FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 24, December, 2026, pp 79 – 87
DOI: https://doi.org/10.33003/fjs-2026-1024-6612

Chemical Profiling of *Phyllanthus Niruri* L. Leaves by Phytochemical Screening and GC–MS Analysis

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Received: 30 Oct. 2026
Accepted: 20 Nov. 2026
Published: 25 Nov. 2026

ABSTRACT
Phyllanthus niruri is a medicinal plant widely used in traditional medicine and is recognized for its diverse pharmacological properties. This study aimed to comprehensively profile the secondary metabolites present in the ethanol leaf extract of *P. niruri* using preliminary phytochemical screening and Gas Chromatography–Mass Spectrometry (GC-MS). Fresh leaves of *P. niruri* were collected, authenticated, air-dried, pulverized, and subjected to ethanol extraction. The resulting extract was evaluated qualitatively for major classes of secondary metabolites, including alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, steroids, glycosides, and other bioactive constituents. GC-MS analysis was employed to separate and identify volatile and semi-volatile compounds based on their retention times and mass spectral fragmentation patterns, while NMR spectroscopy was used to characterize the structural features of prominent metabolites present in the extract. The phytochemical screening indicated the presence of several biologically important secondary metabolites, with the chromatographic and spectroscopic analyses providing further evidence of the chemical complexity of the leaf extract. Flavonoids was heavily detected and had the highest phytoconstituent content (510.23 mg/100g). GC-MS detected 16 compounds with the most abundant being Phytol (19.12%). The identified constituents may contribute to the reported antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and other therapeutic activities. The combined application of phytochemical screening and GC-MS offers a comprehensive approach to profiling the chemical constituents of *P. niruri* leaves. The findings provide a scientific basis for further isolation, structural elucidation, pharmacological evaluation, and potential development of *P. niruri*-derived compounds for therapeutic applications.

Keywords: Ethanol extract, GC-MS, Medicinal plants, NMR spectroscopy Phytochemical screening, Secondary metabolites, Traditional medicine

INTRODUCTION

Medicinal plants remain important sources of structurally diverse secondary metabolites with potential applications in the development of therapeutic agents and functional herbal products (Ibobo *et al*., 2025; Okom *et al*., 2025; Okpoghono *et al*., 2025). Among these plants, *Phyllanthus niruri* L., commonly known as stonebreaker, is a small herb belonging to the family Phyllanthaceae and is widely distributed throughout tropical and subtropical regions (Onakurhefe *et al*., 2025). The plant has a long history of use in traditional medicine, particularly in Ayurvedic, Chinese, Malay and African medicinal practices, for conditions associated with the liver, kidneys, urinary tract, inflammation and infectious diseases (Araújo *et al*., 2024). Scientific investigations have subsequently reported a broad range of biological activities for *P. niruri* extracts, including antioxidant, antimicrobial, antiviral, hepatoprotective, anti-inflammatory, hypoglycaemic and anti-urolithiatic activities (Onakurhefe *et al*., 2025; Shoeb *et al*., 2022; Tiwana *et al*., 2024).

The medicinal value of *P. niruri* has been associated largely with its rich phytochemical composition. Previous investigations have identified several classes of secondary metabolites in different parts of the plant, including flavonoids, lignans, tannins, phenolic compounds, alkaloids, terpenoids, coumarins, saponins and sterols (Hasan *et al*., 2024; Rani and Pooja, 2025). Structural investigations of these metabolites have historically employed spectroscopic techniques including nuclear magnetic resonance, demonstrating the importance of spectroscopic characterization in establishing the chemical identity of *P. niruri* constituents (Mediani *et al*., 2017; Meselhy *et al*., 2020).

Phytochemical screening provides an initial assessment of the major classes of bioactive constituents present in a plant extract (Ekayoda *et al*., 2026; Rani and Pooja, 2025). Studies on *P. niruri* leaves have demonstrated the presence of several of these phytochemical groups, although the qualitative and quantitative composition may vary according to geographical origin, plant age, drying conditions, extraction solvent and extraction procedure (Araújo *et al*., 2024; Hasan *et al*., 2024; Rani and Pooja, 2025). The choice of extraction solvent is particularly important because plant metabolites differ considerably in polarity (Park *et al*., 2025). Ethanol is therefore frequently employed because it can extract a

relatively broad range of polar and moderately non-polar constituents and is also suitable for preparations intended for biological investigation (Lee *et al*., 2024). Conventional phytochemical screening generally provides information at the compound-class level rather than definitive molecular identification. Therefore, chromatographic and spectroscopic techniques are required to obtain a more comprehensive chemical characterization of ethanol extracts of *P. niruri*.

