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Original Research Article

GC-MS Analysis of Five Commonly Used Herbal Formulations Sold in Anambra State, Southeast, Nigeria

Chinedu J. Ikem[1]*, Angus N. Oli[2], Ifunanya Nwaigwe[3], Cyril Ogbiko[4], Charles O. Esimone[2]

[1]*Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.*
[2]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[3]*Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmacy, Madonna University, Elele, River State, Nigeria*
[4]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria*

ABSRTACT

Plant extracts have been known to possess multiple bioactive compounds with therapeutic uses. Gas chromatography-mass spectrometry (GC-MS) was used in the current study to characterize the bioactive constituents of five (5) commercially available herbal formulations. The National Institute of Standard Technology Database was used to analyze the mass spectrum of the five commonly available herbal formulations sold in Anambra State, Southeast, Nigeria. The GC-MS analysis showed the presence of thirty-five (35) bioactive compounds. The identified compounds were listed in the following order; Compound name, peak area (%), molecular weight, molecular formula, and biological activities. The bioactive compounds present include; Quinolone, Oleic acid, Hexadecanoic acid, Phenolic, Dodecanoic acid, and, phthalic acid which are believed to possess antiplasmodial, antimicrobial, antiviral, anticancer, antioxidant, and antifungal activities. This study confirmed that the five herbal formulations have rich bioactive compounds that could benefit mankind, although some of the synthetic compounds isolated are of major health concern.

Keywords: Herbal formulations, Bioactive constituents, Antioxidant, Antiplasmodial, Antimicrobial, Anticancer

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Introduction

For many years natural products have been screened/used by individuals as an alternative treatment for various ailments.[1] This is due to the presence of active substances in plants, which have been touted as having healing properties.[2] In recent times, traditional medicine has played a leading role in the healthcare system, especially in developing countries.[3,4] This practice of traditional medicine in developing nations is generally accepted owing to the expensive price of synthetic drugs and the accessibility of the health care system.[3,4] Aside from the fear of its side effects, those in developing countries are unable to afford their prescription costs continuously.

The use of herbal extracts or formulations that are less expensive but are believed to be more effective with few to no side effects has been greatly encouraged by this condition. In both developing and developed countries, polyherbal formulations are becoming more and more well-liked and accepted as a treatment option.[5]

It is estimated by the World Health Organization (WHO) that 80% of people in developing nations treat common illnesses with herbal remedies. [6,7]

*Corresponding author. E mail: ikemcj@dufuhs.edu.ng, ikemchiedu@gmail.com;
Tel: +2348062996055

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Although plant medicines are generally believed to be safe, some are inherently toxic and dangerous, while others may be toxic at high doses or have potential side effects when used for an extended period. When using herbal formulations, there is a chance of unanticipated side effects, just like with other medications.[5]

The goal of identifying bioactive compounds in plants is to separate irrelevant materials from the active fraction that is therapeutically desired. The purpose of this study was to use gas chromatography and mass spectrometry (GC-MS) to identify the bioactive components in the five herbal formulations.

Materials and Methods

Herbal Formulation

Between March and April of 2021, the five herbal formulations were purchased from drug markets located in Anambra State, in the southeast region of Nigeria. They include ruzu bitters, African Iba herbal mixtures, yoyo cleanser bitters, blood purifiers, and deep root herbal mixtures (Table 1).

Twenty milliliters (20 mL) of each herbal preparation were measured out into a Labconco fast-freeze flask, transferred, and frozen at -20 ^{0}C. The herbal formulations were removed and placed in a freeze dryer (Labconco FreeZone 6, USA) set to -40 ^{0}C and 12 mbar of reduced pressure in order to ensure full dryness.

