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Novel Bioactive Compounds in Methanolic Extract of *Andrographis paniculata* through GC-MS

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KEYWORDS

Andrographis paniculata, gas chromatography–mass spectrometry (GC MS), methanolic extract, phytoconstituents, novel compound.

ABSTRACT:

Introduction

Medicinal plants have garnered significant attention in biotechnology research due to their pivotal role in synthesizing bioactive compounds essential for various pharmaceutical applications. Historically, these plants have been primary sources of numerous biologically active substances, including therapeutic agents, natural dyes, fragrances, and flavour enhancers, especially in India. Herbal remedies often derive from raw plant extracts rich in diverse phytochemicals, predominantly secondary metabolites, which can vary considerably among different plant species. Andrographis paniculata is a well established medicinal herb known for its anti inflammatory, antiviral, and hepatoprotective properties. Despite extensive study of its major diterpenoids, andrographolide, many minor phytochemicals remain unexplored.

Objective

To uncover and profile previously unreported phytoconstituents in A. paniculata leaf extract using gas chromatography–mass spectrometry (GC MS).

Methods

Botanical identity of A. paniculata was validated via morphological and taxonomic criteria. Air dried leaves were pulverized and subjected to methanol extraction by Soxhlet and the concentrated extract underwent. The GC-MS evaluation was performed following standard procedures with a Mass Hunter GC/MS Acquisition B.07.05.2479 system (Agilent Technologies, Inc.).

Result

GC MS profiling identified 14 phytoconstituents, of which one of compounds Dodecanedioic has not been previously reported in A. paniculata based on library match quality and fragmentation patterns.

Conclusion

This study expands the phytochemical repertoire of A. paniculata by reporting one newly compound and fourteen, several compounds of which as fatty acid methyl esters and phenolic ethers contribute to its pharmacological actions. These findings support further bioactivity assays (antioxidant, cytotoxicity, anti inflammatory) to evaluate therapeutic potential and may inform isolation and structure activity relationship studies.

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1. Introduction

Andrographis paniculata commonly known as "Kalmegh" or the “King of Bitters,” is a widely used medicinal herb in traditional healthcare systems as Ayurveda, Siddha, and Traditional Chinese Medicine[8]. Indigenous to India and Southeast Asia, *A. paniculata* has long been employed for the treatment of a broad range of ailments including fever, liver disorders, respiratory infections, and inflammation[1]. The bitter principles in its leaves and aerial parts are associated with its therapeutic potential, making it a staple in herbal preparations and polyherbal formulations[6]. Numerous ethnobotanical reports describe its role in managing gastrointestinal disturbances, skin diseases, and even as a preventive remedy for chronic illnesses. Its longstanding traditional use is supported by a variety of experimental and clinical studies which confirm its bioactivity[9]. However, the specific chemical constituents responsible for these effects have not yet been fully identified or characterized in all extract forms, particularly those obtained through methanolic extraction[5]. The pharmacological value of *A. paniculata* is largely attributed to its rich content of diterpenoids, flavonoids, and polyphenols. Among these, andrographolide a labdane diterpenoid, is the most studied compound known for its anti-inflammatory, anti-viral, antioxidant, anti-cancer, immunomodulatory, and hepatoprotective activities[10]. Other bioactive constituents such as neoandrographolide, andrograpanin, and various flavones also contribute to its medicinal profile[3]. These compounds have shown significant potential in modulating biological pathways related to oxidative stress, immune response, and microbial infection. Despite the wealth of knowledge on these compounds, research is heavily focused on specific extracts or individual molecules, while the broader phytochemical landscape remains underexplored, especially when methanol is used as a solvent for extraction. Gas Chromatography–Mass Spectrometry (GC-MS) is a powerful analytical technique widely used for the separation, identification, and quantification of volatile and semi-volatile organic compounds[4]. While some studies have utilized GC-MS to analyze extracts of *A. paniculata*, they primarily focus on aqueous or ethanolic extractions. The methanolic extract, which often yields a unique and richer spectrum of secondary metabolites, has not been comprehensively studied using GC-MS. As a result, there exists a considerable knowledge gap in the identification of lesser-known or newly emerging phytoconstituents present in methanol-soluble fractions[2]. This underrepresentation has limited our understanding of the full therapeutic potential and chemical diversity of *A. paniculata*. It is essential to extend phytochemical investigations beyond known compounds and standard extraction techniques to uncover the hidden reservoir of bioactive molecules that may contribute to its pharmacological properties[11].