Advanced analytical techniques are necessary to complement preliminary phytochemical screening and provide more detailed information about the chemical constituents of plant extracts. Gas chromatography–mass spectrometry (GC-MS) is particularly useful for profiling volatile, semi-volatile and thermally stable compounds (Frolova *et al*., 2025). The technique combines chromatographic separation with mass spectral detection, allowing compounds to be tentatively identified through their retention characteristics and mass fragmentation patterns. GC-MS investigations of *P. niruri* leaves have demonstrated the presence of diverse phytocompounds, including fatty acids, fatty-acid derivatives, alcohols and other organic compounds, highlighting the usefulness of GC–MS in chemical profiling of the plant (Ahuchaogu *et al*., 2024; Labu *et al*., 2026).

The combination of preliminary phytochemical screening and GC–MS therefore provides a more comprehensive strategy for characterizing an ethanol extract of *P. niruri* leaves. Phytochemical screening establishes broad metabolite classes while GC–MS provides chromatographic and mass-spectral information for suitable volatile or semi-volatile constituents. Integrating these approaches can improve chemical profiling, support identification of marker compounds, facilitate comparison between plant materials and provide a stronger chemical basis for subsequent biological investigations.

Despite the extensive phytochemical literature on *P. niruri*, chemical composition can vary considerably with extraction conditions, plant material and environmental factors. Moreover, identification of a compound by a single analytical platform does not necessarily constitute definitive structural confirmation. Thus, combining complementary analytical techniques and, where possible, authentic standards, retention indices and mass spectral matching is important for reliable compound annotation. The present study therefore focuses on the phytochemical screening and GC–MS characterization of ethanol extract of *P. niruri* leaves, with the aim of establishing its major phytochemical classes and generating a detailed chemical profile that can serve as a basis for understanding its potential biological and pharmacological properties.

MATERIALS AND METHODS

Plant Collection and Extraction

Fresh leaves of *P. niruri* were harvested from a farm in Alihame, Agbor, Delta State, Nigeria and authenticated at the University of Benin, Benin City with voucher number, UBH-P406. The leaves were cleaned by washing with water after which they were air dried at ambient temperature. Dried leaves were ground into fine powder and stored at room temperature in airtight glass containers. Fifty grams (50 g) of the sample were weighed and soaked in 500 mL of ethanol for 48 hours in big amber bottles with periodic shaking. The flasks were sealed with aluminum foil and left to stand at room temperature for 72 hours. The extracts were then filtered using muslin cloth and the filtrate stored at 4°C for further analysis.

Phytochemical Analysis

The ethanol extract of *P. niruri* was subjected to a qualitative phytochemical screening to determine the presence of several bioactive compounds as outlined by Harborne (1973). Among the major tests conducted were: Mayer's test, Dragendorff's test, Wagner's test, Frothing test, Fehling's test, Borntrager's test, Keller Killani's test, Lieberman test, Lieberman-Buchard's test, Salkowski's test, Lead acetate test, Ferric chloride test amd Shinoda's test. The phytochemical contents were quantitatively determined using the methods described by (Chakravarthi, 2016).

GC-MS Analysis of Extract

The sample was prepared for GC-MS analysis by dissolving 10 mg of *P. niruri* leaf ethanol extract in 1 mL of GC-MS-grade ethanol, carefully vortexing the mixture to exclude any insoluble particles. To ensure sample clarity, the clear supernatant was carefully extracted and filtered with a 0.22 µm syringe filter. The GC-MS device was then filled with 1 µL of the filtrate solution for analysis. An Agilent 7890A capillary gas chromatograph (Agilent Technologies) and a 5975C inert XL EI/CI triple-axis mass detector were used to perform the GC–MS study. An HP-5MSI fused silica capillary column (5% phenyl, 95% dimethylpolysiloxane; 0.25 µm film thickness, 90 m length, 0.25 mm internal diameter) was utilized for the separation process. The oven temperature was first set at 90°C (held for 0 minutes), ramped to 200°C at 3°C/min and held for 2 minutes, and then raised to 280°C at 15°C/min and maintained for 2 minutes. It ran for 50 minutes in total. The carrier gas, helium, was utilized at a flow rate of 1.1 mL/min. The auxiliary temperature was set at 280°C, while the intake temperature was kept at 250°C. A quadrupole temperature of 150°C, an ion source temperature of 230°C, and a mass scan range of 50–550 m/z in scan mode were the mass spectrometric parameters. By matching the mass spectra with entries in the NIST-MS Library, chemicals were identified. The peak regions in the total ion chromatogram (TIC) were used to calculate the relative fraction of each detected substance. Compound identification was verified by comparing mass spectra with several entries in the NIST library to ensure analytical reliability. Only compounds with a match quality of at least 90% were noted. Peak area normalization was used for quantitative confirmation.

Statistical Analysis

The triplicate values were displayed using the mean ± Standard Error of the Mean (SEM). The statistical analysis was conducted using SPSS version 22. Every statistical analysis was considered significant when the p-value was less than 0.05.

