

# Gas Chromatography-Mass Spectrometry Analysis Of Chloroform Extracts Of *Andrographis Paniculata*: Identification Of Phytochemicals And Discovery Of Novel Bioactive Compounds

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## Abstract

This study employed Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the chloroform extracts of *Andrographis paniculata*, aiming to identify its phytochemical constituents and discover novel bioactive compounds. The GC-MS analysis revealed a diverse array of phytochemicals, including phenols, aromatic carboxylic acids, esters, and other volatile and semi-volatile compounds. Notably, compounds such as 3,7,11,15-tetramethyl-2-hexadecen-1-ol and eicosanoic acid were identified, which are known for their antimicrobial and anti-inflammatory properties. The presence of these bioactive constituents underscores the therapeutic potential of *A. paniculata*, particularly in developing novel antimicrobial agents. This comprehensive phytochemical profiling enhances our understanding of the medicinal properties of *A. paniculata* and supports its traditional use in herbal medicine. *Andrographis paniculata*, a medicinal herb renowned for its therapeutic properties, was analysed using gas chromatography-mass spectrometry (GC-MS) to elucidate its phytochemical profile from chloroform leaf extracts. The study identified a complex array of volatile and semi-volatile compounds, including key bioactive such as 3,7,11,15-tetramethyl-2-hexadecen-1-ol and eicosanoic acid compounds previously reported to possess antimicrobial and anti-inflammatory activities. In total, multiple terpenoids, fatty acids, esters, and other secondary metabolites were detected, highlighting the extract's chemical diversity. Comparison across different solvent extracts affirmed that chloroform is effective in isolating non-polar phytoconstituents integral to the plant's pharmacological actions. Notably, several minor components not commonly noted in prior literature were also observed, suggesting the possibility of novel bioactive entities deserving further exploration. These results expand on the current understanding of *A. paniculata*'s biochemical composition and reinforce its potential as a source of therapeutic agents. This GC-MS-based profiling lays the groundwork for more targeted bioactivity assays, isolation of unidentified compounds, and structure-activity relationship studies to validate their pharmacological value.

**Keyword:** *Andrographis paniculata*, GC-MS, chloroform extract, bioactive compounds, phytochemical analysis, diterpenoids, flavonoids

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## 1. INTRODUCTION

*Andrographis paniculata* widely known as Kalmegh or the "King of Bitters," has been revered for centuries across traditional medical systems in Asia as Ayurveda, Traditional Chinese Medicine, Siddha, and Unani for treating ailments ranging from fever, respiratory infections, gastrointestinal disorders, and liver dysfunction to snakebites and inflammation (Akbar, S. 2011). In modern times, scientific investigations have substantiated its therapeutic value, attributing a spectrum of pharmacological activities including anti-inflammatory, antioxidant, antimicrobial, antiviral, antidiabetic, hepatoprotective, immunomodulatory, and anticancer effects to its diverse phytoconstituents. Chief among these is 'andrographolide', a bioactive labdane diterpenoid that predominates in the leaves and has been extensively studied for its anti-inflammatory, immune-stimulating, and anticancer properties, alongside numerous other therapeutic benefits (Jayakumar, T. et., 2013). However, most existing research has focused on polar extracts rich in flavonoids and diterpenoid lactones, leaving the nonpolar chloroform fraction known to solubilize terpenoids, fatty acids, esters, and aromatic carboxylic acids relatively underexplored. Preliminary GC-MS analyses of this chloroform-soluble fraction have hinted at a complex mixture of lipophilic bioactive, including neophytadiene, phytol, squalene, campesterol, and various fatty

