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Antinutritive Properties and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *Solenostemon monostachyus* (P. Beauv.) Briq (monkey's potato) Leaf

[1]Chijindu, P. C. & [2]Onyeukwu, O. B.*

[1]Department of Biological Sciences, University of Delta, Agbor, Delta State.

[2]Department of Chemical Sciences, University of Delta, Aghor, Delta State.

***Corresponding author**: benjamin.onyeukwu@unidel.edu.ng

ABSTRACT
Solenostemon monostachyus (Monkey's potato), a vibrant Lamiaceae herb thriving in Nigeria's lush ecosystems, holds a cherished place in traditional medicine for its healing properties. This study investigated its antinutritive factors and chemical composition to validate its ethnomedicinal value. Fresh leaves, collected from Oleh, Delta State, Nigeria, were extracted using ethanolic cold maceration. Antinutritive analysis indicated significant saponins (830.26 ± 3.18 mg/100g), oxalates (766.67 ± 1.209 mg/100g), tannins (188.20 ± 0.727 mg/100g), and phytates (112.54 ± 1.146 mg/100g), suggesting nutritional limitations requiring processing. Gas chromatography-mass spectrometry (GC-MS) identified 21 compounds, with n-Hexadecanoic acid ($C_{16}H_{32}O_2$, 20.90%), Isobornyl acetate ($C_{12}H_{20}O_2$, 11.31%), and Eugenol ($C_{10}H_{12}O_2$, 10.04%) as dominant. The bioactive constituents determined validated the plant's traditional use for managing inflammation, infections, and oxidative stress, while highlighting the need for processing to reduce antinutritive effects. This comprehensive chemical profile positions *Solenostemon monostachyus* as a promising candidate for pharmaceutical development, necessitating further toxicity, bioactivity, and formulation studies to harness its therapeutic potential.

Keywords: Antinutritive properties, *Solenostemon monostachyus,* monkey's potato leaf, phytochemical, GC-MS,

INTRODUCTION
Plants have remained a cornerstone of human survival, providing essential resources such as food, shelter, medicine, and raw materials for thousands of years (Srivastava, 2018). Among their many uses, their role in traditional medicine and nutrition has attracted increasing attention in modern scientific research. In many developing countries, especially across Africa, medicinal plants continue to play a vital role in primary healthcare systems due to their affordability, availability, and cultural acceptance (Nsagha *et al.*, 2020). One of such medicinal plant is *Solenostemon monostachyus* commonly known as monkey's potato, an herbaceous species in the *lamiaceae* family (Afolabi *et al.*, 2022). *S. monostachyus* is an aromatic plant widely distributed in tropical West Africa, including Nigeria, where it thrives in moist environments and often grows wild (Essien *et al.*, 2017). It is traditionally used in herbal remedies to treat a variety of ailments, including fever, convulsions, inflammation, gastrointestinal disturbances, wounds, and skin infections (Edet *et al.*,

2015; Okokon *et al*., 2016). This plant is not only prominent in local medicine but is also known by various indigenous names across different ethnic groups in Nigeria, reflecting its widespread recognition and use. Its common name is African dead nettle and in Efik it is called Ntorikwot, Awakmmon (Edet *et al*., 2015). It occurs as an annual weed in anthropogenic habitat and rocky savannahs. It is slightly succulent, aromatic and grows up to 100cm tall. These medicinal applications are attributed to the presence of biologically active compounds within the plant. Despite its widespread use in folk medicine, there is limited scientific documentation on its antinutritive composition and full chemical profile of the leaves. Hence, the lack of scientific evidence on the antinutritional content of *S. monostachyus also* limits its promotion as a cultivated crop. Currently regarded as a weed in many regions, the plant's potential as a functional food or medicinal resource remains underutilized. Without a thorough understanding of its antinutritional factors and their chemical profiles, stakeholders, including farmers, nutritionists, and policymakers, cannot make informed decisions about its integration into modern dietary and pharmaceutical practices (Samtiya *et al*., 2020).