RESULTS AND DISCUSSION

The qualitative phytochemical screening of ethanol extract of *P. niruri* leaf is presented in Table 1. The results showed that alkaloids, flavonoids, phenolic compounds, reducing sugar, Cardiac glycosides, terpenoids, triterpenoids, steroids, tannins, saponins and anthraquinones were present in the leaves of *P. niruri*. The result revealed that flavonoids, tannins, phenolic compounds, steroids and cardiac glycosides were highly detected, terpenoids was moderately detected while saponins and reducing sugar were in trace in the extract. However, alkaloids, anthraquinones and triterpenoids were absent.

Table 1: Qualitative Phytochemical Screening of Ethanol Extract of *P. Niruri* Leaf

Phytoconstituent	Test Method	Result
Alkaloids	Mayer's Test	-
	Dragendorff's Test	-
	Wagner's Test	-
Saponins	Frothing Test	+
Reducing Sugar	Fehling's Test	+
Anthraquinones	Borntrager's Test	-
Cardiac glycosides	Keller Killani's Test	+++
Terpenoids	Lieberman Test	++
Tritepenoid	Lieberman-Buchard's	-
Steroids	Salkowski's Test	+++
Phenolic Compounds	Lead acetate Test	+++
Tannins	Ferric chloride Test	+++
Flavonoids	Shinoda's Test	+++

Heavily detected: +++; moderately detected: ++; slightly detected: +; not detected: −

Table 2 showed the quantitative phytochemical analysis of ethanol extract of *P. niruri* leaf. Flavonoids had the highest concentration with mean value of 510.23±0.63 followed by phenolic compounds with mean value of 384.38±0.47 while steroids had the least concentration with mean value of 165.36±0.35.

Table 2: Quantitative Phytochemical Screening of Ethanol Extract of *P. Niruri* Leaf

Phytoconstituent	mg/100g of dry sample
Flavonoids	510.23±0.63
Phenolic compounds	384.38±0.47
Steroids	165.36±0.35

Values are expressed as mean ± SEM of triplicate determinations

Table 3 shows the chemical components, the molecular formula, peak area and retention time of *P. niruri* leaf found in the ethanol extract assayed by GC/MS analysis. Sixteen (16) compounds in the *P. niruri* leaf ethanol extract were found by GC-MS analysis. Phytol was the most prevalent compound, with a peak area percentage of 19.12 and a retention time of 16.168. Figure 1 displays the GC-MS chromatogram, which demonstrates the presence of many bioactive chemicals at various peaks. The compounds identified in the *P. niruri* leaf ethanol extract by GC-MS analysis were further validated. Each of the 16 compounds discovered at each peak by GC-MS analysis has three metabolites identified as shown in the various spectra (Supplementary Figure S1-S16).

Table 3: GCMS Analysis of *P. Niruri* Leaves

Peak No.	Retention Time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1	4.152	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144.21	2.34
2	4.495	D-Limonene	$C_{10}H_{16}$	136.23	1.62
3	6.738	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172.26	12.91
4	9.113	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200.32	1.05
5	9.158	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200.32	12.80
6	14.405	N-Acetyl-L-leucine	$C_8H_{15}NO_3$	173.21	0.96
7	14.669	Carbonic acid, decyl 2-ethylhexyl ester	$C_{19}H_{38}O_3$	314.50	6.63
8	14.823	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	11.66
9	15.012	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.56	1.23
10	15.063	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	5.09
11	16.168	Phytol	$C_{20}H_{40}O$	296.54	19.12
12	16.351	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46	2.46
13	16.465	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	13.88
14	16.694	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	3.39
15	16.746	Longifolenaldehyde	$C_{15}H_{24}O$	220.35	3.87
16	17.032	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.53	0.99