acid esters, many of which exhibit promising antimicrobial, anti-inflammatory, antioxidant, and cholesterol-lowering activities (Kumar, S. R. et al., 2021). Therefore, a comprehensive GC-MS investigation targeting the chloroform extract holds significant potential for the discovery of novel bioactive compounds that could broaden the pharmacological landscape of *A. paniculata*. The primary aim of this study is to employ Gas Chromatography-Mass Spectrometry to systematically identify and characterize terpenoids, fatty acids, esters, and aromatic carboxylic acids present in the chloroform extract of *A. paniculata* leaves, thereby enriching our understanding of its chemical and therapeutic potential. For instance, neophytadiene, phytol, squalene, campesterol, hexadecanoic acid methyl ester, and benzyl benzoate identified within the chloroform-soluble fraction have demonstrated inhibitory effects against bacterial enzymes, free radicals, and cholesterol metabolism. Given that the chloroform extract consistently exhibits higher antimicrobial potency compared to other fractions, a detailed GC-MS profiling is justified to uncover novel lipophilic phytoconstituents. Therefore, the aim of this study is to employ GC-MS to comprehensively identify and characterize terpenoids, fatty acids, esters, and aromatic carboxylic acids in the chloroform leaf extract of *A. paniculata*, thereby expanding its phytochemical repertoire and uncovering new candidates for future pharmacological investigation. The objective of this study is to apply GC-MS for the systematic identification and comprehensive characterization of bioactive, non-polar phytoconstituents specifically terpenoids, fatty acids, esters, and aromatic carboxylic acids present in the chloroform extract of *A. paniculata* leaves using GC-MS. Utilizing GC-MS allows for rapid separation, detection, and structural elucidation of volatile and semi-volatile organic compounds by matching their unique mass spectra with established libraries such as NIST, providing high sensitivity and specificity in detecting potentially novel bioactive molecules.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

Fresh leaves of *A. paniculata* were collected from herbal garden of Amity University Chhattisgarh Raipur. The collected leaves were washed with deionized water, after dried- shade and then converted into fine raw material and used for the further experiment.

### 2.2. Sample Preparation

The stock raw material of 15 g of the powdered leaves were packed in a cellulose thimble and extracted in a Soxhlet apparatus with 150 mL chloroform for 8 hours at 60 °C. The plant sample was extracted using the Soxhlet extraction method to obtain bioactive compounds, after completion of cycle, the extract was concentrated to dryness, until resulting in a crude extract with a characteristic Odor and greasy consistency. The final crude extract was weighed for yield calculation and stored at 4 °C in amber vials until for GC-MS analysis.

### 2.3. Phytochemical Screening

Conduct preliminary qualitative tests to detect the presence of various phytochemical groups as alkaloids, flavonoids, terpenoids, saponins, and phenolics mentioned in table 2.

### 2.4. Gas chromatography mass spectroscopy Analysis

Separation and Identification of compounds were carried out by using a GC-MS system (Thermo Scientific Trace GC Ultra coupled with ISQ Single Quadrupole MS) equipped with a TG-5MS fused silica capillary column (0.1 mm film thickness). The analysis used electron ionization at 70 eV, with helium as the carrier gas at a constant flow rate of 1 mL/min. A sample volume of 1 µL was injected, with the injector temperature set at 280°C. The oven temperature was programmed to start at 40°C, increasing at 5°C per minute up to 280°C, holding for 5 minutes at the final temperature. Quantification of components was based on their relative peak area percentages. Tentative identification was achieved by comparing the mass spectra and retention times of the detected compounds with those in the NIST libraries.

**Table 1.** List of Bioactive compounds found in chloroform extract fraction *A. paniculata* of with their retention time and molecular formula

Compound Label (Cpd)	Retention Time (RT)	Compound Name	Molecular Formula
Cpd 1	6.385	Phenol, 2,4-bis (1,1 dimethylethyl)	C <sub>14</sub> H <sub>22</sub> O