Study Objective and Rationale. The primary objective of this study is to perform an in-depth GC-MS analysis of the methanolic extract of *Andrographis paniculata* to identify and characterize its phytoconstituents. The rationale behind this research stems from the need to bridge the existing knowledge gap in the chemical profiling of *A. paniculata* and to expand our understanding of its full phytochemical spectrum.

By applying GC-MS to methanol-extracted samples, this study aims to uncover potentially novel or underreported compounds that may have significant biological relevance[7]. Such profiling can not only validate traditional uses with a scientific basis but also provide insights for future pharmacological studies and drug development efforts. Ultimately, the findings will help to map out a more complete phytochemical profile of this important medicinal plant and pave the way for the discovery of new bioactive agents[12].

2. Materials & Methods

2.1. Plant sample collection and Extraction of crude extract

Fresh, healthy leaves of *Andrographis paniculata* were collected from the medicinal plant garden of Amity University, Chhattisgarh. The plant material was thoroughly washed, shade-dried, and ground into fine powder. 20 grams of powdered material were subjected to extraction using 200 mL of methanol through Soxhlet apparatus for 8 hours. The extract was filtered, and the solvent was evaporated using a rotary evaporator under reduced pressure. The resulting crude extract was stored at 4 °C until further analysis by GC-MS.

2.2. GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed using a Mass Hunter GC/MS Acquisition B.07.05.2479 system (Agilent Technologies, Inc.) system or its equivalent. The analytical column used

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was an Rxi-5Sil MS capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness), coated with 5% diphenyl/95% dimethylpolysiloxane. Helium was employed as the carrier gas at a constant flow rate of 1.0 mL/min. A 1–2 µL aliquot of the methanolic extract was injected into the system in split mode at an injector temperature of 260 °C. The oven temperature was initially held at 80 °C for 4 minutes, followed by a linear ramp of 5 °C/min up to 280 °C, where it was held constant for 6 minutes. The ion source operated in electron impact (EI) mode at 70 eV. The mass detector scanned a mass range of 50 to 500 amu.

2.3. Data Analysis and Compound Identification

Chromatograms were recorded and peaks were analysed based on retention times as per second and corresponding mass spectra. Identification of compounds was carried out by comparing the acquired spectra with reference spectra available in NIST and Wiley libraries. Quantitative data were derived by calculating the relative percentage of each compound based on its peak area in the total ion chromatogram (TIC). Where applicable, retention indices were computed to aid in compound confirmation.

3. Results and Discussion

Compound Identification and GC-MS Chromatogram

The methanolic extract of the plant sample was subjected to compound profiling to identify the phytochemical constituents. The mentioned in table 1 of GC-MS analysis of the methanolic extract revealed the presence of multiple fourteen bioactive compounds, identified based on their retention times and mass spectral data. The chromatogram displayed in figure 1 shows five highest distinct peaks, indicating a complex mixture of phytochemicals within the extract. Among these five identified compounds, the major constituents were observed at retention times corresponding to significant peak areas. The most prominent compounds included Methyl 8-methyl-nonanoate; Dodecanedioic acid; 9,12-Octadecadienoyl chloride, (Z, Z); [1-(3,3-Dimethyloxiran-2 ylmethyl)-3,7-dimethylocta 2,6-dienyl] trimethylsilane, and 9,12-Octadecadiynoic acid, trimethylsilyl ester are reported as known for their pharmacological properties as anti-inflammatory, antioxidant, and antimicrobial activities. Additionally, among of these rest of mentioned table 2 of minor bioactive constituents were identified, which may contribute synergistically to the overall bioactivity of the extract. Notably, a one of the Dodecanedioic acids identified compounds appeared to be either novel and not reported in previous literature from this plant species, indicating the uniqueness of the phytochemical profile under methanol extraction. This Dodecanedioic acid is an alpha, omega-dicarboxylic acid that is dodecane in which the methyl group have been oxidised to the corresponding carboxylic acid. It has a role as an EC 1.1.1.1 (alcohol dehydrogenase) inhibitor and a human metabolite. It is an alpha, omega-dicarboxylic acid and a dicarboxylic fatty acid. It is a conjugate acid of dodecanedioate (2-) it derives from a hydride of a dodecane. Dodecanedioic acid has been reported in Drosophila melanogaster, homo sapiens, and other organism but not yet in A. paniculata confirmed by.