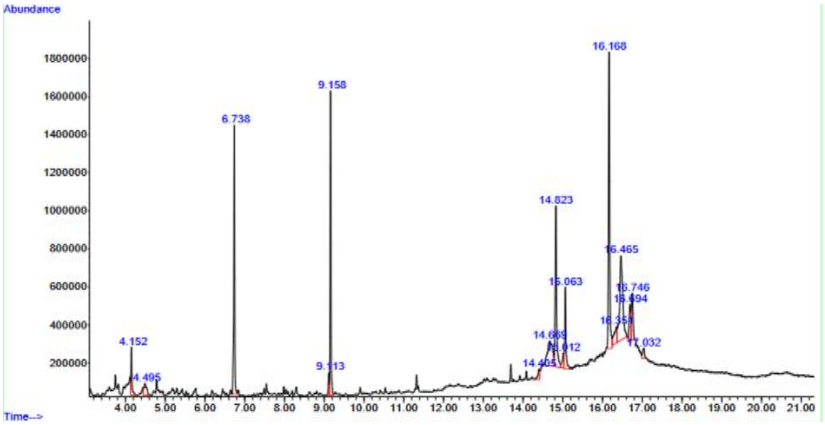


Figure 1: GCMS Chromatogram of *P. niruri* leaf ethanol extract

Discussion

P. niruri is a medicinal plant widely investigated because of its chemically diverse secondary-metabolite profile. Qualitative assay of *P. niruri* leaves have reported alkaloids, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and phenolic compounds (Rani and Pooja, 2025). Importantly, the exact chemical profile depends on the plant part, geographical origin, drying method, extraction solvent and analytical technique. In the present study, the phytochemical screening of *P. niruri* leaves revealed the presence of flavonoids, tannins, phenolic compounds, steroids, cardiac glycosides, terpenoids, saponins and reducing sugar. This profile supports the leaves' use in traditional medicine and remedies. This finding is in line with a recent study (Ojeaburu and Olasehinde, 2025) which also reported substantial quantity of flavonoids, alkaloids, saponins, tannins, anthraquinones phenols, phlobatannins, cardiac glycosides and terpenoids from ethanol extraction of fresh *P. niruri* leaves. Furthermore, this study revealed that alkaloids, anthraquinones and triterpenoids were absent in the ethanol extract of *P. niruri* leaves. This is in agreement with the study of Ojeaburu and Olasehinde (2025), which reported steroids and quinones to be absent in ethanol extract of *P. niruri* leaves. Also, phlobatannins and purine type alkaloids have been reported to be absent in aqueous extract of *P. niruri* leaves (Adebisi *et al*., 2021). The notable inconsistent variability in the types of phytochemicals present in the leaves of *P. niruri* may be attributed to geographical location, plant maturity, extraction solvent and analytical method.

The real concentration of bioactive components in *P. niruri* leaf ethanol extract is measured by quantitative analysis, total flavonoids, phenolics, and steroids were detected in the extract.. Flavonoids are a dominating class of free radical scavengers, as evidenced by their high content. The extract of *P. niruri* leaf was found to have roughly 120.5 mg/g total phenolics, 85.2 mg/g total flavonoids, 45.8 mg/g tannins, 32.1 mg/g saponins, 18.9 mg/g alkaloids, and 22.7 mg/g terpenoids, according to a prior study (Ojeaburu and Olasehinde, 2025).

Ethanol is a useful extraction solvent because it can moderately dissolve polar and some less-polar phytochemicals; moreover, its intermediate polarity permits recovery of a broad range of phenolic and flavonoid compounds as well as other secondary metabolites. In an Nuclear Magnetic Resonance metabolomics study, comparing 0–100% ethanol showed that extraction solvent concentration substantially changes the metabolite profile. In particular, 70–80% ethanol extracts showed relatively high levels of hypophyllanthin, catechin, epicatechin, rutin, quercetin and several phenolic acids, with 80% ethanol extract showing particularly strong metabolite–bioactivity associations (Mediani *et al*., 2017).

Gas chromatography–mass spectrometry (GC–MS) is particularly useful for detecting volatile, semi-volatile and thermally stable compounds. In plant extracts, derivatization may also be required to make polar compounds sufficiently volatile for GC analysis. The present study revealed 16 phytochemical compounds in the *P. niruri* leaves based on ethanol extract and phytol was the most prevalent compound (19.12 %). At the various peaks, the compounds identified in the *P. niruri* leaves ethanol extract by GC-MS analysis were further validated and each of the 16 compounds revealed three metabolites. GC–MS investigation of *P. niruri* leaves identified numerous compounds, demonstrating that the leaf contains fatty acids and their derivatives, alcohols, amines, aromatic compounds and other metabolites (Labu *et al*., 2026). Previous study (Ahuchaogu *et al*., 2024) using ethanolic extract *P. niruri* leaf detected 15 compounds and octadecanoic acid (23.84 %) and hexadecanoic acid methyl ester (18.25 %) were among the most abundant compounds detected. Another study identified approximately 30 phytocompounds in *P. niruri* leaf extracts, demonstrating the chemical diversity that can be detected by GC–MS (Chakravarthi *et al*., 2019). The current study's findings demonstrate that GC-MS analysis of P. niruri leaf ethanol extract reveals a rich profile of bioactive compounds with a variety of potential therapeutic uses. This suggests that varying extract may determine the quantity of bioactive compounds for profile discovery. This chemical profile supports the potential use of *P. niruri* in both traditional and modern medicine.

CONCLUSION

The ethanol extract of *P. niruri* leaf enhanced the identification of a diverse range of phytochemical constituents, including flavonoids, tannins, phenolic compounds, steroids, cardiac glycosides, terpenoids, saponins and reducing sugar. The GC-MS analysis further demonstrated that the ethanol leaf extract contains a range of volatile and semi-volatile compounds, providing a chemical profile that supports the presence of biologically active constituents. The identification of these compounds suggests that *P. niruri* leaves may hosts a potential source of naturally occurring compounds of pharmacological and therapeutic interest. However, the presence of a compound in the GC-MS profile does not by itself establish its biological activity or therapeutic effectiveness. The findings provide scientific support for the traditional and medicinal relevance of *P. niruri* and establish a basis for further studies involving isolation, structural characterization, biological evaluation,

and toxicological assessment of the identified compounds. Further research is therefore recommended to determine the specific activities, mechanisms of action, safety, and potential applications of the major constituents present in the ethanol leaf extract.