Cpd 2	7.726	1,13-Tetradecadien-3-one	C <sub>14</sub> H <sub>24</sub> O
Cpd 3	7.773	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C <sub>20</sub> H <sub>40</sub> O
Cpd 4	7.932	3,7,11,15-Tetramethyl-2 hexadecen-1-ol	C <sub>20</sub> H <sub>40</sub> O
Cpd 5	8.051	3,7,11,15-Tetramethyl-2 hexadecen-1-ol	C <sub>20</sub> H <sub>40</sub> O
Cpd 6	8.366	Methyl 8-methyl-nonanoate	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>
Cpd 7	8.78	Phthalic acid, butyl tridec-2-yn-1-yl ester	C <sub>25</sub> H <sub>36</sub> O <sub>4</sub>
Cpd 8	9.717	Methyl 11,12 octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
Cpd 9	9.754	9-Octadecenoic acid (Z)-, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
Cpd 10	9.79	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>
Cpd 11	9.886	Phytol	C <sub>20</sub> H <sub>40</sub> O
Cpd 12	9.959	Methyl 8-methyl-nonanoate	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>
Cpd 13	11.757	Tetradecanedioic acid	C <sub>14</sub> H <sub>26</sub> O <sub>4</sub>
Cpd 14	13.155	Icosapent	C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>
Cpd 15	13.838	9,12-Octadecadienoyl chloride, (Z, Z)	C <sub>18</sub> H <sub>31</sub> ClO
Cpd 16	13.9	[1-(3,3-Dimethyloxiran-2-ylmethyl)-3,7-dimethylocta-2,6-dienyl] trimethyl silane	C <sub>18</sub> H <sub>34</sub> OSi
Cpd 17	16.999	Icosapent (Z, Z)	C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>
Cpd 18	17.919	Squalene	C <sub>30</sub> H <sub>50</sub>
Cpd 19	18.778	2,2-Dimethyl-6-methylene-1 [3,5-dihydroxy-1 pentenyl] cyclohexan-1 perhydro	C <sub>14</sub> H <sub>24</sub> O <sub>4</sub>
Cpd 20	20.683	cis-5,8,11,14,17 Eicosapentaenoic acid, tert butyldimethylsilyl ester	C <sub>26</sub> H <sub>44</sub> O <sub>2</sub> Si
Cpd 21	20.823	cis-4,7,10,13,16,19 Docosahexaenoic acid, tert butyldimethylsilyl ester	C <sub>28</sub> H <sub>46</sub> O <sub>2</sub> Si
Cpd 22	21.183	cis-5,8,11,14,17 Eicosapentaenoic acid, tert butyldimethylsilyl ester	C <sub>26</sub> H <sub>44</sub> O <sub>2</sub> Si
Cpd 23	22.271	1-Heptatriacotanol	C <sub>37</sub> H <sub>76</sub> O

### 3. RESULTS AND DISCUSSION

#### 3.1. Phytochemical Screening

The qualitative phytochemical screening of chloroform extract of *A. paniculata* showed the presence of alkaloids, flavonoids, terpenoids, steroids, saponins, and glycosides. Alkaloids suggest antimicrobial and antimalarial potential, while flavonoids indicate antioxidant and anti-inflammatory effects. Terpenoids and steroids contribute to immunomodulatory and hepatoprotective activities, and saponins may enhance bioavailability with mild antimicrobial action. Glycosides suggest possible cardiotoxic benefits. Tannins and phenolic compounds showed negligible presence due to their higher solubility in polar solvents. Overall, the extract is rich in non-polar bioactive compounds, supporting its traditional pharmacological uses and warranting further isolation and bioactivity studies.

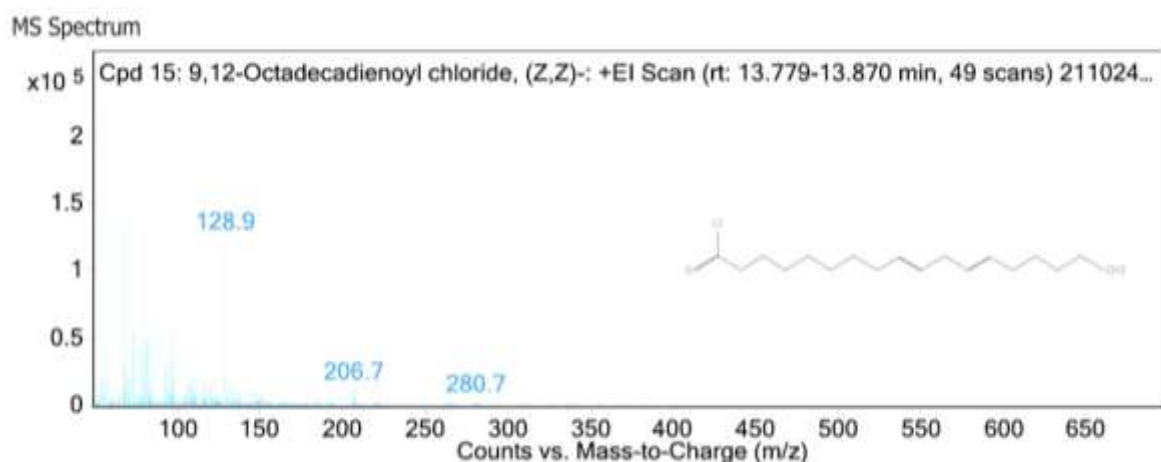
**Table 2.** Confirmation Preliminary Phytochemical Analysis of Bioactive Compounds present in extracts of acetone, chloroform and methanol of *A. paniculate* Positive Outcomes and Comparative Identification of eight Bioactive Compounds.