Gas Chromatography-Mass Spectroscopy Analysis

GC-MS analysis of the herbal formulation was carried out as described by Nwobodo *et al.* (2022).[8] An Agilent 7820A gas chromatograph (Agilent Technologies, USA) connected to an Agilent 5975C inert mass selective detector (MSD) with a triple-axis detector and operating in electron impact (EI) mode at an ionization energy of 70 eV was used to conduct the analysis. For the separation, an HP-5 capillary column (30 m × 250 µm diameter × 0.25 µm film thickness) coated with 5% phenyl

methyl siloxane. At an injection temperature of 300 °C, the sample (1 µL, diluted 1:100 in dichloromethane) was injected in splitless mode. At 0.75 minutes, the purge flow to the split vent was 15 mL/min, for a total flow of 16.654 mL/min. Helium was employed as the carrier gas, with an average velocity of 44.22 cm/sec and an initial nominal pressure of 1.4902 psi. The flow rate of helium was 1 mL/min. The oven was set to start at 50°C for one minute and ramp up to 300°C for ten minutes at a rate of 3°C per minute. With a hold time of 5°C per minute, the run time was 43 minutes. Based on the peak area created in the chromatogram, the percentage of the chemical compounds present in the extract was determined. By comparing the mass spectra of the extract's constituents with those in the National Institute database and using their GC retention time (RT), the components were identified.

Table 1: Showing various herbal formulations, their constituents, Nafdac number

Herbal formulations	Constituents/composition	NAFDAC number
Ruzu Bitters	*Curculigo pilosa, Uvaria chamae, Citrullus colocynthis*.	A7-1102 L
African Iba	*Kigella africana, Nauclea latifolia linn*, water	A7-0476 L
Yoyo Cleanser Bitters	*Aloe vera, Acinos arventis, Citrus aurantifolia, Chenopodium murale, Cinnamomum & Other natural ingrdients.*	A7-1055 L
Blood Purifier	*Aloe barbadenis, Xylopia aethiopica, Gongroneria latifolium, Dichrostschys cinerea*, Water	A7-1390 L
Deep Root Herbal Mixture	*Cymbopogon, citratus, Carica papaya, Mangifera indica, Moringa oleifera, Citrus limonia, Psidium guajava, Allium sativa, Zingiber officinale*, water	A7-0912 L

Results and Discussion

GC-MS analysis

Natural products have been used as an alternative treatment for a variety of ailments by consumers.[1] This is because plants contain bioactive compounds that have been linked to therapeutic effects.[2] Traditional medicine has taken the lead in the healthcare system in recent years, particularly in developing countries. As a result of the high cost of synthetic drugs and the accessibility of the healthcare system, the practice of traditional medicine in developing countries is widely accepted.[1,4] One of the most precise approaches for identifying active metabolites in herbal formulation/crude extract is GC-MS.

The five herbal formulations underwent GC-MS analysis, revealing the presence of 35 significant compounds with a significant percentage composition and quality match ranging from 70 to 99%. In Tables 2, 3, 4, 5, and 6, the identified compounds are listed by compound name, peak area (%), molecular weight, molecular formula, and reported biological activities. The Peak area is directly proportional to the compound concentration in the solvent.

The five herbal formulation revealed presence of 35 major bioactive compounds, they include; Methenamine (26.29), phenol(8.11), Dodecanoic acid(7.11), Decanoic acid(1.79), 3-methylcyclopetane1-2-dione (1.45), Octanoic acid (1.34), 4 (1H)-pyridone (0.50), 1,2-Benzeenediol, 3-methoxy- (0.72), Pentadecanoic acid, 14-methyl-, methyl ester (0.71), Hexadecanoic acid, ethyl ester (0.54), 9-Octadecenoic acid, ethyl ester (1.10), Oleic acid (0.54), Cetene (0.67), Triethyl citrate (0.56), Octadecene (1.35), Phthalic acid (14.57), Behenic alcohol (1.08), Bis (2- ethyl hexyl) phthalate (58.31), Docosene (0.93), Quinolone, 1,2,3,4- tetrahydro-1- (7.67), 2-pyrrolidinone, 1-methy-l (0.65), Triacetin (0.35), 1,2,3-benzenetriol (14.41), Acetophenone, 4- hydroxyl (0.52), Vanillic acid (0.19), Benzoic acid, 3,4-dihydroxyl (0.91), Phthalic acid, isobutyl octyl este (12.47), n-Hexadecanoic acid (2.52), Di-n-octyl phthalate (25.81), Trans-3-undecene-1,5-diyne (0.69), 1-Docosene (0.80), Dimethyl sulfoxide (1.22), Phthalic anhydride (3.69), 1 19-eicosadiene (3.03), 9,12-Octadecadienoic acid (28.39), 3H-Pyrazol-3-one, 4-benzoyl-2,4-di (6.12), Dibutyl phthalate (2.16), and Eicosane (0.93).