Antinutrients are naturally occurring compounds found in plant-based foods that can interfere with the absorption and utilization of essential nutrients. These include tannins, oxalates, phytates, saponins, alkaloids, and cyanogenic glycosides (Singh and Arora, 2023). While some antinutrients exhibit beneficial health effects such as antioxidant, anticancer, and cholesterol-lowering activities at low concentrations, high levels can pose health risks. For instance, phytates can chelate minerals such as iron and zinc, reducing their bioavailability; oxalates can form insoluble calcium oxalate crystals leading to kidney stones; and tannins can inhibit digestive enzymes and protein digestibility (Akande and Fabiyi, 2010; Abera *et al*., 2023). In many African communities, *S. monostachyus* leaves are consumed as a leafy vegetable or incorporated into soups and stews, particularly in rural areas where access to diverse food sources is limited. Despite its nutritional potential, the antinutritional content of the plant has not been thoroughly investigated. Preliminary studies indicate the presence of saponins, tannins, phytates, and oxalates in the leaves (Imathiu, 2021).

In addition to evaluating the antinutritive content of the plant, identifying its bioactive constituents is key to understanding its therapeutic potential. One of the most effective analytical techniques for this purpose is Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS combines the features of gas-liquid chromatography and mass spectrometry to identify different substances in a test sample (Afolabi *et al*., 2021). This method is particularly effective for profiling volatile and semi-volatile compounds, including fatty acids, terpenoids, phenolics, alkaloids, and other secondary metabolites found in plants (Parra-Paz *et al*., 2015). Applying GC-MS to the leaves of *S. monostachyus* could provide detailed information on the specific bioactive compounds responsible for the plant's medicinal properties (Afolabi *et al*., 2021).

Over the past decade, there has been a paradigm shift toward the scientific validation of ethnobotanical knowledge, largely driven by the global demand for alternative and complementary medicines (Carvalho *et al*., 2023). This study aimed to investigate the antinutritive properties and gas chromatography mass-spectrometry analysis of the leaf extract of *Solenostemon monostachyus* (Monkey's potato).

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh leaves of *S. monostachyus* (commonly referred to as Monkey's Potato) were collected from wild-growing populations within the Oleh, Isoko South, Delta State, Nigeria. The harvested leaves were carefully detached by hand and placed in clean, perforated polyethylene bags to allow adequate ventilation during transit. After cleaning, the samples were spread on sterile trays lined with muslin cloth and placed in a well-ventilated laboratory room for drying. Air-drying was done at ambient room temperature (25-30°C) for approximately 10-14 days. The samples were periodically turned to ensure uniform drying and to prevent fungal growth. After drying, the leaves and stems were pulverized separately using an electric grinder (Binatone Model BLG-450) that was pre-sterilized with 70% ethanol. The resulting powders were sieved through a 0.5 mm mesh to obtain uniform particle sizes and stored in sterile, airtight, amber-colored glass containers at 4°C to preserve their phytochemical integrity until extraction and analysis.

Ethanolic Extraction

Cold maceration was employed to extract ethanolic phytochemicals from the powdered leaves and stems of *S. monostachyus*. 50 g of each sample were weighed using an analytical balance and transferred into separate 1000 mL conical flasks. Each sample was soaked in 500 mL of absolute ethanol (99.9% purity). The flasks were sealed with aluminum foil and left to stand at room temperature (25-30°C) for 72 hours. Intermittent shaking (every 8 hours) was performed to increase solvent penetration and phytochemical release. After maceration, the extracts were filtered using sterile muslin cloth followed by Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator at 40°C to remove ethanol, yielding semi-solid crude extracts. These were collected into sterile, amber-colored glass bottles and stored at 4°C for further analysis.

Determination of Antiutritive Properties of *Solenostemon monostachyus*

Determination of Tanin Content

The tannin content of the plant sample was determined using the Folin-Denis colorimetric method as described by AOAC (2005). A 0.5 g portion of the dried and ground leaf sample was extracted with 50 mL of 70% aqueous acetone. The mixture was centrifuged, and 1 mL of the supernatant was mixed with 1 mL of Folin-Denis reagent and 2 mL of 20% sodium carbonate solution. The mixture was diluted to 10 mL with distilled water and incubated for 30 minutes at room temperature. The absorbance was measured at 700 nm using a spectrophotometer. The tannin concentration was estimated by comparing the absorbance with a standard curve prepared using tannic acid.

Determination of Phytate Content

Phytate content was estimated following the Wade reagent Method (Sharma *et al.*, 2020). Briefly, 0.5 g of the plant sample was extracted with 10 mL of 0.2 N hydrochloric acid for 1 hour, followed by filtration. Three milliliters of the filtrate were then mixed with 1 mL of Wade's reagent (containing $FeCl_3$ and sulfosalicylic acid), and the absorbance was read at 500 nm. The phytate concentration was calculated using a calibration curve of sodium phytate standard.

