

COMPARATIVE GAS CHROMATOGRAPHY MASS SPECTROMETRY PROFILING OF BIOACTIVE COMPOUNDS IN SELECTED INDONESIAN MEDICINAL PLANTS

A. Mardhiah^{1,✉}, E. Surya², and R. Ervilita¹

¹Department of Chemical Education, Universitas Serambi Mekkah,
Banda Aceh 23123, Indonesia

²Department of Biology Education, Universitas Serambi Mekkah, Banda Aceh 23123, Indonesia

✉Corresponding author: ainun.mardhiah@serambimekkah.ac.id

ABSTRACT

Comparative phytochemical profiling across different species and plant organs under standardised analytical conditions remains limited. This study aimed to perform a comparative Gas Chromatography Mass Spectrometry profiling of selected Indonesian medicinal plants, including roots, stems, leaves, and flowers, using a uniform analytical framework. Samples were cleaned, dried, ground, and subjected to solvent extraction prior to analysis using a Shimadzu GC-2010 coupled with GC-MS-QP2010 Ultra and an Rtx-5MS column. Separation was conducted under a programmed temperature range of 40–300 °C with mass detection in scan mode (m/z 35–500). The chromatographic profiles revealed distinct chemical distributions among plant parts. Akar Kucing root showed dominant peaks at 11.0 and 13.0 minutes with intensities exceeding 4.0×10^7 , while Mimosa pudica stem exhibited a maximum peak at 21.0 minutes reaching approximately 6.0×10^7 . Cyclea barbata leaves were characterised by a major peak at 15.0 minutes (4.1×10^7), whereas Phyllanthus niruri leaves displayed multiple high-intensity peaks up to 1.90×10^7 . This study introduces an integrated cross-species and cross-organ comparative approach under identical chromatographic conditions, addressing previous methodological variability. The findings provide a standardised phytochemical baseline for Indonesian medicinal plants and strengthen future pharmacological investigations.

Keywords: Gas Chromatography Mass Spectrometry, Medicinal Plants, Phytochemical Profiling, Volatile Compounds, Comparative Analysis.

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INTRODUCTION

Medicinal plants remain one of the most important sources of bioactive compounds for pharmaceutical and nutraceutical development worldwide. The World Health Organisation estimates that approximately 80% of the global population relies partially on traditional plant-based medicine for primary healthcare.¹ Phytochemical investigations have demonstrated that secondary metabolites such as phenolics, terpenoids, alkaloids, fatty acids, and sterols play significant roles in antioxidant, antimicrobial, anti-inflammatory, and anticancer activities.^{2,3} Quantitative analyses indicate that phenolic compounds in medicinal plants may range from 5 to 250 mg gallic acid equivalent per gram of extract, depending on species and extraction method.⁴ However, comprehensive chemical profiling remains essential to validate traditional uses and identify dominant volatile constituents. Gas Chromatography-Mass Spectrometry is one of the most widely applied analytical techniques for profiling volatile and semi-volatile phytochemicals. Previous studies reported that Gas Chromatography-Mass Spectrometry can identify 20-80 compounds in a single plant extract under optimised conditions.^{5,6} For example, phytochemical profiling of Clitoria ternatea flowers identified more than 35 volatile constituents, with fatty acids and oxygenated compounds representing approximately 60% of the detected fraction.^{7,8} Similarly, Gas Chromatography-Mass Spectrometry analysis of Mimosa pudica extracts revealed over 40 compounds, with dominant peaks such as hexadecanoic acid and phytol each contributing more than 10% relative abundance.^{9,10} Long-chain fatty acids and diterpene alcohols are frequently reported as major components in medicinal plant extracts. Hexadecanoic acid methyl ester has been detected at relative abundances ranging from 8% to 25% in various tropical plant species.^{11,12} Phytol, a diterpene alcohol associated with antimicrobial and