These bioactive compounds were narrowed down to a selected few that exhibit antiplasmodial, antimicrobial, antioxidant, cytotoxic, and phytochemical activities.

Some major bioactive compounds isolated from these herbal formulations are believed to be an important antioxidant career; Hexadecanoic acid, ethyl ester, 9-octadecenoic acid, ethyl ester, Oleic acid, and others. Also, Phthalic acid is known to possess antimicrobial potential while oleic acid is generally known to exhibit anticancer activity.[19-20,29,35]

Synthetic compounds were also isolated from these herbal formulations; DMSO, Methenamine, and others, this could be the issue encountered during the handling and processing of the various plant extracts

Essential oils accounted for the majority of the active compounds identified by GC-MS analysis in this study, which may have contributed to the antioxidant and antiplasmodial properties observed, as well as the antimicrobial activity observed against susceptible strains of pathogenic bacteria and fungi.[31]

The bioactive substances found in this study were also found in earlier studies using single plant extracts and were found to have various biological characteristics.[31-39]

The identified bioactive compounds were verified by comparing each herbal formulation's computed linear retention index to corresponding indices found in published works and online resources. Deep root and Ruzu bitter herbal formulations were observed to produce the greatest number of important bioactive compounds among the five herbal formulations studied. Twelve important bioactive compounds were identified from both formulations. The compounds identified include: Methenamine (26.29), Phenol (8.11), Dodecanoic acid (7.11), Decanoic acid (1.79), Hexadecanoic acid, ethyl ester (0.54), 9-Octadecenoic acid, ethyl ester (1.10), 1,2,3-benzenetriol (14.41), Phthalic acid (14.57), Bis (2- ethyl hexyl) phthalate (58.31), Docosene (0.93), and Oleic acid (0.54).

African iba herbal showed the presence of the bioactive compound Quinolone which is believed to be responsible for antiplasmodial activity.[27]

Deep root herbal mixture had promising results due to the essential oil and also phytochemical properties of the mixture. Bioactive compounds identified including; phenol, Methenamine, Hexadecanoic acid, ethyl ester, and oleic acid are believed to be responsible for their antioxidant, phenolic, antibacterial antiviral, and antiplasmodial activities.[9,12,20]

There had not been any previously documented research on the GC-MC analysis of these five herbal formulations but most of the extracts used in the formulation of its mixture have been proven to contain these valuable bioactive compounds including *Magnifera indica*, *Uvaria chamae*, *Cymbopogon citratus*.[41] The most common bioactive compounds identified from these formulations include; Hexadecanoic acid, n-hexadecanoic acid, 9,12-octadecanoic acid, and Phthalic acid, this is in line with the earlier research conducted by Beulah *et al*. (2018), GC-MS determination of bioactive compounds of *Dentrophthoe falcate* (L.F) Etting sh; an Epiphytic plant.[29]

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Table 2: Compounds identified from Deep root herbal formulation