Determination of Oxalate Content ($KMnO_4$ Titration Method)

Oxalate content was determined using the potassium permanganate titration method. Two grams of the sample were boiled with 190 mL of distilled water and 10 mL of 6

M hydrochloric acid for one hour. The mixture was cooled and filtered, and 25 mL of the filtrate was titrated against 0.05 N potassium permanganate solution until a persistent pink color appeared. The oxalate concentration was then calculated, where 1 mL of 0.05 N $KMnO_4$ corresponds to 2.2 mg of oxalate (Karamad *et al.*, 2019).

Determination of Saponin Content (Gravimetric Method)
The saponin content was determined by the gravimetric method as described by Shaik *et al.* (2019). Five grams of the sample were extracted with 50 mL of 20% ethanol by heating in a water bath for four hours. The extract was filtered and re-extracted. The combined filtrates were partitioned with diethylether (to remove non-polar compounds), and the aqueous layer was then extracted with n-butanol. The butanol layer was washed with 5% NaCl, evaporated to dryness, and the residue was dried to constant weight. The percentage saponin content was calculated using the formula: Saponin (%) = (Weight of residue / Weight of sample) × 100.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *S. monostachyus*
The GC-MS analysis of the plant extract was conducted using an Agilent 5977B GC/MSD system, which was coupled with an Agilent 8860 auto-sampler. This system comprised a gas chromatograph interfaced with a mass spectrometer and was equipped with an Elite-5MS capillary column, composed of 5% diphenyl and 95% dimethylpolysiloxane. The column had dimensions of 30 meters in length, 0.25 micrometers internal diameter (ID), and 0.25 micrometers film thickness (df). For GC-MS detection, an electron ionization technique was employed, operating in electron impact mode at an ionization energy of 70 electron volts (eV). High-purity helium gas (99.999%) served as the carrier gas, maintained at a constant flow rate of 1 milliliter per minute. The injection of the sample was done using a 1 microliter (µL) volume with a split ratio of 10:1 to ensure optimal separation and sensitivity. A five-point serial dilution calibration was carried out using standard solutions of 1.25, 2.5, 5.0, and 10.0 parts per million (ppm), all prepared from a 40-ppm stock solution, to calibrate the GC-MS instrument. During the analysis, the injector temperature was set to 300 °C while the ion source was maintained at 250 °C. The oven temperature was initially programmed to hold at 100 °C for 0.5 minutes (isothermal), followed by a temperature ramp at a rate of 20 °C per minute until it reached 280 °C, where it was held for 2.5 minutes. Mass spectral data were acquired with a scanning interval of 0.5 seconds, covering a mass-to-charge (m/z) range from 45 to 450 Daltons. A solvent delay of 0 to 3 minutes was applied to prevent solvent peaks from interfering with the detection of analytes. The total runtime for each GC-MS analysis was 21.33 minutes (Odion *et al.*, 2024). The identification of bioactive components present in the extract was carried out by interpreting the mass spectra generated during the GC-MS analysis. The identification process utilized reference data from the National Institute of Standards and Technology (NIST) database, which contains more than 62,000 mass spectral patterns, and was supplemented with resources from the National Center for Biotechnology Information (NCBI).

The spectra of the unknown compounds obtained from the extract were compared against those in the NIST library. Only compounds with significant spectral matching were considered as valid phytoconstituents present in the sample (Odion *et al.*, 2024).

Data Analysis
Data obtained from the study were expressed as mean ± standard deviation and analyzed using the GraphPad Prism version software, version 10. Comparisons among groups for all measured parameters were performed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION
Figure 1 shows the composition of anti-nutritive constituents, with saponins (830.26 mg/100g) and oxalates (766.67 mg/100g) being significantly higher when compared to tannins (188.20 mg/100g) and phytates (112.54 mg/100g). The high saponin content is consistent with their role in plant defense, as well as their potential antimicrobial and cholesterol-lowering effects (Shi *et al.*, 2004; Timilsena *et al.*, 2023). However, high saponin levels can reduce nutrient bioavailability by forming complexes with proteins and minerals (Francis *et al.*, 2002), which may limit the nutritional value of *S. monostachyus* leaves if consumed in large quantities. The elevated oxalate content is a concern, as oxalates can bind to calcium, forming insoluble complexes that reduce mineral absorption and may contribute to kidney stone formation (Huynh *et al.*, 2022). Tannins may contribute to astringency and reduced protein digestibility, though their antioxidant properties are also beneficial. Phytates are the least abundant anti-nutritive constituent but still pose a risk of mineral chelation, particularly for iron and zinc (Sandberg, 2002). These anti-nutritive factors suggest that processing methods, such as boiling or fermentation, may be necessary to reduce their impact in dietary applications (Soetan and Oyewole, 2009).