Overall, the mention mass spectrum in figure 2 methanol extract demonstrated a rich chemical profile, suggesting its suitability for further biological screening and potential pharmaceutical applications. These findings suggest that methanol is an effective solvent for extracting a broad spectrum of bioactive compounds, making it suitable for further pharmacological and functional studies.

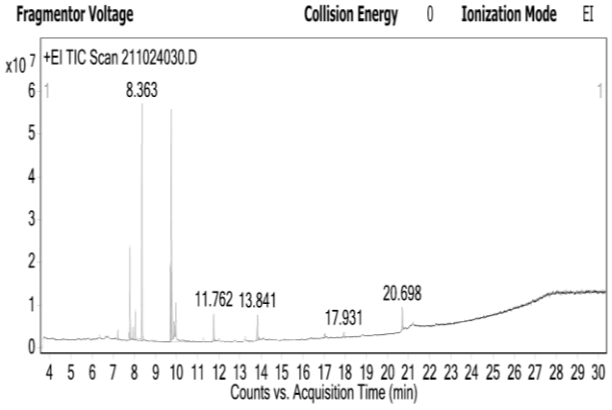

Figure 1. GC–MS analysis of high prominent peak of compounds:

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Table 1. Fourteen Compounds identified by GC–MS are listed by retention time (RT), peak height, area, % area (relative to total), symmetry factor, and peak width. The table shows RT (min), height (counts), area (counts), normalized area (%), symmetry (front/rear area ratio), and width (area/height). Data are arranged in ascending RT to aid clear visual comparison of chromatographic performance

Compund Label	Retention Time	Height	Height %	Area	Area %	Area Sum %	Symmetry	Width
Cpd 1	7.226	2348175.66	4.22	3163584.33	2.56	0.86	0.97	0.056
Cpd 2	7.796	22207661.81	39.9	30039689	24.28	8.14	2	0.077
Cpd 3	7.943	2984674.55	5.36	3106579.6	2.51	0.84	1.02	0.038
Cpd 4	8.056	7152704.48	12.85	8799543.59	7.11	2.39	0.74	0.06
Cpd 5	8.363	55665247.99	100	103595467	83.74	28.08	1.55	0.14
Cpd 6	9.72	18511776.5	33.26	23585606.5	19.06	6.39	0.59	0.045
Cpd 7	9.756	54187175.13	97.34	123712853	100	33.54	1.84	0.119
Cpd 8	9.893	3856618.81	6.93	7770700.83	6.28	2.11	1.64	0.071
Cpd 9	9.967	7926398.33	14.24	11915874.3	9.63	3.23	1.14	0.055
Cpd 10	11.762	6539455.82	11.75	14086817.6	11.39	3.82	1.15	0.143
Cpd 11	13.841	6059374.53	10.89	17972176.3	14.53	4.87	0.97	0.178
Cpd 12	17.027	1087134.47	1.95	2481565.16	2.01	0.67	0.91	0.082
Cpd 13	17.931	1300137.77	2.34	3087314.99	2.5	0.84	0.66	0.101
Cpd 14	20.698	5330163.72	9.58	15564378.8	12.58	4.22	1.65	0.129

Table 2. List of identified Compound names, molecular formula, and retention time identified in GC-MS analysis.