Part of Plant	Phytochemical compound	Methanol Extract	Acetone Extract	Chloroform Extract
Leaf	Alkaloids	+	+	+
	Flavonoids	+	+	+
	Glycosides	+	+	+
	Phenols	+	+	-
	Tannins	+	+	+

	Triterpenoids	+	+	+
	Quinones	+	-	+
	Saponins	+	+	+

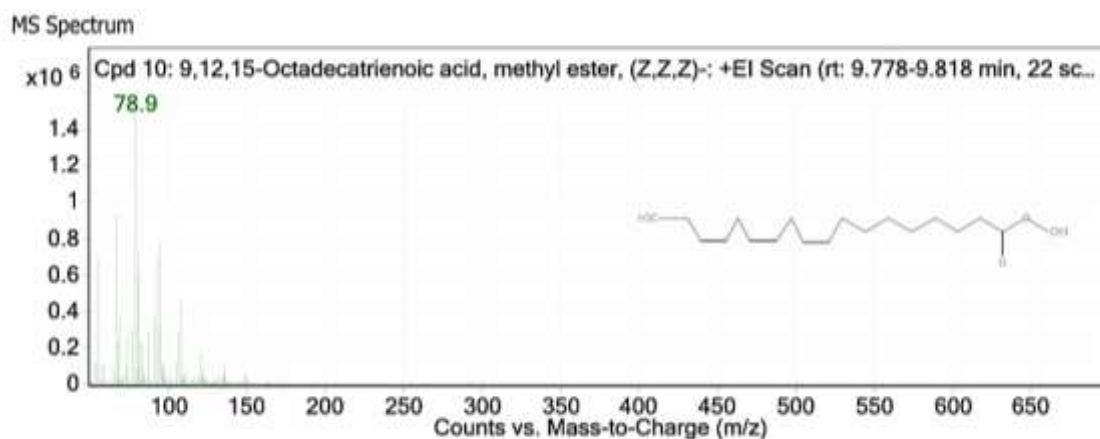
### 3.2. Gas chromatography-mass spectrophotometer Analysis composition of chloroform *A. paniculata*

In the present study, the chloroform extract of *Andrographis paniculata* was analyzed via GC-MS, through which twenty-three bioactive compounds were identified. Each of these compounds was characterized by its molecular formula, molecular weight, percentage composition (derived from peak areas), and retention time, as illustrated in figure 5. Chloroform-based extract of *A. paniculata* has shown the presence of the major twenty-three different bioactive compounds. The spectra of these bioactive compounds were matched and identified that was reported in *A. paniculata* but out of these four major bioactive compounds was not be found or reported confirmed with NIST library's software of GC-MS. The Newly four compounds were identified are (9,12-Octadecadienoyl chloride, (Z, Z)); (9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)); (Tetradecanedioic acid); and 1-(Heptatriacotanol). The first bioactive compound 9,12-Octadecadienoyl chloride, (Z, Z) is a methyl ester derivative of the essential omega-3 fatty acid  $\alpha$ -linolenic acid, characterized by its three cis-configured double bonds. In nature, it functions both as a plant metabolite and as an attractant for insects. Chemically related to its parent fatty acid, it has been detected in several species, including *Mikania goyazensis* and *Anchietea salutaris*, highlighting its occurrence across diverse organisms but not be found or reported in *A. paniculata* data were mentioned in figure 1.