No	Compound name	Peak area (%)	M.W	M.F	Compound nature and Biological activities
1	Phenol	8.11	94.4	C_6H_5OH	Phenolic compound, antiviral, antibacterial and antifungal activities [9]
2	3-methylcyclopentane-1 2-dione	1.45	112.13	$C_6H_8O_2$	No report found
3	Octanoic acid	1.34	I44	$C_8H_{16}O_2$	Antimicrobial activities [10]
4	4 (1H) –pyridone	0.50	95	C_5H_5	Antimalarial activities [11]
5	Methenamine	26.29	140.18	$C_6H_{12}N_4$	Antibacterial activities [12]
6.	1,2- Benzeenediol, 3-methoxy-	0.72	140.13	$C_7H_8O_3$	Antimicrobial activities [13]
7	Decanoic acid, methyl ester	1.79	186	$C_{11}H_{22}O_2$	Antimicrobial activities [14]
8	Pentadecanoic acid, 14-methyl-, methyl ester	0.71	256	$C_{16}H_{32}O_2$	Antibacterial and antifungal activities [15]
9	Dodecanoic acid, methyl ester	7.11	200	$C_{12}H_{24}O_2$	Antimicrobial, Anticancer activities [16]
10	Hexadecanoic acid, ethyl ester	0.54	284	$C_{18}H_{36}O_2$	Antioxidant, nematicide, pesticide [17]
11	9-Octadecenoic acid, ethyl ester	1.10	310	$C_{20}H_{38}O_2$	Antibacterial [18]
12	Oleic acid	0.25	282	$C_{18}H_{34}O_2$	Antimicrobial, Antioxidant, Anticancer, Antiplasmodial activities [19-20].

Table 3: Compounds identified from African iba herbal mixture

No	Compound name	Peak area (%)	M.W	M.F	Compound nature and Biological activities
1	Cetene	0.67	224	$C_{16}H_{32}$	Antimicrobial, Antioxidant [20-21]
2	Triethyl citrate	0.56	276	$C_{12}H_{20}O_7$	No report found
3	Octadecene	1.35	252	$C_{18}H_{36}$	Antimicrobial activities [22]
4	Phthalic acid	14.57	166	$C_8H_6O_4$	Antimicrobial activities [23].
5	Behenic alcohol	1.08	326	$C_{22}H_{46}O$	Antimicrobial, Anti-inflammatory, Anticancer, Antifouling and Anti-arthritic activities [24]
6.	Bis (2- ethyl hexyl) phthalate	58.31	390	$C_{24}H_{38}O_4$	Antimicrobial activities [25]
7	Docosene	0.93	308.6	$C_{22}H_{44}$	Antimicrobial activities [26]
8	Quinolone, 1,2,3,4-tetrahydro-1-	7.67	138	$C_9H_{11}N$	Anticancer, Antimalarial, Antimycobacterial, Antimicrobial, Anticonvulsant activities [27]

Table 4: Compounds identified from Ruzu bitters herbal mixture

No	Compound name	Peak (%)	M.W	M.F	Compound nature and Biological activities
1	2-pyrrolidinone, 1-methyl	0.65	99	C_5H_9NO	Antimicrobial [28]
2	Triacetin	0.35	218	$C_9H_{14}O_6$	Triester of glycerin and acetic acid (no report found)
3	1,2,3-benzenetriol (pyrogallol)	14.41	126	$C_6H_6O_3$	Antimicrobial, Anti-inflammatory, Antioxidant, Analgesic, Insecticide, Anticancer, Cytotoxic activities [29]
4	Acetophenone, 4- hydroxyl	0.52	136	$C_8H_8O_2$	Antimycobacterial, Antibacterial activities [30]
5	Vanillic acid	0.19	168	$C_8H_8O_4$	Antioxidant, Antimicrobial, Anti-inflammatory, and Neuroprotective activities [29]
6	Triethyl citrate	0.70	276	$C_{12}H_{20}O_7$	Not reported

