

GC-MS Profiling of Phytoconstituents from *Bauhinia variegata* Linn. bark: Identification of Triterpenoids and Bioactive Secondary Metabolites.

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ABSTRACT

The identification of bioactive secondary metabolites is crucial for the pharmacological validation of medicinal plants. In this study, an Agilent 7890A/5975C GC-MS system equipped with a DB-5MS column was employed to profile *Bauhinia variegata* Linn. bark extract. The oven program ranged from 60 °C to 300 °C, and compounds were identified via the NIST 4.0 library. Chromatographic analysis resolved 21 distinct phytoconstituents. The major component was identified as 9,19-Cyclolanost-24-en-3-ol (12.29%), followed by significant concentrations of pentacyclic triterpenoids, including alpha-amyrin (6.85%) and beta-amyrin (1.86%). The method effectively separated isomeric triterpenes and late-eluting sterols like gamma-sitosterol (3.29%). The results demonstrate a diverse chemical profile rich in anti-inflammatory markers, providing a standardized chemical baseline for future therapeutic investigations.

KEYWORDS: *Bauhinia variegata* Linn., Secondary metabolites, GC-MS, chemical profile.

1. INTRODUCTION

Historically, medicinal plants have served as the cornerstone of global healthcare and the primary source for the development of modern pharmacopoeias. Despite advancements in synthetic chemistry, botanical sources continue to account for roughly one-fourth of all contemporary therapeutic agents [1]. These plants act as sophisticated biological systems, producing a diverse range of secondary metabolites—including terpenes, alkaloids, and phenolic derivatives—that demonstrate potent antimicrobial, antioxidant, and anti-inflammatory properties [2].

However, the inherent chemical diversity of plant matrices presents a significant analytical hurdle. Crude extracts often contain hundreds of volatile and semi-volatile compounds, requiring high-resolution techniques to accurately link traditional therapeutic applications with specific chemical profiles [3]. In this context, Gas Chromatography-Mass Spectrometry (GC-MS) represents the definitive standard for profiling volatile organic compounds and lipophilic constituents [4]. By merging the separation efficiency of chromatography with the sensitive detection of mass spectrometry, GC-MS enables the definitive identification of metabolites through comparison with standardized spectral databases like NIST and Wiley [5].

2. MATERIALS AND METHODS

2.1. Plant Material and Extraction

The taxonomically authenticated *Bauhinia variegata* Linn. bark powder purchased from Triphal consumer products pvt ltd. Uttar Pradesh. 2g of powder is extracted with 50 ml of solvents such as petroleum ether with gentle stirring for 72 hours. The sample is kept in dark for 72 hours with intermittent shaking. The resulting isolate was filtered through Whatmann No.1 filter paper and prepared for gas chromatography-mass spectrometry (GC-MS) analysis.

2.2. Instrumentation and Chromatographic Conditions

The chemical characterization was performed using an Agilent 7890A Gas Chromatograph coupled with an Agilent 5975C Mass Spectrometer. Separation was achieved using an Agilent DB-5MS capillary column 30m / 0.25mm internal dia / 0.25micron film thickness. High-purity helium (99.999%) was employed as the carrier gas at a constant flow rate of 1 mL/min. The sample 1 µL was introduced via an automated injector in split mode (10:1 ratio), with the injector temperature maintained at 250 °C.

2.3. Oven Temperature Program

The GC oven temperature was precisely controlled to ensure optimal separation of phytoconstituents. The program commenced at an initial temperature of 60 °C (held for 1 min), followed by a linear ramp of 15 °C/min until reaching a final temperature of 300 °C. This final temperature was maintained for an isothermal hold of 30 min to ensure the elution of high-molecular-weight sterols and triterpenoids.

2.4. Mass Spectrometry Parameters

The mass spectrometer was operated in positive electron ionization (EI) mode with an ionization energy of 70 eV. The ion source temperature was set to 200 °C. To protect the detector filament, a solvent delay was programmed. The MS was configured to operate in full scan mode, recording fragments across a mass-to-charge ratio range of m/z 35 to 500 Da.