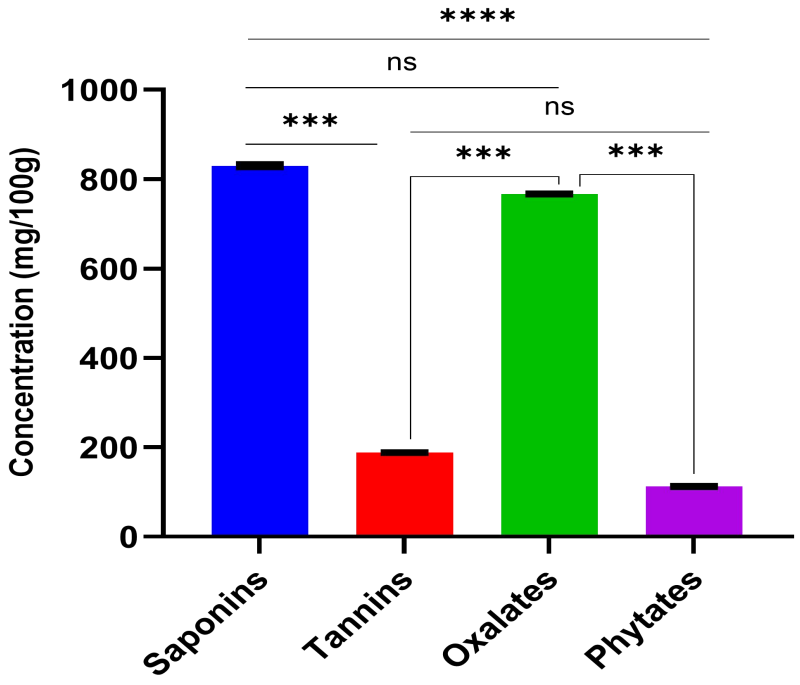

Figure 1: Anti-nutritive constituents of *S. monostachyus*. (Monkey's potato) Leaves.
ns = not significant, ✱✱✱ = significant at p <0.05

Table 1 and Figure 2 provide a detailed profile of the volatile and semi-volatile compounds in *S. monostachyus* leaf extract, identifying 21 compounds with peak

areas ranging from 1.52% to 20.90%. The most abundant compound, n-Hexadecanoic acid ($C_{16}H_{32}O_2$, 256.42 g/mol, 20.90% peak area, Rt 14.795), is a saturated fatty acid commonly found in plant extracts and known for its anti-inflammatory and antimicrobial properties (Aparna *et al.*, 2012, Onyeukwu *et al.*, 2025). Its high abundance suggests a significant contribution to the plant's bioactivity, particularly in topical or systemic applications. Isobornyl acetate ($C_{12}H_{20}O_2$, 196.29 g/mol, 11.31%, Rt 8.975) and Eugenol ($C_{10}H_{12}O_2$, 164.20 g/mol, 10.04%, Rt 8.758) are the next most abundant compounds, both of which are associated with the *Lamiaceae* family. Eugenol, a phenolic compound, is documented for its analgesic, antiseptic, and antioxidant properties (Pramod *et al.*, 2010). Isobornyl acetate, a terpenoid ester, contributes to the plant's aromatic profile and potential antimicrobial activity (Bakkali *et al.*, 2008).

The presence of terpenoids such as Caryophyllene ($C_{15}H_{24}$, 204.35 g/mol, 2.85%, Rt 9.519), Humulene ($C_{15}H_{24}$, 204.35 g/mol, 1.52%, Rt 9.908), and Phytol ($C_{20}H_{40}O$, 296.53 g/mol, 2.70%, Rt 16.111) are known for their anti-inflammatory, anticancer, and antimicrobial effects, making them valuable in pharmaceutical applications (Thoppil and Bishayee, 2011, Siddiqui *et al.*, 2024). The identification of Phenol, 3-pentadecyl ($C_{21}H_{36}O$, 304.51 g/mol, 5.14%, Rt 17.541) further corroborates the phenolic content, while compounds such as Linoelaidic acid ($C_{18}H_{32}O_2$, 280.45 g/mol, 3.74%, Rt 16.362) and Ethyl 9,12,15-octadecatrienoate ($C_{20}H_{34}O_2$, 306.48 g/mol, 6.53%, Rt 16.437) indicate the presence of fatty acid derivatives with potential nutritional and therapeutic benefits (Simopoulos, 2002).