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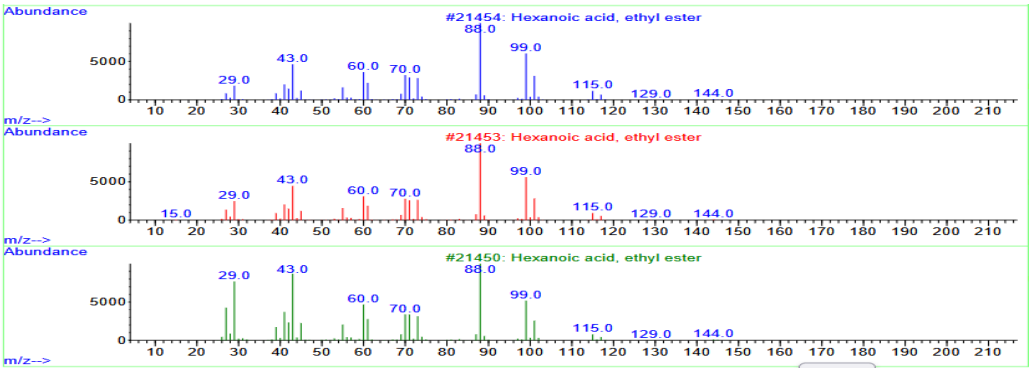