2.5. Identification and Quantification

Data acquisition and processing were conducted using LabSolution software. Phytoconstituents were identified by comparing their resulting mass spectra and retention indices with the NIST 4.0 (National Institute of Standards and Technology) library database. The relative concentration of each identified component was expressed as a percentage of the total peak area, calculated using the following formula:

$$\text{Relative concentration (\%)} = \text{Area of specific peak} / \text{Total area of all peaks} \times 100$$

RESULTS AND DISCUSSION

3.1. Chromatographic Profiling and Chemical Composition

The GC-MS analysis of the plant extract, utilizing an Agilent 7890A/5975C system equipped with a DB-5MS column, yielded a complex phytochemical profile. A total of 20 distinct compounds were resolved and identified based on their mass spectra and retention indices. The extract was characterized by a diverse range of secondary metabolites, predominantly comprising pentacyclic triterpenoids, phytosterols, and fatty acids.

The chromatographic conditions provided excellent resolution, particularly for structurally similar triterpene isomers. For instance, beta-Amyrin (Rt: 11.597 min) and alpha-Amyrin (Rt: 15.197 min) were clearly baseline-separated, demonstrating the efficiency of the DB-5MS stationary phase for isomeric differentiation.

3.2. Major Phytoconstituents

The most abundant component identified was 9,19-Cyclolanost-24-en-3-ol, representing 12.29% of the total relative peak area (Area: 1,113,570). Given its high concentration, this tetracyclic triterpene may serve as a primary bioactive marker for this species.

The extract also exhibited a significant concentration of pentacyclic triterpenoids, including alpha-Amyrin (6.85%), Friedelan-3-one (4.44%), and beta-Amyrin (1.86%). These compounds are well-regarded in pharmacological literature for their potent anti-inflammatory and antioxidant activities.

3.3. Sterol and Organic Acid Content

Significant phytosterol content was observed, characterized by Lanosterol (4.72%) and gamma-Sitosterol (3.29%). The presence of gamma-Sitosterol, which eluted late in the run at 22.396 min, confirms the plant-based origin of the extract and contributes to its lipid-stabilizing potential. Furthermore, minor constituents including Nonanoic acid (Rt: 7.442 min) and n-Hexadecanoic acid (Rt: 12.997 min) were detected at concentrations below 1%, adding to the overall chemical complexity of the isolate.

The 23-minute oven program was sufficient to elute high-molecular-weight, non-polar constituents without evidence of peak tailing or column overload. The 1 μ L injection volume at a 10:1 split ratio ensured high signal-to-noise ratios, facilitating accurate integration and relative quantification.

Table 1: Detailed Chromatographic Profile of Isolated Phytoconstituents

Peak No.	Retention Time (Rt)	Peak Area	Area %
1. (Cyclohexane carbonyl chloride)	4.442 min	8602	0.09
2. (1-methyl-2-Pyrrolidinone)	5.220 min	20414	0.23
3.(2,3-dihydro-6-nitro-1,4-Phthalazinedione)	5.376 min	2700	0.03
4. (l-Tyrosinol)	6.464 min	3827	0.04
5. (Nonanoic acid)	7.442 min	8542	0.09
6. (1-(4,5-dihydro-2-thiazoyl)-Ethanone)	8.353 min	2040	0.02
7. ((E)-3-Eicosene)	9.397 min	4977	0.05
8. (Diisopropyl-, methyl ester Phosphinic acid)	9.453 min	5825	0.06
9.(2,5-bis(1,1-dimethylethyl) Phenol)	9.686	4610	0.05
9. (4-ethoxy-, ethyl ester Benzoic acid)	9.864 min	12747	0.14
10. (beta-Amyrin)	11.597 min	169010	1.86
11. (n-Hexadecanoic acid)	12.997 min	45593	0.50
12. (E)-9-Eicosene	14.141 min	47558	0.52
13.9,19-Cyclolanost-24-en-3-ol	14.730 min	1113570	12.29