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7	Benzoic acid, 3,4-dihydroxy	0.91	154	$C_7H_6O_4$	Antimicrobial, Preservative [29]
8	Phthalic acid, isobutyl octyl este	12.47	334	$C_{20}H_{30}O_4$	Antimicrobial, Antifouling [29]
9	n-Hexadecanoic acid	2.52	256	$C_{16}H_{32}O_2$	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Antimicrobial, Flavor, Hemolytic,5-Alpha reductase inhibitor [29]
10	Di-n-octyl phthalate	25.81	390	$C_{24}H38O_4$	Antimicrobial, Antifouling [29]
11	Trans-3-undecene-1,5-diyne	0.69	146	$C_{11}H_{14}$	Antioxidant [32]
12	1-Docosene	0.80	308	$C_{22}H_{44}$	Antimicrobial [18]

Table 5: Compounds identified from Blood purifier herbal mixture

No	Compound name	Peak(%)	M.W	M.F	Compound nature and biological activities.
1.	Dimethyl sulfoxide	1.22	78	C_6H_6OS	Antibacterial [33]
2	n-Hexadecanoic acid	2.25	256	$C_{16}H_{32}O_2$	Fatty Acids. Antioxidant, Anticancer, Pesticide, Lubricant, Antiandrogenic, Antimicrobial, Flavor, Hemolytic,5-Alphareductase inhibitor [29]
3	Phthalic anhydride	3.69	148	$C_8H_4O_3$	Antimicrobial [34]
4	1 19-eicosadiene	3.03	278.5	$C_{20}H_{38}$	No activity reported
5	9,12-Octadecadienoic acid	28.39	270	$C_{19}H_{34}O_2$	Poly unsaturated fatty acid. Antioxidant, Antimicrobial [35]
6	Oleic acid	10.29	282	$C_{18}H_{34}O_2$	Antimicrobial, Antioxidant, Anticancer, Antiplasmodial activities [19-20]
7	3H-Pyrazol-3-one, 4-benzoyl-2,4-di	6.12	264	$C_{17}H_{16}N_2O$	Flavonoids, Anti-inflammatory, analgesic, antimicrobial activity [36]

Table 6: Compounds identified from Yoyo Cleanser bitters herbal mixture

No	Compound name	Peak(%)	M.W	M.F	Compound nature and biological activities
1	Phthalic acid, isobutyl octyl este	10.4	334	$C_{20}H_{30}O_4$	Antimicrobial, Antifouling [29]
2	Dibutyl phthalate	2.16	278	$C_{16}H_{22}O_4$	Antibacterial [37]
3	Eicosane	0.93	282	$C_{20}H_{42}$	Antifungal [38]
4	Octadecane	0.86	254	$C_{18}H_{38}$	Antibacterial, Antioxidant, Anticancer [22,39]
5	Docosane	0.96	408	$C_{20}H_{60}$	Antibacterial [40]
6	Bis (2- ethyl hexyl) phthalate	58.64	390	$C_{24}H_{38}O_4$	Antimicrobial activities [25]

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Conclusion

Our study has validated the claims that the five herbal formulations have varying activities as proposed by the manufacturers, but the identification of some synthetic products is of major concern because with the name tag 'herbal formulations' it is believed that every bioactive compound used should come from a natural source. The five (5) herbal formulations contain several significant bioactive substances that have a variety of promising biological effects, including antiplasmodial, antimicrobial, antioxidant, and anticancer properties, although more research studies including; using advanced analytical techniques in characterization, bioactivity, antimicrobial assay and safety profiling.