Figure S1: Chromatogram of metabolites in peak number 1

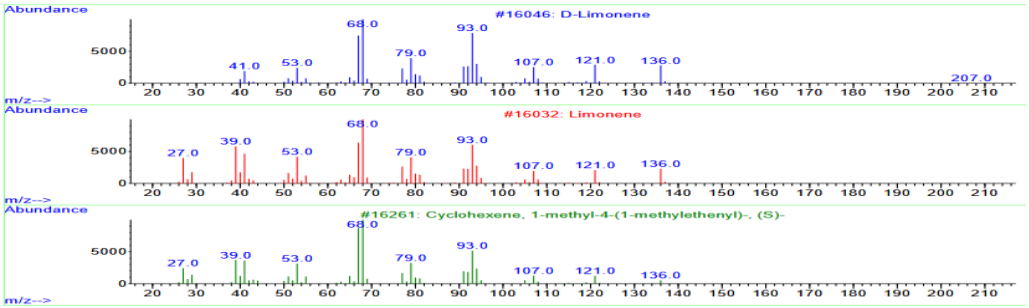


Figure S2: Chromatogram of metabolites in peak number 2

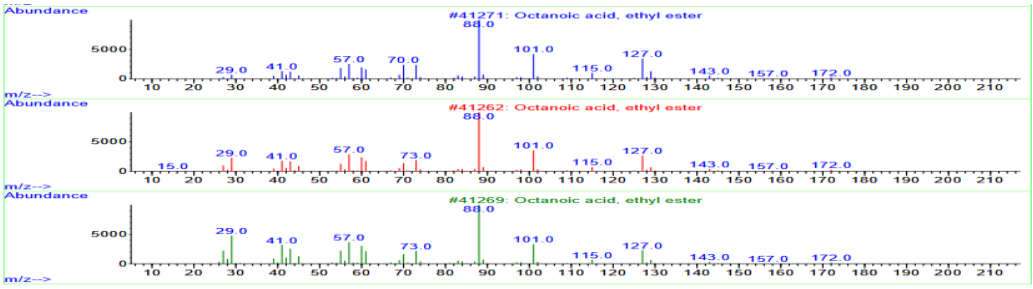


Figure S3: Chromatogram of metabolites in peak number 3

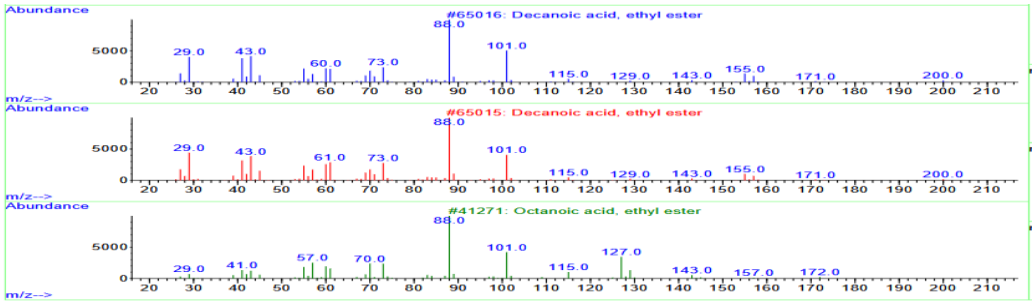


Figure S4: Chromatogram of metabolites in peak number 4

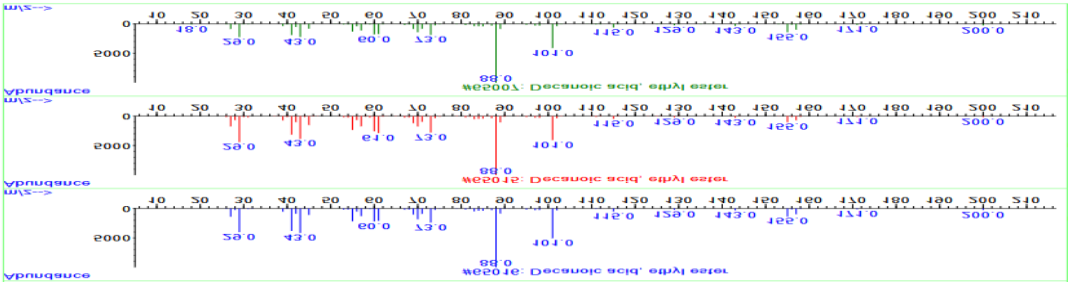


Figure S5: Chromatogram of metabolites in peak number 5

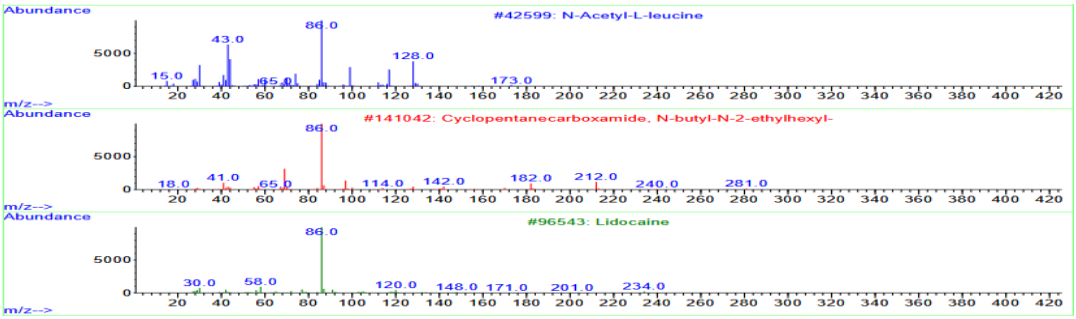


Figure S6: Chromatogram of metabolites in peak number 6