Compound Label	Retention Time	Compound Name	Molecular Formula
Cpd 1	7.226	13,16-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$
Cpd 2	7.796	3,7,11,15-Tetramethyl-2 hexadecen-1-ol	$C_{20}H_{40}O$
Cpd 3	7.943	17-Octadecynoic acid	$C_{18}H_{32}O_2$
Cpd 4	8.056	3,7,11,15-Tetramethyl-2 hexadecen-1-ol	$C_{20}H_{40}O$
Cpd 5	8.363	Methyl 8-methyl-nonanoate	$C_{11}H_{22}O_2$
Cpd 6	9.72	Methyl 11,12 octadecadienoate	$C_{19}H_{34}O_2$
Cpd 7	9.756	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$
Cpd 8	9.893	Phytol	$C_{20}H4_0O$

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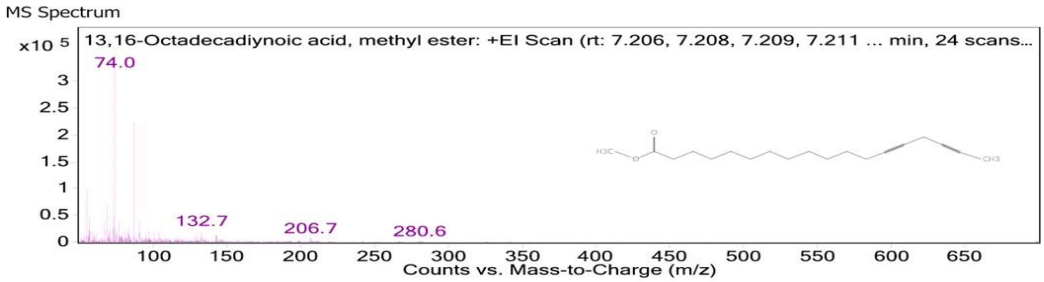
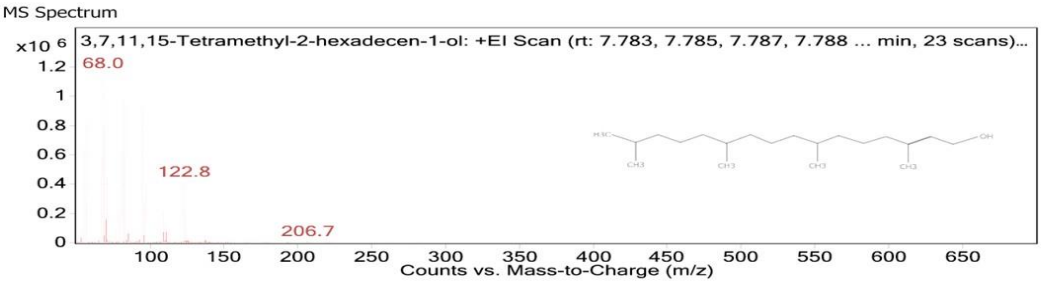
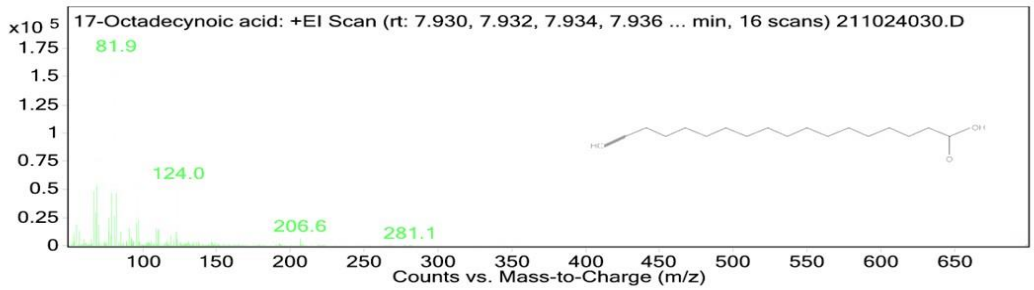
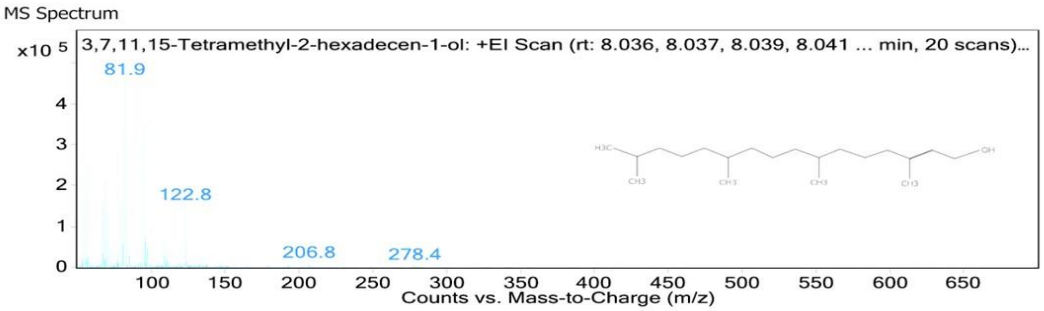
Cpd 9	9.967	Methyl 8-methyl-nonanoate	$C_{11}H_{22}O_2$
Cpd 10	11.762	Dodecanedioic acid	$C_{12}H_{22}O_4$
Cpd 11	13.841	9,12-Octadecadienoyl chloride, (Z,Z)	$C_{18}H_{31}ClO$
Cpd 12	17.027	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1 [[(trimethylsilyl)oxy]methyl]et hyl ester, (Z,Z,Z)	$C_{27}H_{52}O_4Si_2$
Cpd 13	17.931	[1-(3,3-Dimethyloxiran-2 ylmethyl)-3,7-dimethylocta 2,6-dienyl]trimethylsilane	$C_{18}H_{34}OSi$
Cpd 14	20.698	9,12-Octadecadiynoic acid, trimethylsilyl ester	$C_{21}H_{36}O_2Si$