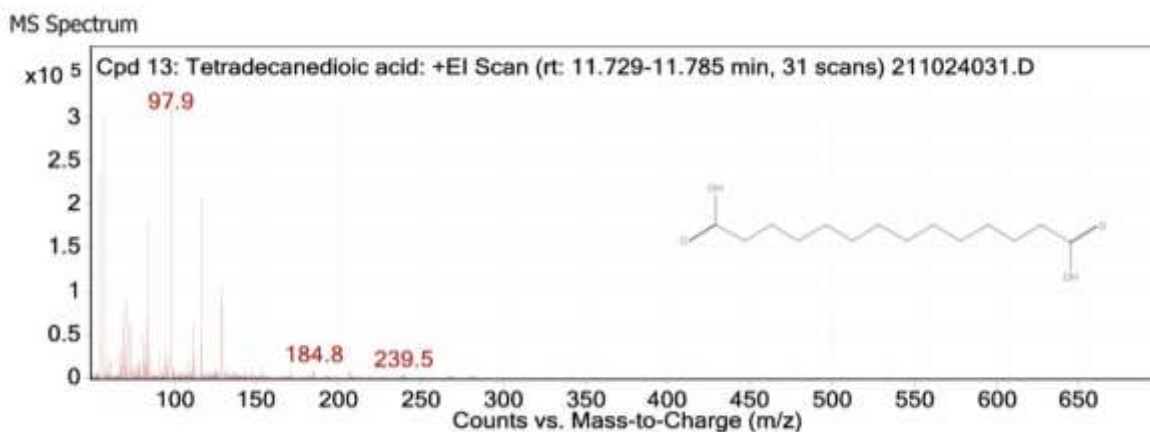
**Figure 1.** Electron ionization (EI) mass spectrum of 9,12-Octadecadienoyl chloride (Z, Z) obtained by gas chromatography-mass spectrometry (GC-MS). The total ion chromatogram (TIC) shows a prominent peak at a retention time of approximately 20.7 minutes, corresponding to the compound's elution. In the mass spectrum, the molecular ion peak at  $m/z$  298 represents the intact  $[C_{18}H_{31}ClO]^+$  fragment ( $M^+$ ), enabling confident confirmation of the compound's identity.

The second bioactive compound figure 2 were identified, methyl  $\alpha$ -linolenate (9,12,15-octadecatrienoic acid methyl ester), is a polyunsaturated fatty acid methyl ester formed from  $\alpha$ -linolenic acid, a common plant metabolite. It has the molecular formula  $C_{19}H_{32}O_2$  and a molecular weight of 292.46 g/mol. This molecule consists of an 18-carbon chain with three cis double bonds located at positions 9, 12, and 15, giving it its (Z, Z, Z) configuration, and it terminates with a methyl ester group. Methyl  $\alpha$ -linolenate is widely used in biochemical studies, particularly for fatty acid profiling, due to its high purity and standardization. It also serves important roles in plant systems and has been identified as an insect attractant. Physiochemically, it is characterized by its oily, nonpolar nature, exhibiting low solubility in water, a high octanol-water partition coefficient, and a boiling point near 723 K. This compound has been found in various species, including *Mikania goyazensis* and *Solanum tuberosum*, reflecting its distribution across multiple plant sources.



**Figure 2.** Gas chromatography–mass spectrometry (GC–MS) characterization of methyl 9,12,15-octadecatrienoate. The chromatogram displays a distinct peak at the compound’s retention time (5 sec, based on non-polar column data) Its corresponding mass spectrum (electron ionization) reveals the molecular ion ( $M^+$ ) and diagnostic fragment ions (prominent base peak fragments typically observed for such methyl esters), confirming its identity by matching known fragmentation patterns.

