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PHYTOCHEMICALS AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS OF *Rauvolfia vomitoria Afzel* COLD AND HOT WATER LEAVES EXTRACT

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ABSTRACT

Phytochemicals, which are bioactive compounds found in medicinal plants, have been attributed to the therapeutic properties of these plants. Qualitative and quantitative phytochemicals of cold and hot leaf extracts of *Rauvolfia vomitoria Afzel* were determined using standard methods and GC-MS technique was used to examine specific bioactive constituents. The qualitative results showed the presence of of cardiac glycosides, alkaloids, terpenes, flavonoids, phenols, and tannins whereas phlobatannins, thiols and steroids were absent in the cold and hot leaf extracts. The extract from hot water had a significantly higher ($p < 0.05$) value in phytochemical content than the cold water in all total phytochemicals determined with flavonoids (4.808 ± 0.099, 2.596 ± 0.045) having the highest concentration in the hot and cold water extract respectively while phenol (1.382 ± 0.010, 1.356 ± 0.002) had the least concentration in the hot and cold water extract respectively. The GC-MS result showed the following: n-Hexadecanoic acid, 9,12-Octadecadienoic acid, methyl ester, (E, E), 11-Octadecenoic acid, methyl ester, Octadecanoic acid, methyl ester, 2-hydroxy-1,3-propanediyl ester, 9-Octadecenamide, (Z), 9-Tetradecenal, (Z), 9-Octadecenoyl chloride, (Z), Tridecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, methyl ester, (E, E), 9-Octadecenoic acid, Octadecanoic acid, methyl ester, etc. The following are a few of the phytochemicals' pharmacological characteristics: antioxidant, antiviral, anticarcinogenic, antimicrobial, antibiotic, antiallergic and anti-inflammatory. The results of this investigation provide insight into the plant's medicinal usefulness, especially the hot extract, which had higher levels of phytochemicals. Hence, *R. vomitoria* plant may be a true source of medicinal agents to manage and treat diseases.

Keywords: *Rauvolfia vomitoria*, Phytochemicals, Bioactive constituents, Therapeutic, Pharmacology

INTRODUCTION

Plant-based pharmaceuticals have been accepted recently due to their higher biocompatibility and lower side effects compared to synthetic drugs. This has led to searches for plant species that may serve as sources of therapeutic chemicals (Vaisakh and Pandey, 2012; Aziz et al., 2020). Medicinal plants are an important part of nature's biodiversity and have been used for managing and treating a variety of medical ailments since ancient times (Falodun, 2010; Omotoso et al., 2024). A great deal of research has been done on the application of various parts of plant extract for medicinal purposes (Onakurhefe et al., 2022; Lokwutor et al., 2024; Chinonye et al., 2021). Due to the disparity in the proportions of essential components of oxidative metabolism, oxidative stress is the root cause of many diseases (Fatima et al., 2021). Oxidative stress sets in, when a system is unable to efficiently eliminate reactive oxygen species and useful metabolites (Goodarzi et al., 2018). A variety of bioactive compounds known as phytochemicals are typically responsible for the therapeutic properties of medicinal plants, which may be connected to specific plant parts or the entire plant (Omotoso et al., 2020; Olivia et al., 2021). Phytochemicals are naturally occurring bioactive molecules that are beneficial in pharmacology and medicine because of their disease-preventive and protective properties (Egharevba et al., 2019; Shedrach et al., 2020; Singayina et al., 2022). A crucial stage in developing new drugs from medicinal plants is phytochemical screening (Starlin et al., 2019).

Rauvolfia vomitoria Afzel is a family of Apocynaceae, a rainforest tree that normally reaches a height of about 12 meters. Its remarkable crimson globose fruits and whorled trunk are its defining features (Ekarika et al., 2020). The tree features oval leaves with straight veins and clusters of blooms. Commonly referred to as deadly devil's pepper, swizzle stick, or serpent wood, it is extensively grown across Bangladesh, India, China, and Africa (Yu et al., 2012; Zhan et al., 2020a; 2020b). The Yoruba call it asofeyeje, whereas the Ukwuani call it Akanta in Nigeria. Different portions and extracts from this herb have demonstrated anti-inflammatory, anti-cancer, antibacterial, antidiabetic, antioxidant, and antipsychotic properties due to their bioactive compounds (Surendran et al., 2021; Yu and Chen, 2014; Fannang et al., 2011; Bemis et al., 2006). Several studies employing various extraction solvents have been conducted on the leaves, stem, and root of *R.* vomitoria (Chinonye et al., 2021; Ekarika et al., 2020; Surendran et al., 2021) but no research has been done to compare the phytochemical and GC-MS examination of the bioactive components of cold and hot water extract since the majority of people use either the hot or cold extract of the plant for medicinal purposes. Therefore, the goal of this research is to evaluate the phytochemicals of *Rauvolfia vomitoria Afzel's* cold and hot water extracts using GC-MS to identify the extract that may have greater medicinal or therapeutic potential.