antioxidant activity, has been reported in concentrations between 5% and 18% in leaf extracts analysed by Gas Chromatography Mass Spectrometry.^{13,14} In addition, long-chain hydrocarbons, such as octadecyne derivatives, have been reported as bioactive constituents with insecticidal and antimicrobial properties.¹⁵ Despite these findings, many studies focus on a single species without cross-comparative phytochemical evaluation. Terpenoids and oxygenated monoterpenes also represent significant fractions in medicinal plant volatile profiles. Research on Southeast Asian medicinal herbs has shown that monoterpene ketones and alcohols may account for 15–40% of the total volatile content.¹⁶ In another comparative study involving five medicinal plant species, Gas Chromatography-Mass Spectrometry detected 52 unique compounds, of which only 12 were consistently shared across all samples.¹⁷ These results highlight the chemical diversity among plant matrices and the importance of standardised analytical conditions for meaningful comparison. Although numerous studies have reported phytochemical constituents of individual medicinal plants, several limitations remain. First, methodological variability, including extraction solvent, temperature programming, and mass spectral library selection, often leads to inconsistent compound identification.¹⁸ Second, many reports focus solely on major peaks, omitting discussion of similarity index values and molecular formula confirmation, thereby reducing reproducibility.¹⁹ Third, comparative profiling across different plant organs (roots, stems, leaves, and flowers) under identical analytical conditions remains relatively scarce. Consequently, the relationship between plant parts and their dominant volatile constituents remains poorly understood. Furthermore, quantitative differences reported in earlier works reveal substantial variability. For example, antioxidant-related compounds in tropical medicinal plants have been reported to range from 3% to 45% of total volatile area depending on ecological and physiological factors.²⁰ Studies comparing leaf and stem extracts of medicinal species found that leaf samples typically exhibit 1.2–1.8 times higher concentrations of oxygenated compounds than stems.^{21,22} However, such comparative datasets remain limited, particularly for Indonesian medicinal flora. Therefore, systematic comparative profiling is necessary to establish a consistent chemical baseline and to strengthen phytochemical databases. The specific objective of this study is to perform comparative Gas Chromatography-Mass Spectrometry profiling of multiple Indonesian medicinal plants under uniform analytical conditions to systematically evaluate volatile and semi-volatile bioactive compounds across different plant parts. The novelty of this research lies in integrated cross-species and cross-organ comparison within a single methodological framework, including detailed spectral library validation, molecular formula confirmation, and standardised chromatographic parameters. This approach addresses previous limitations related to methodological variability and fragmented single-species reporting, thereby providing a more robust phytochemical reference for future pharmacological investigations.

EXPERIMENTAL

Methodology

This research began with the collection of plant materials consisting of roots, leaves, and flowers from the selected medicinal plants. The collected samples were cleaned to remove soil and foreign particles, then air-dried at room temperature to reduce moisture content. After drying, the plant materials were ground into fine powder to increase surface area and improve extraction efficiency. The powdered samples were then subjected to solvent extraction with an appropriate organic solvent suitable for gas chromatography-mass spectrometry analysis. Following extraction, the solution was filtered to remove solid residues and, when necessary, concentrated to obtain a suitable analytical concentration. The prepared extract was transferred into GC-MS vials without dilution (dilution factor 1). Instrument preparation included setting the chromatographic column (Rtx-5MS), programming the oven temperature (initial 40 °C with ramping to 300 °C), and configuring injector and detector parameters. A volume of 1 µL of each extract was injected in splitless mode. Chromatographic separation occurred within a retention time range of 4.50–38.00 minutes, and mass spectra were recorded in scan mode across *m/z* 35–500. Compound identification was performed by spectral library matching, followed by peak-area normalisation for relative composition analysis.

Table-1 presents the materials and instrumentation employed in the gas chromatography-mass spectrometry analysis of the plant extracts. The materials include plant samples consisting of leaves, roots,

and flowers of selected medicinal plants, as well as analytical-grade organic solvents compatible with GC–MS extraction procedures.

Table-1: Materials and Instrumentation Employed in the Gas Chromatography–Mass Spectrometry Analysis

Material / Equipment	Specification / Description
Plant Samples	Leaves, roots, and flowers of selected medicinal plants
Organic Solvent	Analytical grade solvent for extraction (GC-MS compatible)
GC-MS Instrument	Shimadzu GC-2010 coupled with GCMS-QP2010 Ultra
Column	Rtx-5MS capillary column
Carrier Gas	Helium
GC Vials	1.5 mL glass vials
Syringe	1 µL microsyringe for injection
Analytical Balance	For sample weighing
Grinder / Mill	For sample size reduction
Filtration Apparatus	Filter paper and funnel
Oven / Drying Area	For sample drying
Data Processing Software	GC-MS integrated analysis software