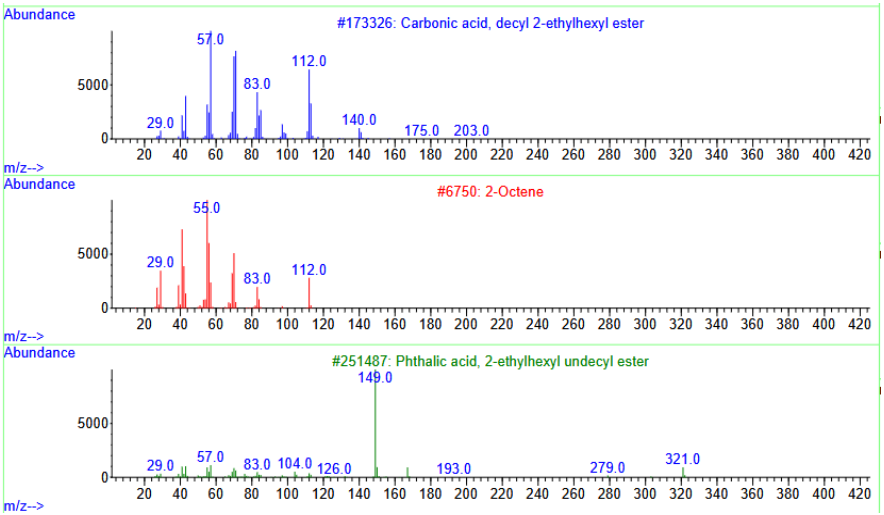


Figure S7: Chromatogram of metabolites in peak number 7

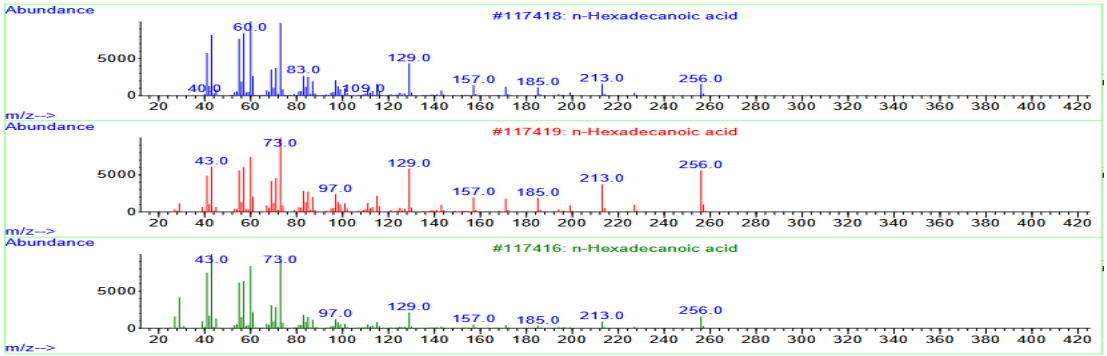


Figure S8: Chromatogram of metabolites in peak number 8

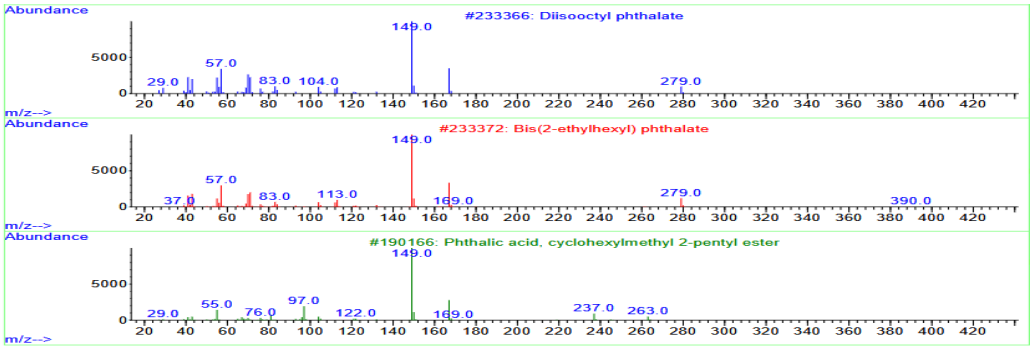


Figure S9: Chromatogram of metabolites in peak number 9

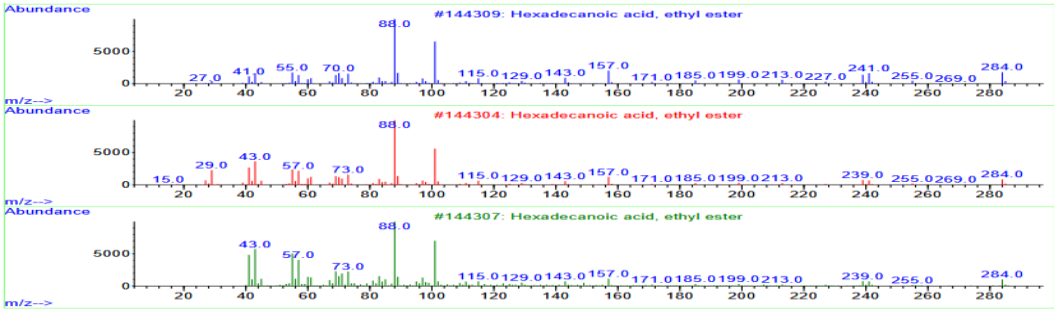


Figure S10: Chromatogram of metabolites in peak number 10

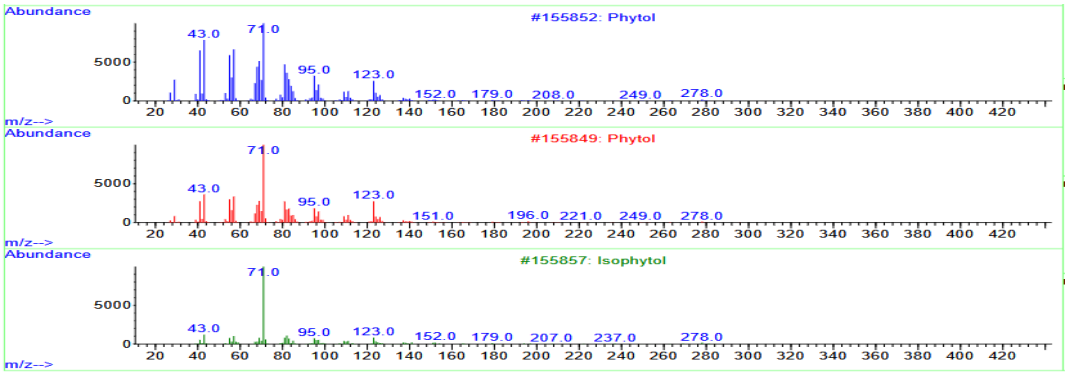


Figure S11: Chromatogram of metabolites in peak number 11

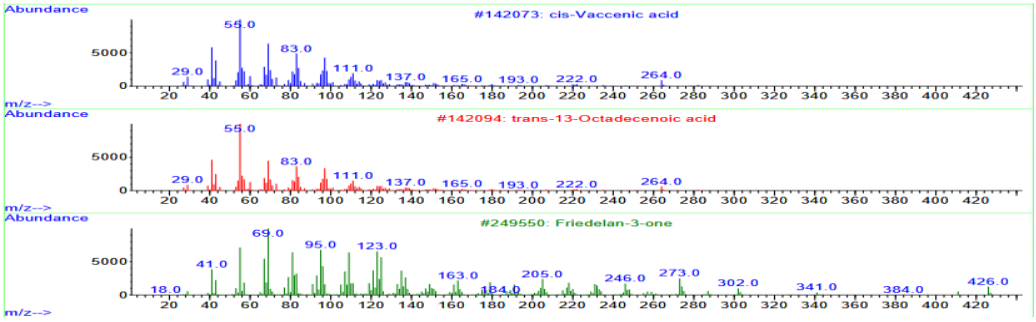