MATERIALS AND METHODS

Collection, identification and sample preparation

Fresh *R. vomitoria Afzel* plant was collected from a nearby bush in Delta State University, Abraka (5°48'2.1" N, 6°7'21.4" E.). The plant was identified with voucher number (UBH-R421) at the Plant Biology and Biotechnology Department Herbarium Unit, University of Benin, Edo State. After gathering more fresh stock of the study plant, distilled water was used to rinse detached leaves to get rid of any leftover debris.

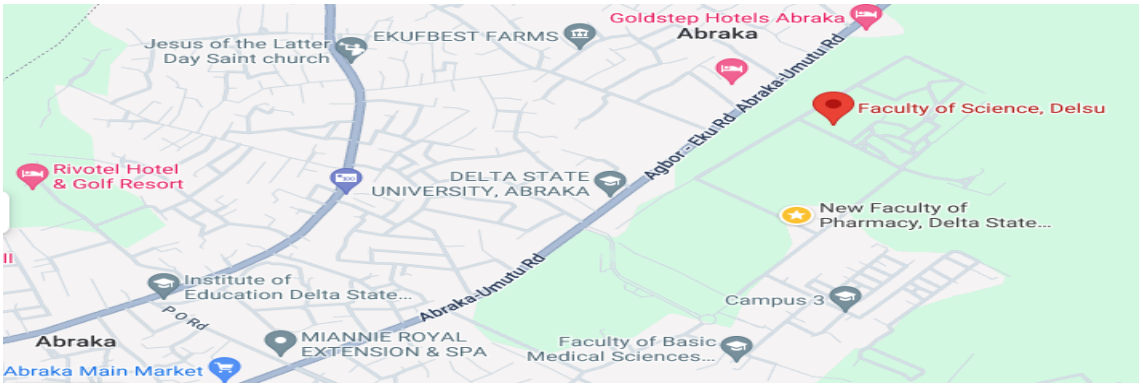

Figure 1: Map of sampling location. (Source: Google map).

Preparation of cold and hot water extracts

Cold water extract

The homogenization process was used to extract the *R. vomitoria Afzel* leaf in cold water (Onyeukwu et al. 2024). Pestle and Mortar were employed to blend 250 g of fresh leaves, in 1000 mL of distilled water (25% w/v). The extract was run through a clean, double-folded sieve cloth, and the cold water filtrate was used in the study.

Hot water extract

The decoction process was used to extract the *R. vomitoria Afzel* leaf in hot water (Onyeukwu et al., 2024). After adding 250 g of fresh leaf to 1000 mL of boiling water (25% w/v), the mixture was left for fifteen minutes. After that, the extract was filtered through double-folded muslin fabric, the hot water filtrate was then cooled and used in the study.

Chemicals and Reagents

Gallic and tannic acid were from Sigma Aldrich Spruce Street Louis, USA. Catechin was from Central Drug House (P) Ltd, New Delhi, India. Atropin was from Loba Chemie Pvt Ltd, Mumbai, India. All other analytical grade chemicals were from SCP Pure Chem Products Ltd Needham Market Suffolk-England, JHD Shantou, Guangdon, China, Loba Chemie Munbai, India, May & Baker Ltd Dageham England, Sigma Aldrich, USA, Kermel Tianjing City Jinnan District, China, Titan Biotech Ltd, Bhiwadi-Rajasthan, India.

Phytochemicals

Qualitative phytochemical screening

The qualitative investigation of the cold and hot water leaf extract of *R. vomitoria* was conducted using conventional procedures as outlined by Borokini and Omotayo (2012) and Njoku and Obi (2009).

Saponin test

The Frothing test was conducted by measuring 30ml of the filtrate and shaking it vigorously. The sample was then examined for the presence of froth formation.