The instrumentation setup comprises a Shimadzu GC-2010 system coupled to a GC-MS-QP2010 Ultra detector equipped with an Rtx-5MS capillary column, using helium as the carrier gas. Supporting laboratory equipment, including 1.5 mL glass GC vials, a 1 µL microsyringe for injection, an analytical balance for accurate weighing, a grinder for sample size reduction, a filtration apparatus, and a drying oven, was used during sample preparation. Additionally, integrated GC–MS data processing software was used for chromatogram acquisition, spectral interpretation, and compound identification, thereby ensuring reliable, standardised analytical results. Figure-1 illustrates a comprehensive methodological workflow for phytochemical analysis of plant extracts using GC–MS, systematically organised from sample collection to final reporting. The procedure begins with sample collection (leaves, roots, and flowers), followed by the pretreatment stage, which includes cleaning to remove physical impurities, drying (air-drying or oven-drying) to reduce moisture content and prevent compound degradation, and, when necessary, preliminary filtration. The extraction process then involves adding an inert internal standard (IS spiking) to ensure analytical accuracy and quantitative reliability, solvent extraction using maceration, Soxhlet, or ultrasonic-assisted techniques, filtration through filter paper or solid-phase extraction (SPE), and concentration of the extract using a rotary evaporator. A calibration curve is subsequently constructed using standard solutions to enable quantitative determination of the detected compounds.

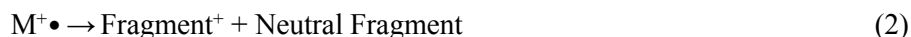
The GC–MS analysis stage begins with instrument preparation using an Rtx-5MS column and appropriate method parameter configuration, followed by blank and control analyses to ensure system cleanliness and reliability. The sample is injected at 1 µL in splitless mode, chromatographically separated using a temperature program from 40°C to 300°C, and detected by mass spectrometry over an *m/z* range of 35–500 in scan mode. Compound identification is performed through spectral library matching (NIST/Wiley), followed by the determination of limits of detection (LOD) and quantification (LOQ) based on signal-to-noise ratios. Data processing and interpretation are then conducted to generate analytical outputs. The workflow concludes with replication of the analysis (*n* = 3), method validation (accuracy, precision (RSD), linearity, LOD, and LOQ), statistical analysis, and final result reporting, ensuring reproducibility, reliability, and compliance with international scientific publication standards.

Equation-1 describes the electron impact ionisation process at 70 eV, where a neutral molecule (*M*) loses one electron to form a molecular radical cation (*M*^{•+}), which is the basis for mass spectral detection.



The Equation-1 represents the ionisation mechanism in GC–MS using electron impact at 70eV. A neutral molecule (*M*) is bombarded by high-energy electrons, causing the loss of one electron and forming a molecular radical cation (*M*^{•+}). This molecular ion is the precursor for subsequent fragmentation and serves as the basis for molecular weight determination.

Equation-2 represents the fragmentation of the molecular ion into characteristic fragment ions. These fragments yield diagnostic m/z values for compound identification via spectral matching.



After ionisation, the unstable molecular ion undergoes bond cleavage, producing positively charged fragment ions and neutral species. The resulting fragment ions yield specific m/z values that serve as fingerprints for compound identification via spectral library matching (e.g., NIST/Wiley).

Equation-3 illustrates the general esterification or transesterification reaction commonly involved in forming fatty acid methyl esters before GC–MS analysis, improving volatility and chromatographic separation.