The diversity of compounds, including fatty acids, terpenoids, phenolics, and heterocyclic compounds such as 6-Methyl-benzo[4,5]imidazo[1,2-c]quinazoline ($C_{14}H_{10}N_4$, 238.26 g/mol, 2.12%, Rt 12.643), suggests a complex chemical profile. This complexity is consistent with the GC-MS profiles of other *Lamiaceae* species, such as *Ocimum basilicum*, where similar classes of compounds are reported (Hussain *et al.*, 2008). The presence of minor compounds, such as 3-Chlorophenyl isothiocyanate (C_7H_4ClNS, 169.63 g/mol, 3.54%, Rt 15.476) indicates trace amounts of halogenated compounds, which may have specific bioactivities but require further investigation (Gribble, 2004).

The phytochemical profile of *S. monostachyus* as shown by GC-MS analysis suggests significant potential for medicinal applications, particularly due to its high levels of phenolic compounds, flavonoids, and terpenoids, which are well-established for their antioxidant, anti-inflammatory, and antimicrobial activities. The documented anti-inflammatory and analgesic effects in murine models support traditional uses in treating infections, inflammation, and oxidative stress-related conditions (Okokon *et al.*, 2016.). However, the notably high levels of anti-nutritive constituents especially saponins and oxalates suggest that dietary consumption of *S. monostachyus* leaves may require processing to mitigate their impact on nutrient bioavailability. Studies on leafy vegetables (e.g., *Colocasia*, Chinese cabbage) demonstrate that household techniques like boiling, soaking, steaming, and fermentation can reduce oxalate and tannin content by up to 80%, while saponin content is lowered through soaking, blanching, and fermentation (Kumar *et al.*, 2023) For instance, boiling for an hour reduced oxalic acid by nearly 97% in a study on *Colocasia* leaves, and fermentation also contributed to oxalate removal. Such methods could be similarly effective in improving the safety and nutritional value of *S. monostachyus*. This study confirms that *S. monostachyus* is a phytochemically rich plant with a wide array of bioactive

compounds and a complex chemical profile, indicating its potential as a source of natural therapeutic agents. Previous ethnobotanical surveys and pharmacological evaluations have reported that *S. monostachyus* exhibits antimicrobial, anti-inflammatory, antinociceptive, antipyretic, and antiplasmodial activities (Okokon *et al.*, 2016). In addition, essential oils derived from the plant have demonstrated insecticidal and larvicidal properties, further broadening its applicability (Chukwura and Iheukwumere, 2013; Atiko *et al.*, 2022). These findings collectively reinforce the relevance of *S. monostachyus* as a promising medicinal plant and justify further pharmacological investigation and standardization efforts to harness its full therapeutic potential.

Table 1. GC-MS Spectral Analysis of *S. monostachyus* Leaves

S/N	Rt	Name of Compound	Peak Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	3.162	1-Dodecanol, 2-methyl-, (S)-	1.60	$C_{13}H_{28}O$	200.36
2	8.758	Eugenol	10.04	$C_{10}H_{12}O_2$	164.20
3	8.975	Isobornyl acetate	11.31	$C_{12}H_{20}O_2$	196.29
4	9.519	Caryophyllene	2.85	$C_{15}H_{24}$	204.35
5	9.908	Humulene	1.52	$C_{15}H_{24}$	204.35
6	10.629	Benzoic acid, 4-ethoxy-, ethyl ester	2.27	$C_{11}H_{14}O_3$	194.23
7	12.220	Benzene, 2-(butenyl)-5-(1,1-dimethylethyl)-1,3-dimethyl	1.76	$C_{16}H_{24}$	216.36
8	12.643	6-Methyl-benzo[4,5]imidazo[1,2-c]quinazoline	2.12	$C_{15}H_{11}N_3$	233.27
9	12.866	Sulfurous acid, 2-ethylhexyl tetradecyl ester	1.60	$C_{22}H_{46}O_3S$	390.66
10	14.795	n-Hexadecanoic acid	20.90	$C_{16}H_{32}O_2$	256.42
11	15.476	3-Chlorophenyl isothiocyanate	3.54	C_7H_4ClNS	169.63
12	15.550	3-Fluorophenol, TBDMS derivative	6.92	$C_{12}H_{19}FOSi$	226.36
13	15.636	2-Thiophenecarboxylic acid, 4-methyl-5-nonadecyl-, methyl ester	4.40	$C_{26}H_{46}O_2S$	422.71
14	16.111	Phytol	2.70	$C_{20}H_{40}O$	296.53
15	16.362	Linoelaidic acid	3.74	$C_{18}H_{32}O_2$	280.45
16	16.437	Ethyl 9,12,15-octadecatrienoate	6.53	$C_{20}H_{34}O_2$	306.48
17	16.649	5-Hepten-1-yne, 6-methyl	2.14	C_8H_{12}	108.18
18	17.541	Phenol, 3-pentadecyl	5.14	$C_{21}H_{36}O$	304.51
19	18.377	8-Methyloctahydrocoumarin	2.94	$C_{10}H_{16}O_2$.	168.23
20	20.047	2-Propenoic acid, 2-methyl-, [1-(3-methoxypropyl)-1H-imidazol-2-yl]methyl ester	1.59	$C_{13}H_{20}N_2O_3$	238.28
21	20.368	4-Amino-5-{carboxy[2-(1H-indol-3-yl)ethyl]amino}-5-oxopentanoic acid	4.41	$C_{19}H_{24}N_4O_6$	333.34