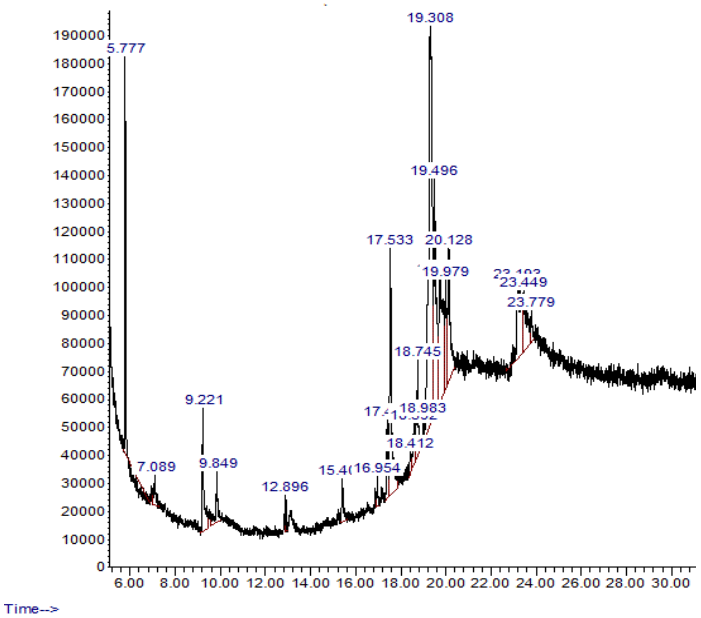

Figure 1: GC-MS chromatogram showing Bioactive compounds present in Blood Purifier.

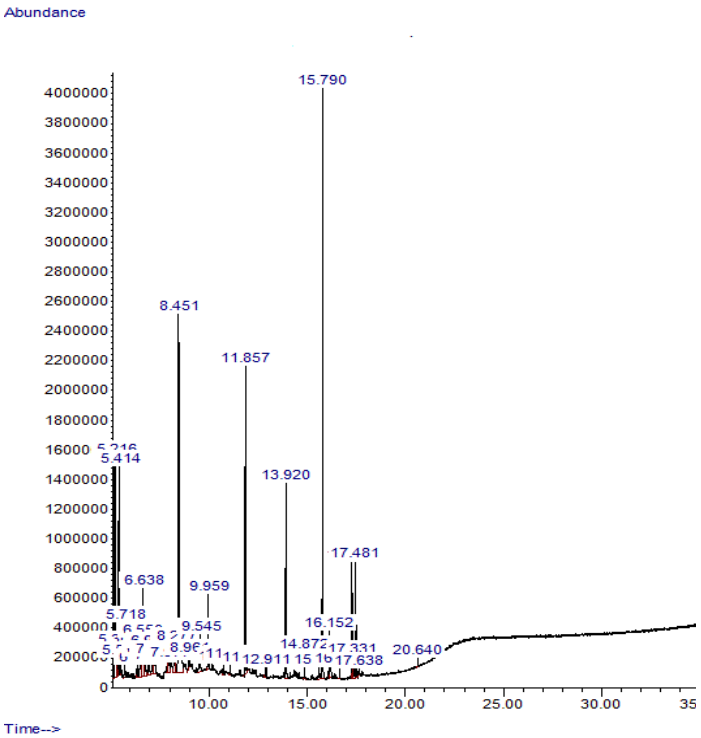

Figure 2: GC-MS chromatogram showing Bioactive compounds present in Deep Root Herbal Mixture.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

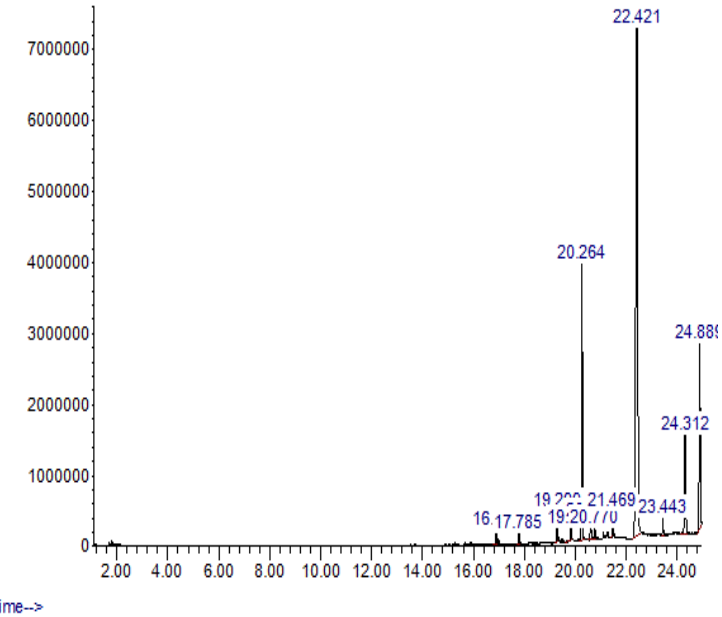

Figure 3: GC-MS chromatogram showing Bioactive compounds present in African Iba Herbal Mixture.