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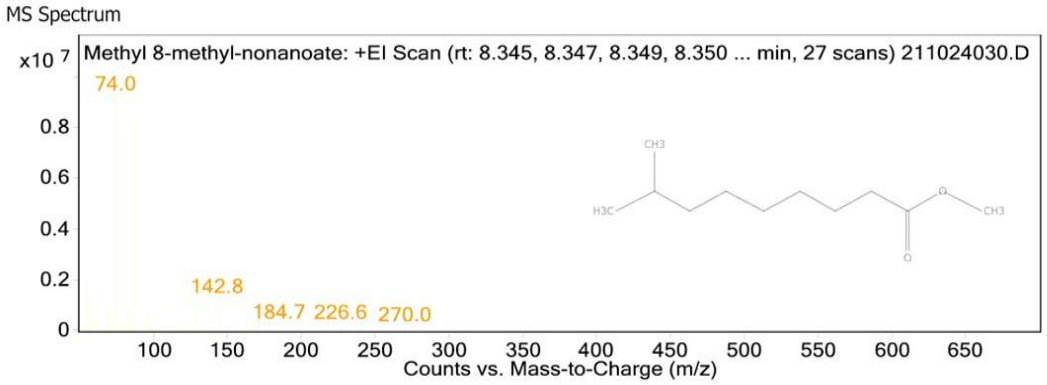