Phlobatanin test

2ml of the filtrate were heated with 2ml of a 2% hydrochloric acid solution. The formation of a red precipitate signified the presence of phlobatanin.

Cardiac glycoside test

The Keller-Killani method was employed for the test. A test tube was filled with 5ml of the sample, followed by the addition of 2ml of glacial acetic acid. One drop of 2% ferric chloride solution was then added, and finally, 1ml of concentrated sulfuric acid was included. A positive result for cardiac glycosides was indicated by the formation of a brown interface, a violet ring, and a greenish ring at the bottom of the tube.

Flavonoid detection test

The Shinoda test was performed by adding 10 drops of concentrated HCl and small pieces of magnesium to a test tube containing 0.5ml of the sample. The mixture was then heated for 5 minutes. A reddish-pink color change in the solution indicated the presence of flavonoids

Tannin detection test

The Ferric chloride test was performed by adding a few drops of a 5% aqueous ferric chloride solution to 2.0ml of the sample extract. A bluish-black color appeared, which faded after the addition of a small amount of dilute sulfuric acid, leading to the formation of a yellowish-brown precipitate. This reaction confirmed the presence of tannins.

Phenol test

The ferric chloride test was employed for detection. To 1.0ml of the sample extract, 2ml of distilled water was added, followed by a few drops of a 10% aqueous ferric chloride solution. The appearance of a blue color signified the presence of phenolic compounds.

Steroid test

A 0.5 ml sample extract was added to 2 ml of acetic anhydride, followed by the addition of 2 ml of sulfuric acid. The appearance of a blue color confirmed the presence of steroids.

Terpenes/terpenoids

A test for terpenes/terpenoids was conducted using the Salkowski method. In this procedure, 5ml of the sample was combined with 2ml of chloroform, and 3ml of concentrated sulfuric acid was added to create a distinct layer. The appearance of a reddish-brown color confirmed the presence of terpenes/terpenoids.

Alkaloid Test

1ml sample of the extract was mixed with 2ml of 1% hydrochloric acid, followed by the addition of 1ml of Drangendoff's reagent. The formation of an orange-red precipitate indicates a positive result.

Thiols

To 0.5ml of the sample extract, ammonium sulfate was added until the solution became saturated. Then, 2 to 4 drops of 5% sodium nitroprusside were introduced, followed by one or

more drops of concentrated nitric acid. A temporary magenta color appears when thiols are present.

Quantitative phytochemical analysis

The method of Singleton and Rossi (1965), Jia *et al.* (1999), Shamsa *et al.* (2008) was employed to estimate the total phenols and total tannin, total flavonoids and alkaloids respectively.

Total phenols and tannins

1.0ml of extract was combined with 1.0ml of Folin-Ciocalteu phenol reagent. After 3 minutes, 1.0ml of saturated sodium carbonate (Na_2CO_3) solution was added, and the mixture was diluted to a final volume of 10 mL using distilled water. The mixture was allowed to incubate in the dark for 90 minutes before its absorbance was measured at 725 nm. A calibration curve was prepared using varying concentrations of gallic acid for phenols (20–100 µg/mL) and tannic acid for tannins (20–100 µg/mL). The results were reported as milligrams of gallic acid equivalents (GAE) and tannic acid equivalents (TAE) per gram of extract.

Total flavonoids

½ mL of each extract was introduced into test tubes containing 1.25 mL of distilled water and 0.075 mL of 5% NaNO2 solution. The mixture was left to stand for 5 minutes. Then, 0.15 mL of 10% AlCl3 solution was added, followed by the addition of 0.5 mL of 1.0 M NaOH after 6 minutes. The solution was diluted with 0.275 mL of distilled water, and the absorbance was measured immediately at 510 nm. Catechin was used as a reference standard (ranging from 20–100 µg/mL). The total flavonoid content was calculated as milligrams of catechin equivalent (CAE) per gram of extract.

Total alkaloids

1 mL of each extract was mixed with 5 mL of phosphate buffer at pH 4.7, followed by the addition of 5 mL of bromocresol green solution. The mixture was then shaken with 4 mL of chloroform. The resulting solution was transferred to a 10-mL volumetric flask, and the volume was adjusted with chloroform. The absorbance of the resulting complex in chloroform was measured at 470 nm, with a blank sample prepared in the same way but without the extract. Atropine, used as a standard, was employed to compare the assay results, and the alkaloid content was expressed in Atropine equivalents (40-120 µg/mL).