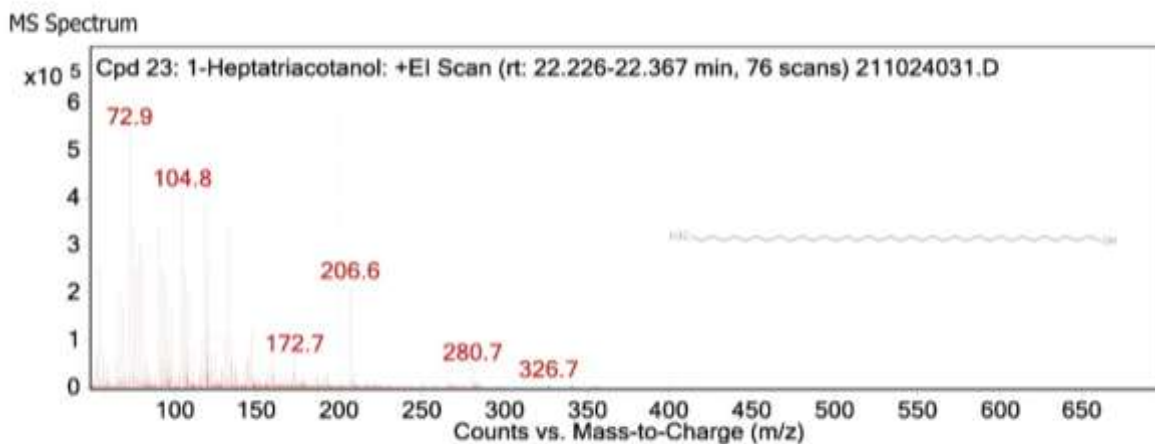
Tetradecanedioic acid, also known as 1,14-tetradecanedioate is a long-chain  $\alpha$ ,  $\omega$ -dicarboxylic fatty acid ( $C_{14}H_{26}O_4$ ) formed by oxidizing both terminal methyl groups of tetradecane. It functions as an endogenous human metabolite and represents the conjugate acid of the tetradecanedioate dianion. This molecule appears naturally in species such as *Drosophila melanogaster*, *Arabidopsis thaliana*, and *Pinus radiata*. Deriving from the hydride of tetradecane, it belongs to the chemical class of long-chain dicarboxylic acids details were mentioned in figure 3.



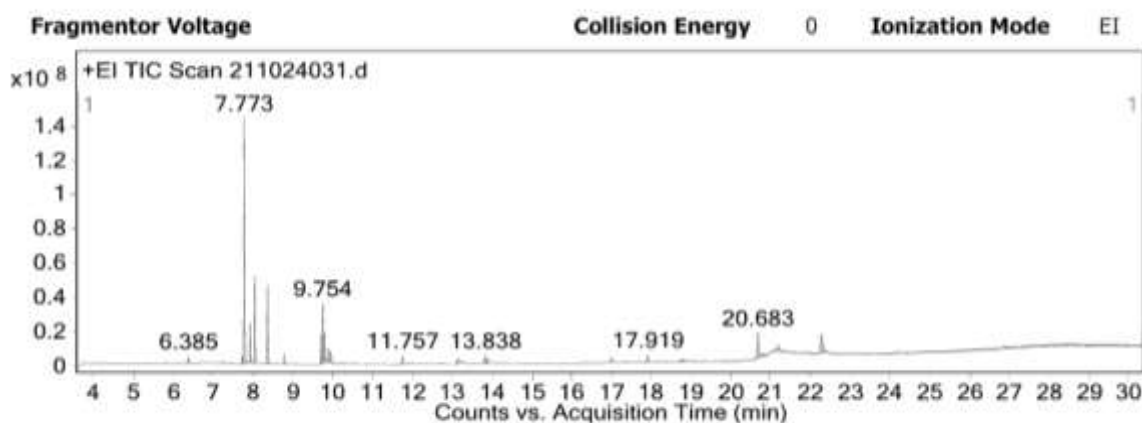
**Figure 3.** Gas chromatography–mass spectrometry (GC–MS) analysis of tetradecanedioic acid, derivatized as the bis-TMS (trimethylsilyl) ester (2 TMS derivative,  $C_{14}$  derivative formula  $C_{20}H_{42}O_4Si_2$ , molecular weight 402.72 Da), using a 5 % phenyl–95 % dimethylpolysiloxane capillary column (based on retention index of  $\sim 2282.49$ ). The total ion chromatogram (TIC) displays the retention time for the derivatized molecule, while the corresponding mass spectrum shows characteristic fragment peaks resulting from electron-ionization (EI) fragmentation of the bis-TMS derivative