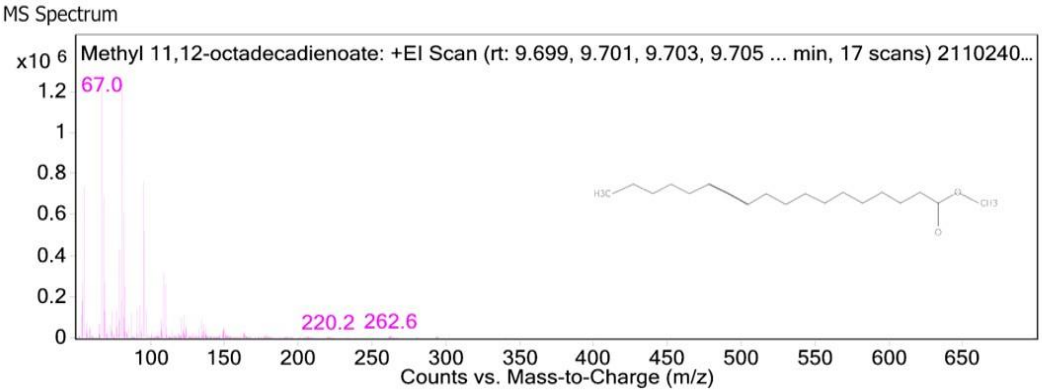

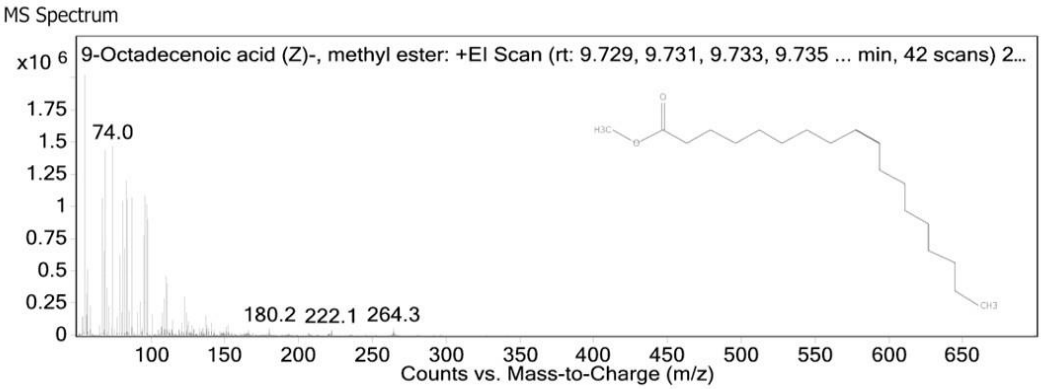

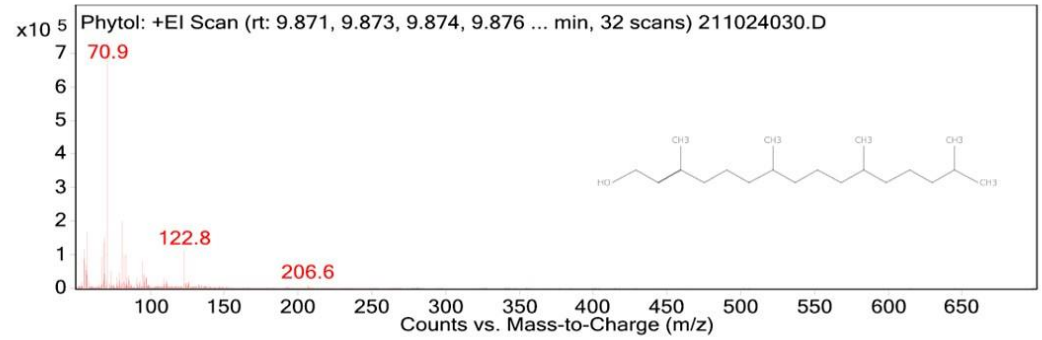

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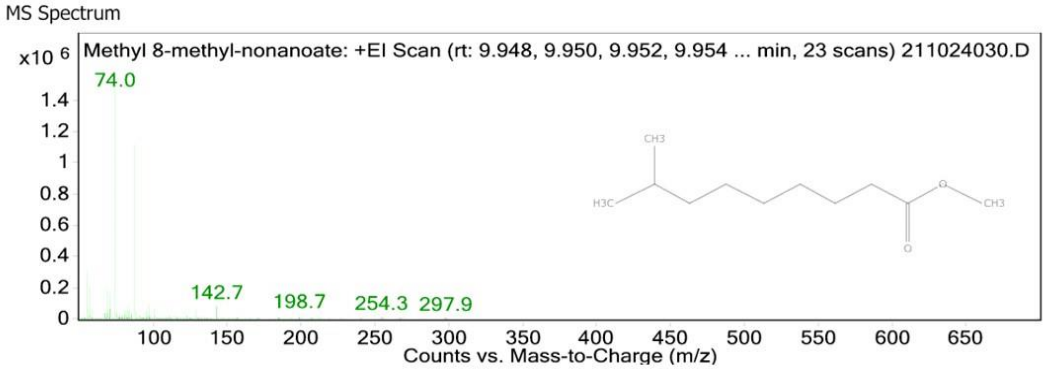