GC-MS Analysis

A GC-MS (Model: QP2010 PLUS Shimadzu, Japan) with an AOC-20i autosampler and chromatograph interfaced within a mass spectrophotometer (GC-MS) was used to perform the GC-MS analysis. The instrument was fitted with a VF 5 ms fused silica capillary column measuring 30 m in length, 0.25 mm in diameter, and 0.25 µm in film thickness. The column oven temperature was maintained at 80.0 °C, while the injection temperature was set to 250.0 °C. A split injection mode was used with a 1.0 split ratio. Flow control was set to linear velocity, with a pressure of 108.0 kPa, total flow rate of 6.2 mL/min, column flow rate of 1.58 mL/min, linear velocity of 46.3 cm/sec, and a purge flow of 3.0 mL/min. The ion source temperature was set at 200.0 °C, and the interface temperature was 250.0 °C. The solvent cut time was 2.50 minutes. A relative detector gain mode was applied with a gain of 0.00 kV and a threshold of 2000. Data acquisition occurred in scan mode from 3.00 to 30.00 minutes, with an event time of 0.50 seconds, a scan speed of 1666, and a mass range of 40.00 to 800.00 m/z. Mass spectrum interpretation was done using the National Institute of Standards and Technology (NIST) database, which includes over 62,000 spectra. The identity, molecular weight, and structure of the components in the test sample were determined by referencing the known compound spectra stored in the NIST library.

Statistical analyses

The data was reported as mean ± standard deviation. The results of the analyses of variance were compared using the least significant difference test. Statistical significance ($p< 0.05$) was determined while microsoft excel was used to plot the standard linear graph.

RESULTS AND DISCUSSION

Results

Tables 1 and 2 show the qualitative and quantitative phytochemicals of cold and hot water leaf extract of *R. vomitoria*. The findings showed that cardiac glycosides, alkaloids, terpenes, flavonoids, phenols, and tannins were present in the cold and hot water leaf extract of *R. vomitoria* while phlobatannins, thiols and steroids were absent in both extracts. Four phytochemicals (Phenols, Tannins, Flavonoids and Alkaloids) were quantitatively determined. In all phytochemicals determined, the hot water extract of *R. vomitoria* was significantly higher than the cold water extract at $p< 0.05$. The result revealed that flavonoid composition was highest in both the hot and cold water extract although the hot water extract (4.808 ± 0.099 mg/g CAE) was higher than the cold water extract (2.596 ± 0.045 mg/g CAE). This was followed by tannins, with 1.890 ± 0.014 mg/g TAE in the hot water extract and 1.852 ± 0.003 mg/g TAE in the cold water extract. Phenol had the lowest content with 1.382 ± 0.010 mg/g GAE and 1.356 ± 0.002 mg/g GAE in the hot and cold water extract respectively.

Table 1: Qualitative phytochemicals of cold and hot water leaf extract of *R. vomitoria*

Phytochemicals	RVH	RVC
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	+
Phenol	+	+
Terpenes	+	+
Saponins	+	+
Phlobatannins	-	-
Thiols	-	-
Cardiac glycosides	+	+
Steroids	-	-

Note: + = Present, - = Absent, RVH- *R. vomitoria* hot water extract, RVC- *R. vomitoria cold* water extract.

Table 2: Quantitative phytochemicals of cold and hot water leaf extract of *R. vomitoria*

Phytochemicals	RVH	RVC	Standard equation
Phenol (mg/g GAE)	1.382 ± 0.010^{a}	1.356 ± 0.002^{b}	Y = 10.2x + 0.02
Tannin (mg/g TAE)	1.890 ± 0.014^{c}	1.852 ± 0.003^{d}	Y = 7.175x + 0.074
Flavonoid (mg/g CAE)	4.808 ± 0.099^{e}	2.596 ± 0.045^{f}	Y = 4.565x + 0.020
Alkaloids (mg/g ATE)	1.561 ± 0.040^{g}	1.136 ± 0.032^{h}	Y = 0.0625 + 0.024

Note: Results are shown as Mean ± SD, significant differences ($p < 0.05$) exist between values with different letters in the same column and row. GAE- Gallic acid equivalent, TAE- Tannic acid equivalent, CAE- Catechin acid equivalent, ATE- Atropin acid equivalent.