Figure S12: Chromatogram of metabolites in peak number 12

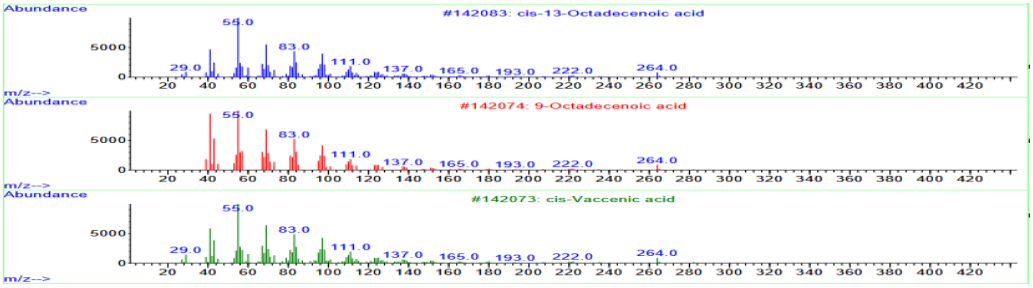


Figure S13: Chromatogram of metabolites in peak number 13

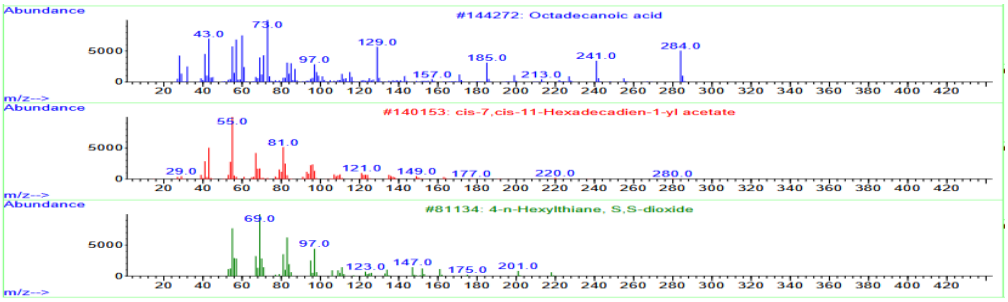


Figure S14: Chromatogram of metabolites in peak number 14

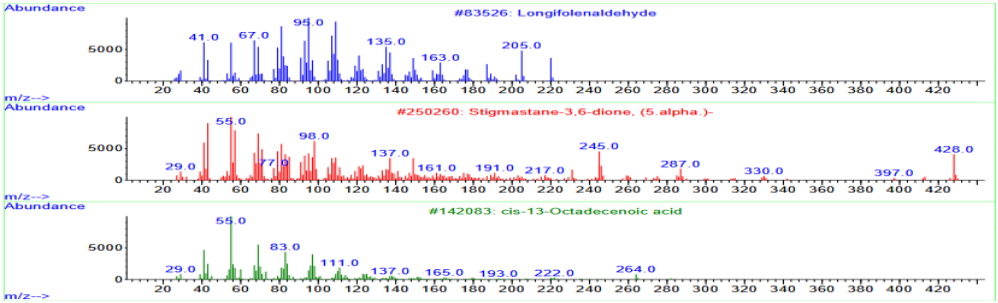


Figure S15: Chromatogram of metabolites in peak number 15

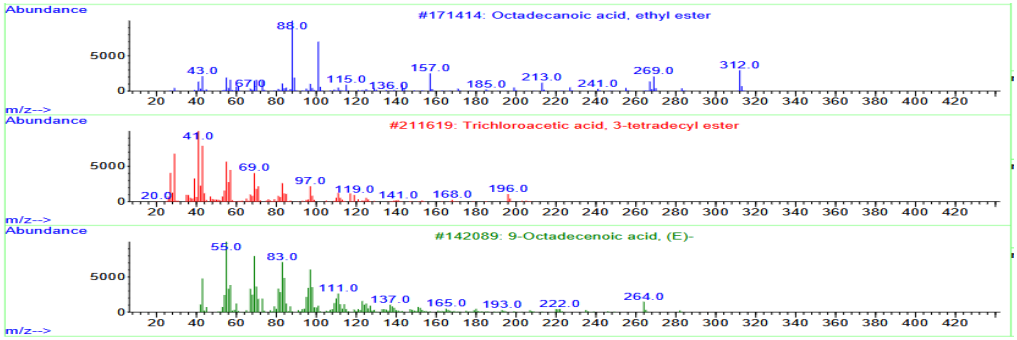


Figure S16: Chromatogram of metabolites in peak number 16

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