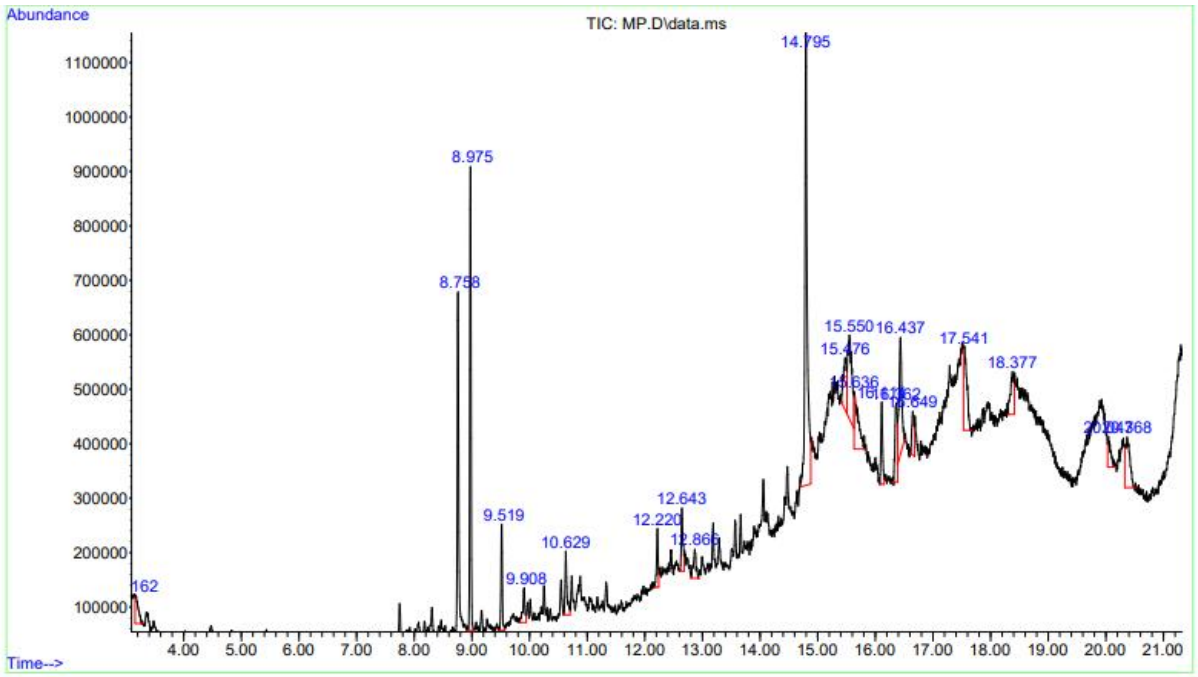

Figure 2. GC-MS Spectral Chromatogram of *S. monostachyus* Leaves

CONCLUSION

The findings in this study validate the traditional medicinal uses of *S. monostachyus* as a potent source of phytochemicals with significant therapeutic potentials. The GC-MS analysis revealed the complexity of its phytochemical profile, which includes several bioactive compounds of pharmaceutical interest that supports its antioxidant, antimicrobial, and anti-inflammatory claims in folk medicine. However, the presence of notable levels of anti-nutritional factors such as saponins and oxalates highlights the need for proper processing techniques to reduce these compounds before dietary use. Overall, *S. monostachyus* holds great promise for future pharmacological research and nutraceutical applications, provided its anti-nutritional factors are addressed during processing.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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