GC-MS analysis of cold and hot water extract of R. vomitoria leaves

The list of bioactive compounds from GC-MS analysis of cold and hot water extract of *R. vomitoria* leaves are presented in Tables 3 and 4 respectively with the gas chromatograms in Figures 2 and 3. The cold water extract revealed 7 absorption peaks. n-Hexadecanoic acid also known as Palmitic acid ($C_{16}H_{32}O_2$) with RT (17.053) has a peak area (9.45%), 9-Octadecenoic acid (Oleic Acid) ($C_{18}H_{34}O_2$) with RT (20.134) has peak area (66.50), Stearin ($C_{39}H_{76}O_5$) with RT (21.718) has peak area (1.48). Other compounds are: 9-Octadecenamide, (Z) (Andogen), 9-Tetradecenal, (Z), 9-Octadecenoyl chloride, (Z) (Oleoyl chloride) etc. In the cold water extract, Oleic acid had the highest peak area while stearin had the lowest peak area. The hot water extract, revealed 13 absorption peaks. Peak 1, Tridecanoic acid, methyl ester ($C_{14}H_{28}O_2$) with RT (19.026) has a peak area (6.37), followed by n-Hexadecanoic acid ($C_{16}H_{32}O_2$) with RT (20.314) has a peak area (24.34), Linolelaidic acid ($C_{19}H_{34}O_2$) with RT (22.207) has peak area (4.57), 11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$) with RT (22.299) has peak area (8.98), Stearic acid, methyl ester ($C_{19}H_{38}O_2$) with RT (22.670) has peak area (3.36), 9-Octadecenoic acid (Z) (Oleic acid) ($C_{18}H_{34}O_2$) with RT (23.221) has peak area (31.45) etc. In the hot water extract, Oleic acid had the highest peak area while Z-10-Octadecen-1-ol acetate had the lowest peak area.

Table 3: Bioactive compounds from GC-MS analysis of cold water extract of *R. vomitoria* leaves

Peak	RT (min)	Area (%)	Molecular formula	Name of compound	M. weight
1.	17.053	9.45	$C_{16}H_{32}O_2$	n-Hexadecanoic acid (Palmitic acid)	256
2.	20.134	66.50	$C_{18}H_{34}O_2$	Oleic Acid (9-Octadecenoic acid)	282
3.	21.718	1.48	$C_{39}H_{76}O_5$	Stearin	624
4.	22.730	2.03	$C_{18}H_{35}NO$	9-Octadecenamide, (Z) (Andogen)	281
5.	23.135	2.80	$C_{18}H_{33}ClO$	9-Octadecenoyl chloride, (Z) (Oleoyl chloride)	300
6.	23.587	9.79	$C_{14}H_{26}O$	9-Tetradecenal, (Z)	210
7.	25.745	7.94	$C_{15}H_{28}O_2$	Z-8-Methyl-9-tetradecenoic acid	240