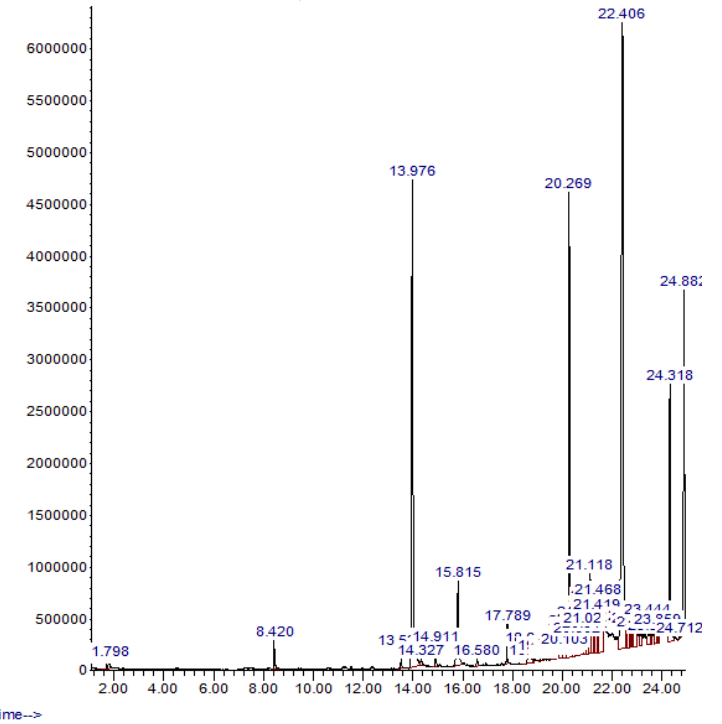

Figure 4: GC-MS chromatogram showing Bioactive compounds present in Ruzu Bitters

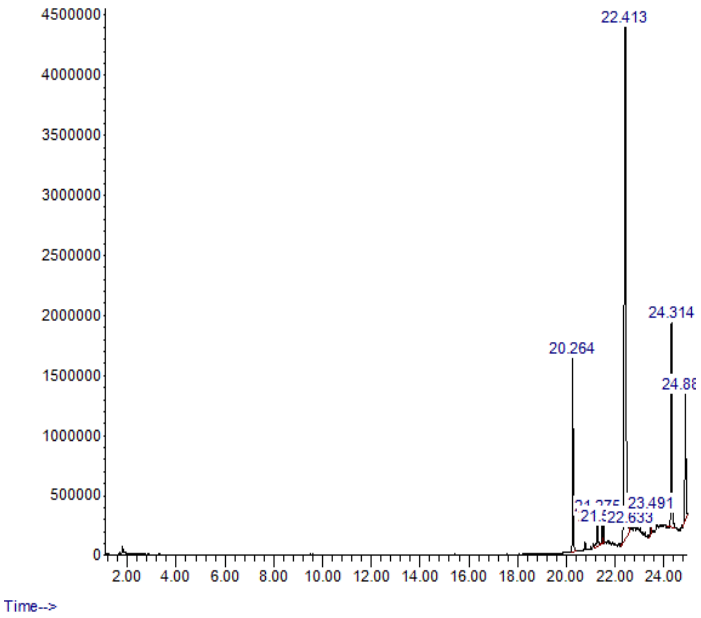

Figure 5: GC-MS chromatogram showing Bioactive compounds present in Yoyo Cleanser Bitters

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