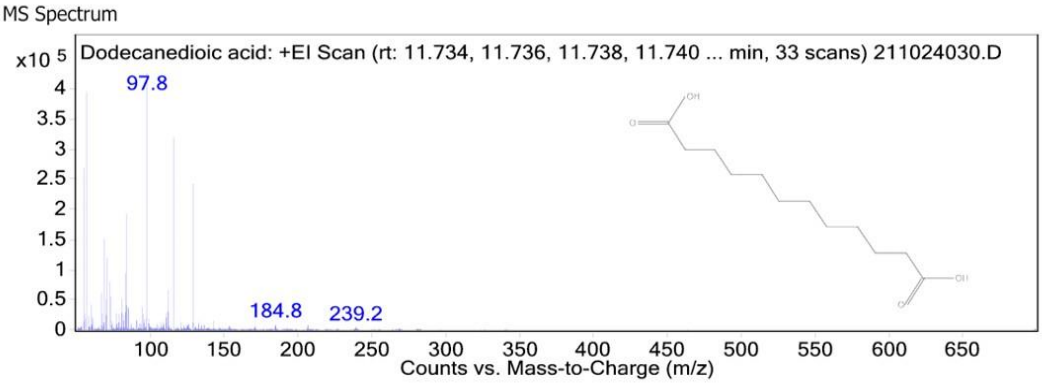

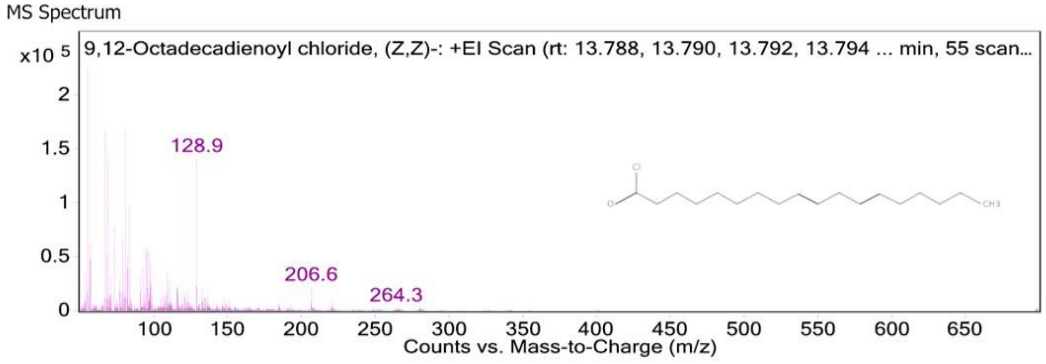

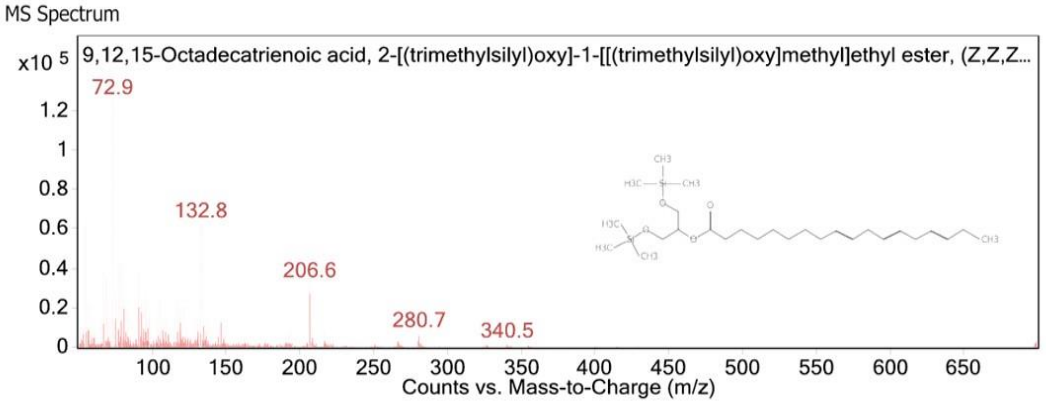

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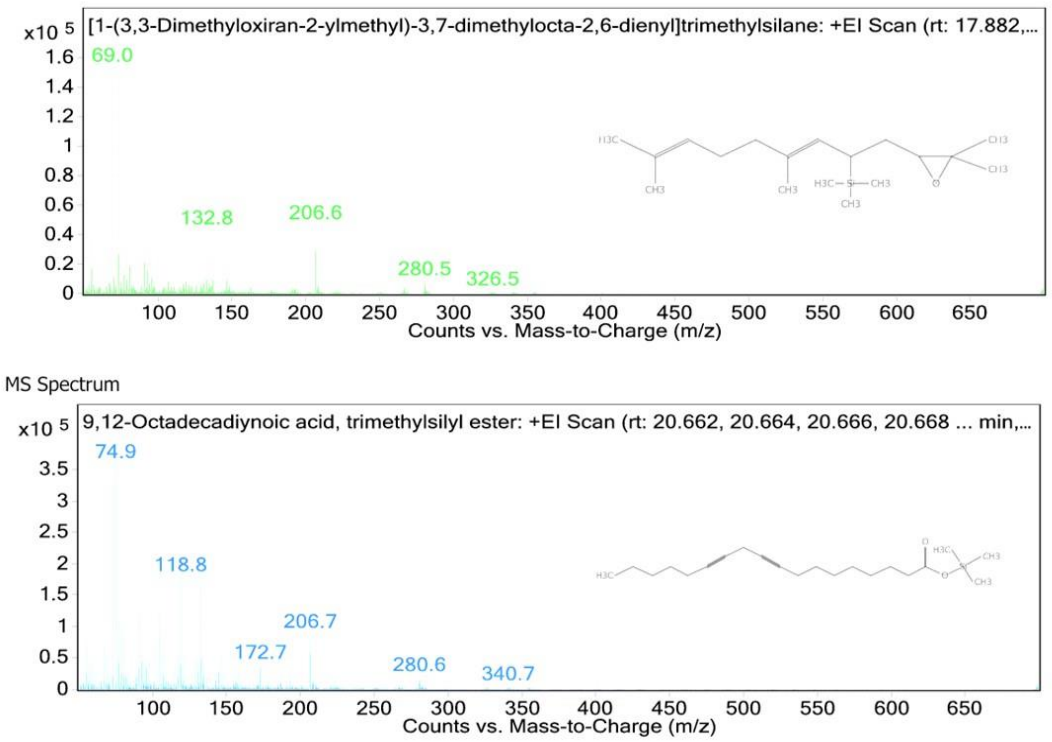

Figure 2: Mass spectra of fourteen compounds identified in the methanolic extract of *A. paniculata*."

4. Conclusion

The present study successfully employed GC-MS profiling to uncover the phytochemical landscape of the methanolic extract of *A. paniculata*. Among these fourteen identified constituents, one of them previously unreported bioactive compound was detected, alongside thirteen known bioactive compounds with various pharmacological potentials. The discovery of the novel compound adds a new dimension to the therapeutic value of *A. paniculata*, highlighting its promise as a reservoir of unexplored medicinal agents. These findings not only contribute to the existing phytochemical database of the plant but also provide a foundation for future pharmacological investigations and potential drug development based on its bioactive constituents.

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