RT: Retention time, M. weight: Molecular weight

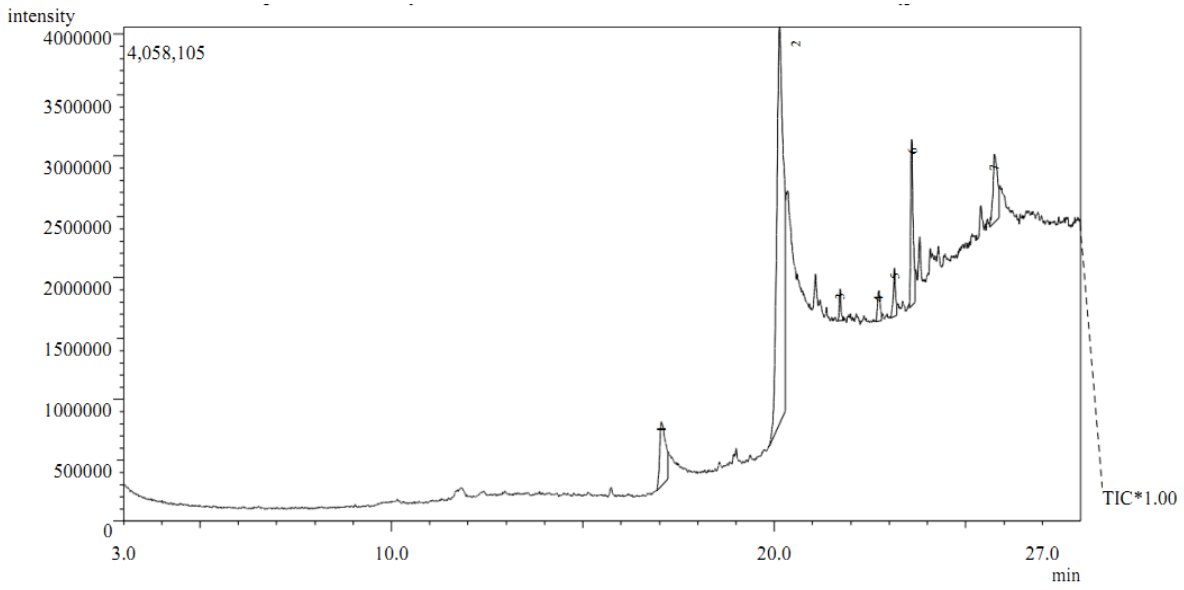

Figure 2: Gas chromatogram of cold water extract of *R. vomitoria* leaves

Table 4: Bioactive compounds detected from GC-MS analysis of hot water extract of *R. vomitoria* leaves

Peak	RT (min)	Area (%)	Molecular formula	Name of compound	M. weight
1	19.026	6.37	$C_{14}H_{28}O_2$	Tridecanoic acid, methyl ester	228
2	20.314	24.34	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	256
3	22.207	4.57	$C_{19}H_{34}O_2$	Linolelaidic acid	294
4	22.299	8.98	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	296
5	22.670	3.36	$C_{19}H_{38}O_2$	Stearic acid, methyl ester	298
6	23.221	31.45	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z) (Oleic Acid)	282
7	23.488	10.10	$C_{22}H_{44}O_2$	Docosanoic acid (Hydrofol)	340
8	24.305	1.89	$C_{15}H_{32}O_2$	2-Dodecyl-1,3-propanediol	244
9	24.846	1.89	$C_{20}H_{40}O$	Octadecane, 1-(ethenyloxy)	296
10	26.235	0.65	$C_{20}H_{38}O_2$	Z-10-Octadecen-1-ol acetate	310
11	26.682	3.93	$C_{18}H_{34}O$	9-Octadecenal	266
12	26.910	0.76	$C_{39}H_{76}O_5$	Stearin	624
13	28.988	1.71	$C_{18}H_{34}O$	2-Octylcyclopropene-1-heptanol	266