The 1-Heptatriacontanol, also known as hentriacontan-1-ol ( $C_{37}H_{76}O$ ), is a long-chain saturated fatty alcohol commonly identified in plants like *Nigella sativa*, *Foeniculum vulgare*, and *Blumea laciniata*. This compound, with a molecular weight around 537 g/mol and a log P value of approximately 13, is characterized by its highly hydrophobic, paraffinic structure containing a single terminal hydroxyl group. Due to its large size and lipophilicity, 1-heptatriacontanol exhibits various biological activities. Research indicates its potential anticancer effects, particularly from plant-based extracts, and its structural profile suggests possible benefits in regulating lipid metabolism, including anti-hypercholesterolemic actions. Although it remains less explored compared to shorter-chain fatty alcohols, its occurrence in multiple medicinal herbs and promising pharmacological properties highlight its value for future studies on

bioactive compounds. Additionally, this mentioned figure 4. fatty alcohol has been reported in *Halocharis hispida*, further supporting its distribution in nature.



**Figure 4.** Gas chromatography–mass spectrometry (GCMS) analysis of 1-heptatriacontanol. The chromatogram exhibits a single prominent peak corresponding to 1-heptatriacontanol, confirming its retention behavior in the GC phase. The inset mass spectrum reveals the characteristic molecular ion peak and fragment patterns typical of a long-chain alcohol, facilitating molecular identification and structural confirmation. The seven highly concentration of bioactive compound were identified as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol was found to be highest peak mentioned in figure 5 and reported for their pharmaceutical application. The chemical name 3,7,11,15-tetramethyl-2-hexadecen-1-ol refers to a molecule also known as phytol. It is a diterpenoid alcohol, meaning it is a type of alcohol derived from four isoprene units. Phytol is a colourless to yellow oily liquid, soluble in organic solvents but insoluble in water. It's a component of chlorophyll and can be obtained by alkaline hydrolysis of chlorophyll. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol has been reported in *Acanthospermum australe*, *Lindera glauca* and other organism with data available also reported for natural product. The 9-Octadecenoic acid (Z)-, methyl ester Methyl 9-octadecenoate is a fatty acid methyl ester has been reported in *Antrodia cinnamomea*, *Beilschmiedia erythrophloia* and other organism with data available. The cis-5,8,11,14,17 Eicosapentaenoic acid, tert butyldimethylsilyl ester the chemical name 3,7,11,15-tetramethyl-2-hexadecen-1-ol refers to a molecule also known as phytol. It is a diterpenoid alcohol, meaning it is a type of alcohol derived from four isoprene units. Phytol is a colorless to yellow oily liquid, soluble in organic solvents but insoluble in water. It's a component of chlorophyll and can be obtained by alkaline hydrolysis of chlorophyll. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol has been reported in *Acanthospermum australe*, *Lindera glauca* and other organism with data available reported for natural product. Other 2,4-Di-tert-butylphenol (2,4-DTBP), also known as 2,4-bis(1,1-dimethylethyl) phenol, is a lipid-soluble phenolic compound commonly found in nature and used industrially. It naturally occurs in numerous plants (*Pinus granatum*, *Corymbia citriodora*) as well as fungi and bacteria, and is also detected in water and sediment due to pesticide degradation. In the lab and field, 2,4-DTBP has demonstrated strong antioxidant, antimicrobial, and antifungal activities. However, it can mimic estrogen, raising concerns about potential hormone-disrupting effects Squalene, also known as trans-squalene, is a slightly yellow, clear liquid with a faint odour and a density of approximately 0.858 g/cm<sup>3</sup>. Chemically, it is a triterpene composed of a 30-carbon structure with six double bonds positioned at carbons 2, 6, 10, 14, 18, and 22, all in the (E) configuration. Squalene functions as a metabolite in humans, plants, yeast (*Saccharomyces cerevisiae*), and mice. Naturally sourced from shark liver oil, squalene acts as an intermediate in cholesterol biosynthesis and belongs to the isoprenoid family. It is resistant to lipid peroxidation, which contributes to its protective role in skin health. In the human body, it is widely distributed across tissues and is generally transported in the bloodstream linked with very low-density lipoproteins (VLDL). Additionally, squalene is under investigation as a supportive therapy in cancer treatment due to its biological properties. It has been identified in plants such as *Erythrophleum fordii* and *Amaranthus hybridus* among others. According to the Yeast Metabolome Database (YMDB) and natural products occurrence data, squalene is a known metabolite of *Saccharomyces cerevisiae* and other organisms, and in analytical studies, it has been detected along with trace levels of other natural triterpenes.