14.Lanosterol	15.052 min	427875	4.72
15. alpha-Amyrin	15.197 min	620813	6.85
16. Stigmast-4-en-3-one	16.096 min	110344	1.22
17.4,4-dimethyl-Cholest-5-en-3-one	16.185 min	332817	3.67
18.Friedelan-3-one	16.419 min	402722	4.44
19. Hexacosane	16.708 min	63365	0.70
20.1,12-Bis(2-nitrophenoxy)dodecane	17.096 min	256434	2.83
21.gamma.-Sitosterol	22.396	297813	3.29

Fig 1: Detailed Chromatographic Profile of Isolated Phytoconstituents

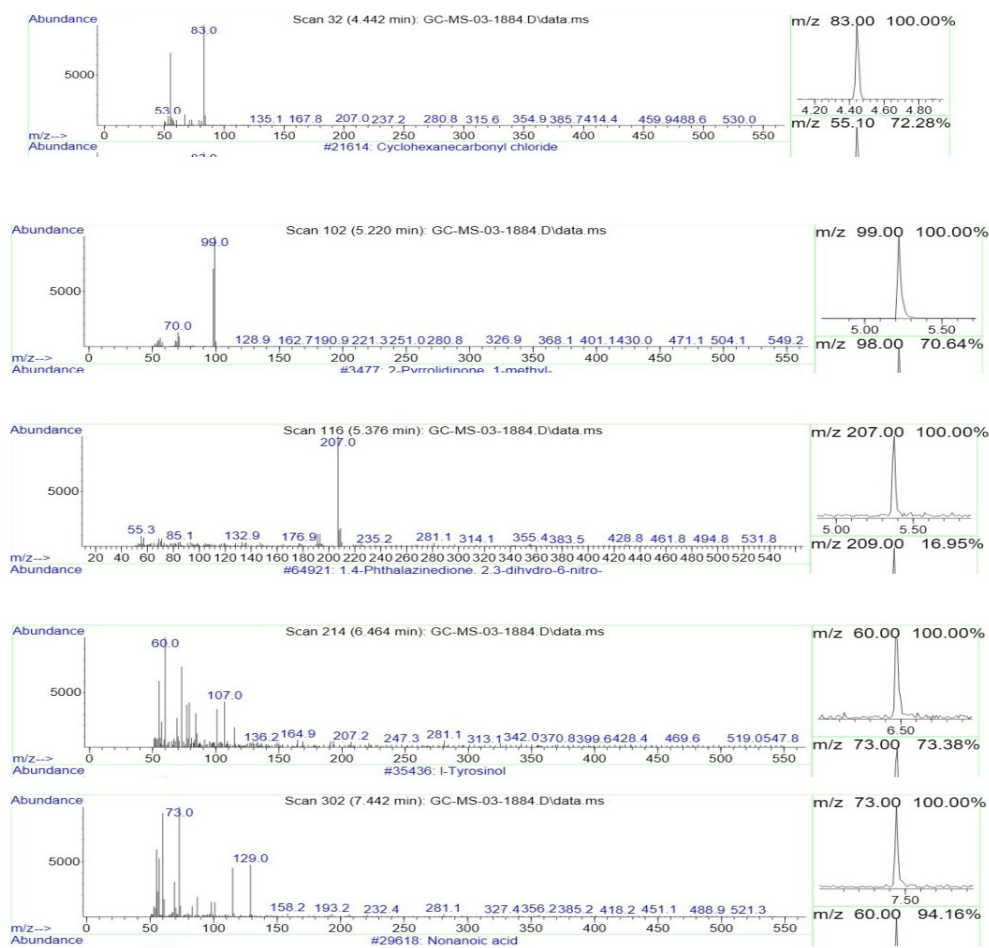


Fig 2: Detailed Chromatographic Profile of Isolated Phytoconstituents

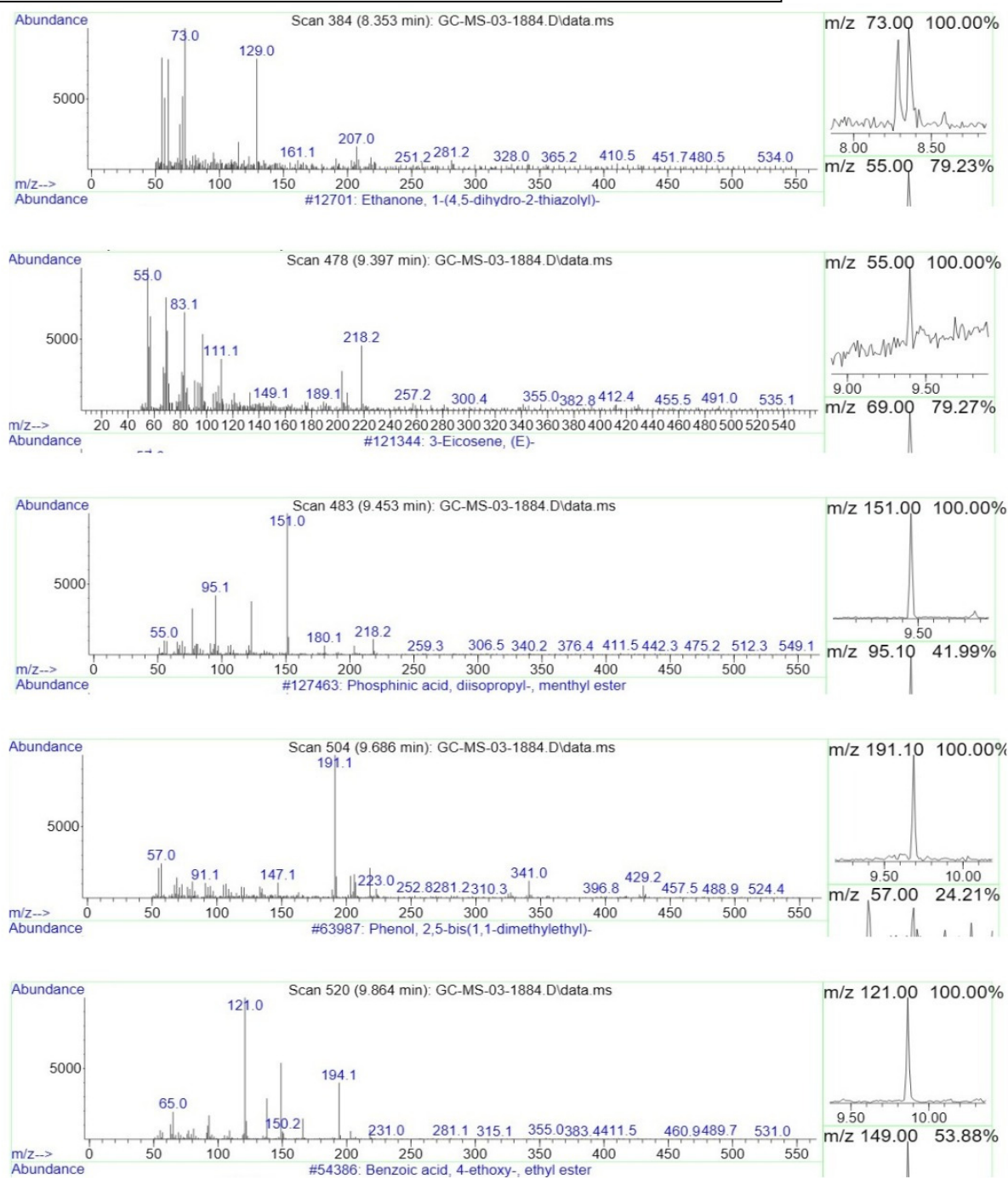


Fig 3: Detailed Chromatographic Profile of Isolated Phytoconstituents

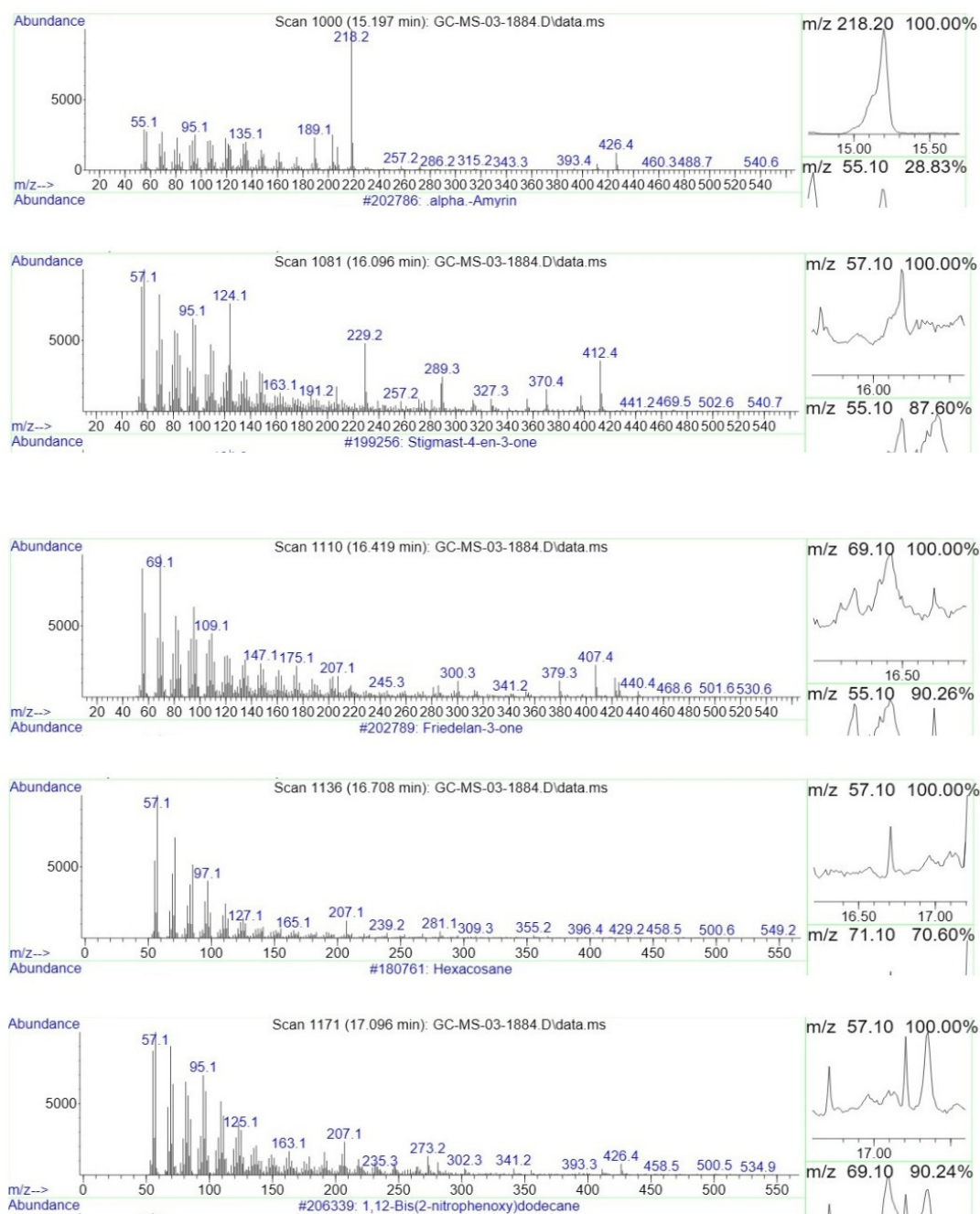
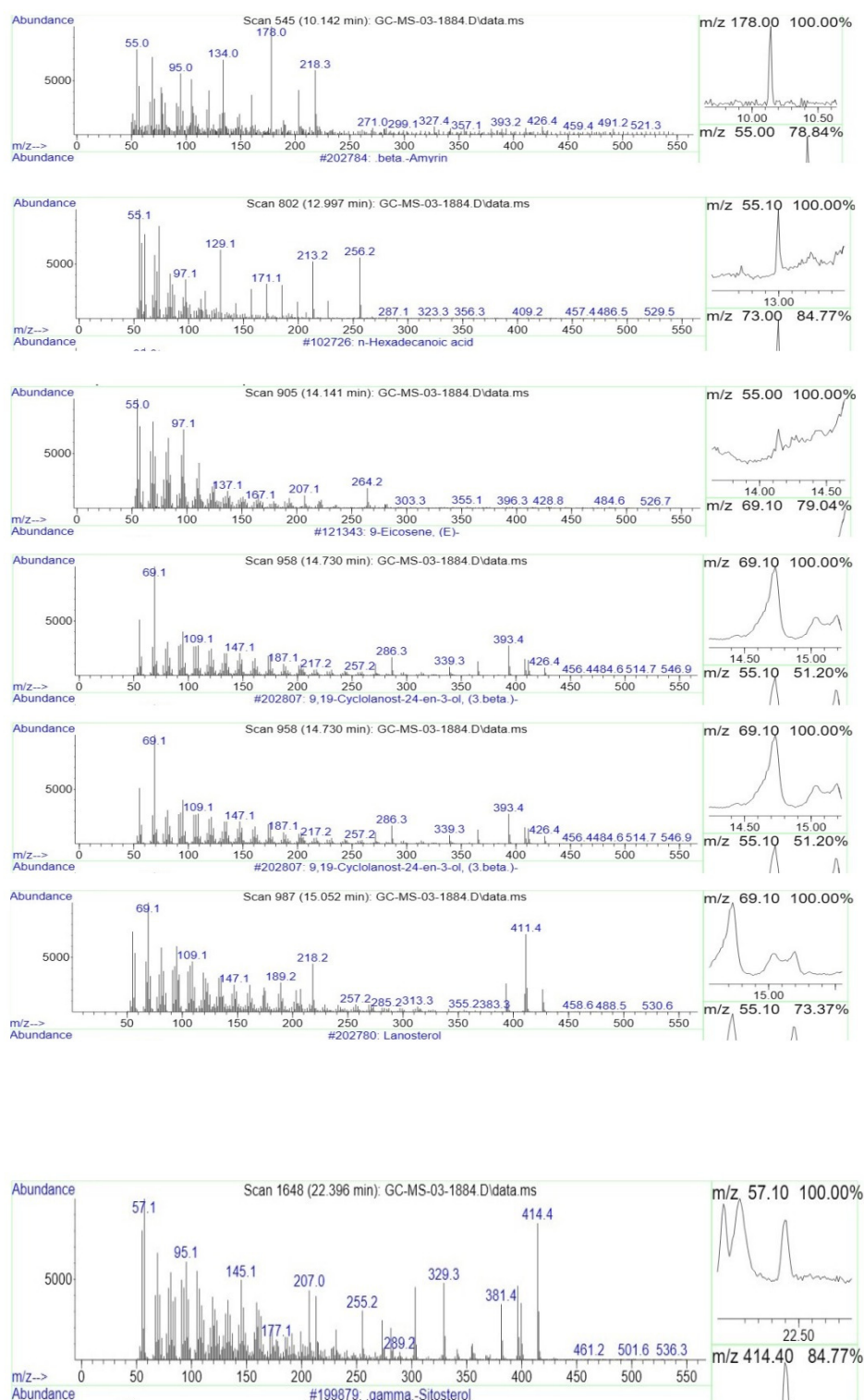


Fig 4: Detailed Chromatographic Profile of Isolated Phytoconstituents



CONCLUSION

The current study successfully established a comprehensive chemical fingerprint of the plant extract using an optimized GC-MS protocol. The methodology allowed for the identification of 21 phytochemicals, with 9,19-Cyclolanost-24-en-3-ol emerging as the major constituent (12.29%).

The high resolution provided by the DB-5MS column enabled the distinct separation of critical isomers like alpha- and beta-Amyrin. The coexistence of diverse triterpenoids and sterols suggests that the extract may possess significant medicinal value, particularly regarding anti-inflammatory and antioxidant pathways. Ultimately, this GC-MS profiling provides a standardized analytical baseline for future pharmacological screening and quality control of this botanical resource.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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