RT: Retention time, M. weight: Molecular weight

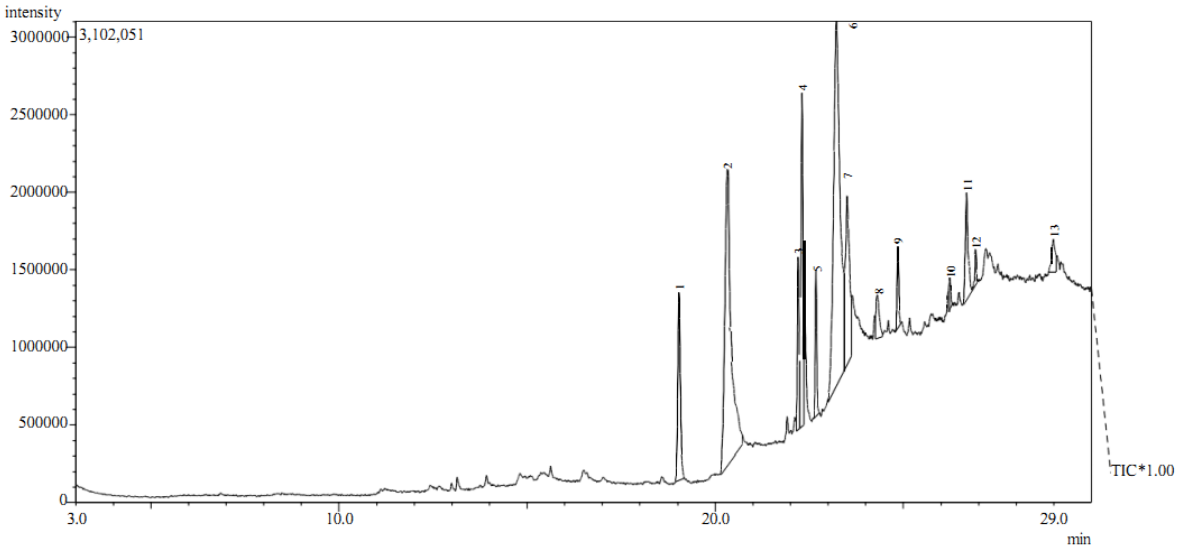

Figure 3: Gas chromatogram of hot water extract of *R. vomitoria* leaves

Discussion

The presence of phytochemicals in a plant as shown in Tables 1 and 2 indicates the presence and varying amounts of bioactive compounds that may have therapeutic value for human health. This agrees with the work of Agbodjogbé et al. (2022) which reported the presence of phytochemicals in *R. vomitoria.* This outcome is also consistent with the findings of Oluyemi and Ademoye (2019) which indicated that this plant has phytochemical elements. Ugwu et al. (2019) also reported the presence and varying amounts of phytochemicals, although contrary to our work, they reported alkaloids to be the highest. It has been demonstrated that flavonoids, a broad class of polycyclic compounds distinguished by their benzo pyrone ring structure, possess antioxidant qualities across a range of biological systems (Okwu and Aluwuo, 2008). In addition to their ability to scavenge free radicals, flavonoids also show a wide range of other biological activities, including the inhibition of phospholipase H2, cycloxygenase, glutathione reductase, and xanthine oxidase, as well as vasodilatory, anti-carcinogenic, anti-allergic, and oestrogenic properties (Okwu, 2004). Flavonoids promote lactogenecity; hence, these properties warrant the use of the plant extract in the treatment of cancer (Asoegwu et al., 2006). According to Emmanuel et al. (2017), alkaloids which have several therapeutic applications, are found in the majority of green leafy plants. Most alkaloids have been used in medicine because of their antimalarial, antibiotic, antibacterial, antifungal, and antiparasitic qualities. Most samples containing alkaloids are used in Nigeria to treat malaria and fever (Iwu et al., 2018). According to Ugwu et al. (2019), tannins inhibit the oxidation of wine and have an astringent effect on living tissues. Saponins were found to be present in *R. vomitoria* leaves. The ability of saponins to bind cholesterol, their haemolytic action, and their capacity to generate foam in aqueous solutions are some of their common traits (Okwu, 2005). Saponins are a natural antibiotic that supports the body's defenses against infections, microbial invasion and are great choices for treating fungal and yeast infections because of their inherent ability to repel germs (Iwu et al., 2016).

The GC-MS analysis of *R. vomitoria* detected the presence of specific bioactive compounds (Tables 3 and 4). The GC-MS analysis of *R. vomitoria* using a range of extraction solvents, such as water, ethanol and methanol has been reported (Ajayi, 2021; Akpojotor and Ebomoyi, 2021). One of the several components that have been identified is n-Hexadecaneoic acid, which has been described as an acidulant, acidifier, an inhibitor of arachidonic acid and uric acid synthesis, as well as an increaser of aromatic acid decarboxylase activity (Akpojotor and Ebomoyi, 2021). For patients with achlorhydria to effectively digest their meals, they must take acidifiers, which are drugs that lower the pH of the body (Nwakudu et al., 2017). These plant compounds will be beneficial since they increase the amount of acid in the

stomach when ingested. According to Chinonye et al. (2021), isomers of oleic acid have potential applications in the management of cancer, inflammatory conditions, heart disease, and bolstering the immune system. According to Rajeswari et al. (2012), hexadecanoic acid and its ethyl ester have anti-inflammatory, haemolytic, nematicide, hypocholesterolemic, and antioxidant properties. The methyl ester of tridecanoic acid has anti-oxidant, lubricating, nematicide, pesticide, and hypocholesterolemic properties (Ogwuche and Adeyemi, 2016). Many household products contain stearic acid, sometimes referred to as octadecanoic acid. It is a substance that permits the blending of water and oil and can be employed as a emulsifier, lubricant or hardener (Ekarika et al., 2020).

CONCLUSION

Various phytochemicals with significant pharmacological activities were found in both the hot and cold water extracts of *R. vomitoria Afzel* leaves, based on the GC-MS and phytochemical study conducted in this work. The hot water extract of *R. vomitoria Afzel* leaves contained more phytochemical components than the cold water extract. For medicinal purposes, it is therefore recommended to employ hot water leaf extracts of *R. vomitoria Afzel* rather than cold water leaf extracts.

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