**Figure 5.** Gas Chromatography-Mass Spectrometry (GC-MS) chromatogram of chloroform extract of *A. paniculata*. The prominent peak at retention time 5 minutes corresponds to detected compound representing the highest nine intensity peak in the spectrum.

### 3.2.1. Nature and biological activity of identified compound from chloroform-based extract of *A. paniculata*

The detection of rest of nine-teen bioactive compounds in the chloroform-based extraction of *A. paniculata* supports its chemical diversity and biological activities, consistent with findings from earlier studies table 2.

**Table 2.** List of bioactive compounds reported and founded in *A. paniculata*

S.No.	Bioactive Compounds	Class of Compounds	Bioactive Properties	References
1	1,1 Tetradecadien-3-one	ketone	Termite defensive secretion component	(Chuah et al., 1990)
2	Methyl 8-methyl-nonanoate	fatty acid	Anti-inflammatory activity	Sirilertpanich et al. (2024), and (Shrivastava et al., 2025)
3	Phthalic acid, butyl tridec-2-yn-1-yl ester	phthalic acid	Antibacterial, antifungal properties	(Shkondrov, A. et al., 2021)
4	Methyl 11,12 octadecadienoate	fatty acid	Antioxidant Activity	(Kibungu, W. C. et al., 2021)
5	Phytol	Acyclic diterpenoids	Antioxidant & Pro-inflammatory Modulation	(Islam et al. 2018),
6	Methyl 8-methyl-nonanoate	Fatty acid	anti-obesity activity	(Kaypetch, R., et al., 2023)
7	Icosapent	ethyl	Anti-Inflammatory & Pro-Resolving Actions	(Ballantyne, C. M et al., 2012).
8	[1-(3,3-Dimethyloxiran-2-ylmethyl)-3,7-dimethylocta-2,6 dienyl] trimethylsilane	Epoxide	anti-cytomegalovirus (CMV) activity	(Noha, K., et al. 2018)
9	Icosapent (Z, Z)	fatty acids	Anti-Inflammatory & Pro-Resolving Actions	(Souza, P. R et al., 2016).
10	2,2-Dimethyl-6-methylene-1 [3,5-dihydroxy-1 pentenyl]cyclohexan-1 perhydro	terpenoid	antibacterial, anti-inflammatory, cytotoxic, antioxidant,	(Stevenson, P. C et al., 2007)

11	cis-4,7,10,13,16,19 Docosahexaenoic acid, tert butyldimethylsilyl ester	fatty acid	Anti-cancer, Anti-inflammatory / Immunomodulatory	(Dumont, A et al., 2019)
12	cis-5,8,11,14,17 Eicosapentaenoic acid, tert butyldimethylsilyl ester	fatty acids	Cardiovascular Benefits, Anti-inflammatory Effects, Precursor to Prostaglandins and Thromboxanes	(Muller, T et al., 2019)

## 5. CONCLUSION

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of chloroform extracts of *A. paniculata* has led to the identification of several bioactive phytochemicals with notable pharmacological properties. Compounds as hexadecanoic acid and 2,4-di-tert-butylphenol exhibit antioxidant activities, while others hexadecanoic acid methyl ester demonstrate antimicrobial properties. These findings underscore the therapeutic potential of *A. paniculata* in addressing various health conditions, including microbial infections, inflammation, and diabetes mellitus. The study's outcomes highlight the significance of GC-MS as a robust analytical tool for phytochemical profiling, facilitating the discovery of novel compounds with potential medicinal applications. The presence of diverse bioactive constituents in the chloroform extract of *A. paniculata* supports its traditional use in herbal medicine and paves the way for future research into its clinical efficacy and potential drug development. In conclusion, the GC-MS analysis of *Andrographis paniculata* chloroform extracts has successfully identified multiple phytochemicals with promising bioactivities. These findings contribute to the growing body of evidence supporting the plant's medicinal value and encourage further exploration into its therapeutic applications.

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