol	239860	000119-13-1	81
			.delta.-Tocopherol	239861	000119-13-1	59
			.delta.-Tocopherol	239859	000119-13-1	58

Equation/Formula
The below formula was used to calculate the relative abundance

%RA = Relative Abundance (1)

$$\%RA = \left(\frac{PKA}{PKAN}\right) X100\% \quad (2)$$

Where PKA = Peak Area of Compound
PKAN = Peak Area of all Identified Compounds (3)

6. Conclusion and Future Scope

The outcome of the research showed that apart from the 23 chemical compounds of known spectrum that were identified from the aqueous seed extracts of *Vigna subterranea*, using the GC-MS analyzer, 13 other chemical compounds of unknown chemical compounds were discovered. The impact of this would be the possibility of newly discovered chemical compounds, their functions, possible uses and benefits in the health industry, and their addition to existing libraries, The result of analysis shows that the seeds of *Vigna subterranea* consist of steroids, tocopherol, saturated and unsaturated fatty acids. The known compounds formed about 40% of the total chemical compounds identified through GC-MS, and were identified between 13 to 20 minutes during separation.

The future scope would involve more indepth analysis using other polar solvents like methanol, dichloromethane, and ethyl acetate as extraction solvents Other methods like fluorometry can also be applied. This would help in further identifying other chemical compounds to determine their structure and possible classification. If the unknowns are completely new compounds, this would result in a breakthrough and addition to existing libraries following standard protocols and processes.
The incidental discovery for improvement of eye sight when used by locals would also be further reviewed to determine the possibility of efficacy against eye defects and what type of eye defect is being addressed. This would require pre-clinical trials using lab mice in a controlled environment.

Author's Statement

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Authors' Contributions - Yemisi Otasanya reviewed the literature, conceived the study, developed the protocol, conducted the research, and handled data analysis and documentation.

Conflict of Interest - The author declares that there are no financial, personal, or other relationships that could have influenced, or be perceived to have influenced, the conduct or outcomes of this research.

Data Availability - Data associated with this research was obtained during the analysis of the aqueous seed extracts of *Vigna subterranea*, using Agilent 8860 Gas Chromatography. Data can be accessed through the author"s ORCID
Study Limitations: The only library used for comparison was the NIST library

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