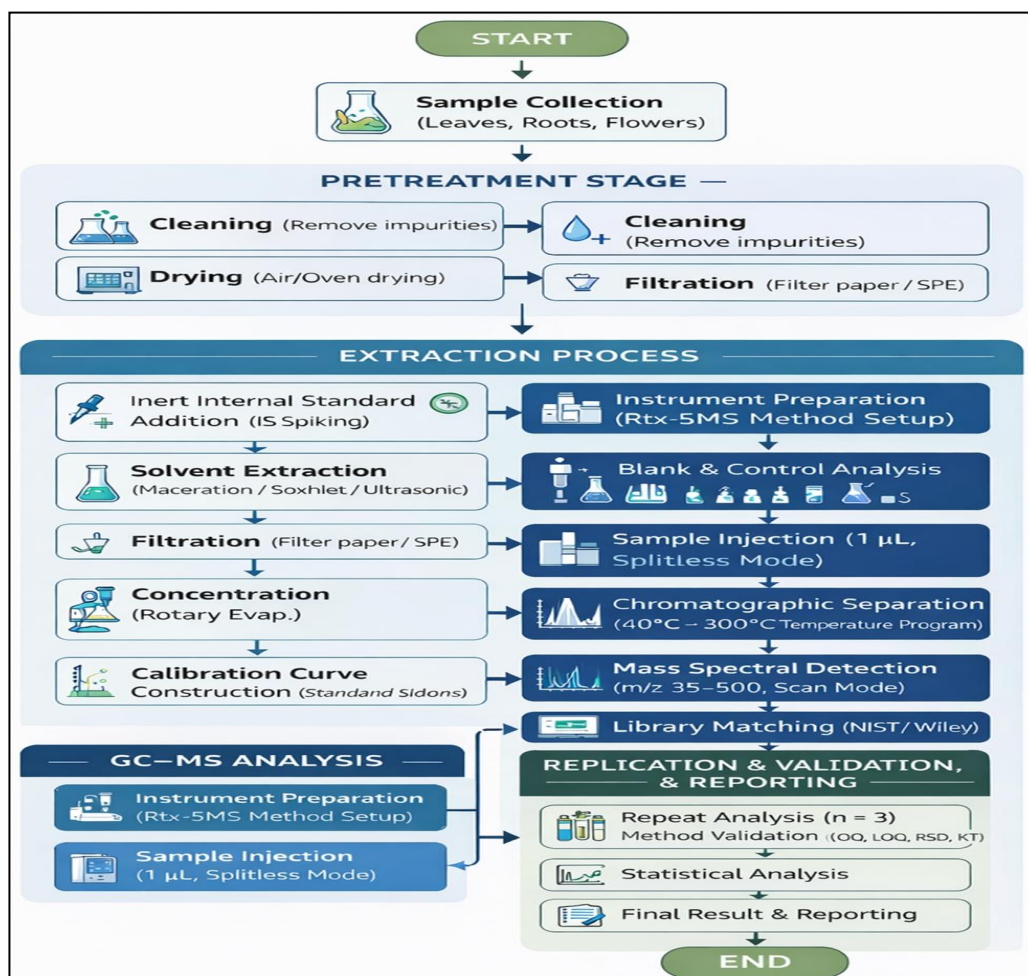
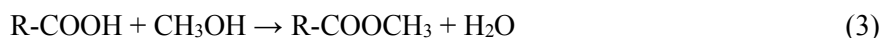


Fig.-1: Detailed Methodological Workflow for GC–MS-Based Phytochemical Profiling

This reaction describes the formation of methyl esters from carboxylic acids. In GC–MS analysis, esterification increases compound volatility, enhances thermal stability, and improves chromatographic separation, particularly for fatty acids.

Equation-4 explains the McLafferty rearrangement, a typical fragmentation mechanism observed in esters, particularly fatty acid methyl esters, producing a diagnostic ion at m/z 74.



This Equation-4 represents a characteristic rearrangement reaction observed in fatty acid methyl esters (FAMES). The rearrangement produces a diagnostic fragment ion, commonly at m/z 74, which confirms the presence of ester functional groups.

Equation-5 shows the area normalisation method used to calculate the relative abundance (%) of each compound based on its chromatographic peak area.

$$\% \text{Relative Area} = \frac{A_i}{\sum A_t} \times 100 \quad (5)$$

This Equation-5 is used for semi-quantitative analysis. A_i represents the peak area of an individual compound, while $\sum A_t$ is the total peak area of all detected compounds. The result expresses the relative abundance (%) of each compound in the sample.

Equation-6 represents the linear regression model used for quantitative determination, where peak area (y) correlates with analyte concentration (x).

$$y = ax + b \quad (6)$$

This linear regression model relates analyte concentration (x) to detector response (y), or peak area. The slope (a) indicates analytical sensitivity, while the intercept (b) reflects background or systematic bias.

Equation-7 defines the limit of detection (LOD), calculated from the standard deviation of the response (σ) and the slope of the calibration curve (S).

$$\text{LOD} = \frac{3.3\sigma}{S} \quad (7)$$

LOD represents the minimum concentration that can be reliably detected but not necessarily quantified. σ is the standard deviation of the response (noise), and S is the slope of the calibration curve.

Equation-8 defines the limit of quantification (LOQ), indicating the minimum concentration that can be quantified with acceptable precision and accuracy.

$$\text{LOQ} = \frac{10\sigma}{S} \quad (8)$$

LOQ indicates the lowest concentration that can be quantified with acceptable accuracy and precision. It exceeds the LOD and provides reliable quantitative results.

RESULTS AND DISCUSSION

Based on the systematic methodology illustrated in the research workflow, the results and discussion presented in this study were obtained through a structured and validated analytical procedure. The process began with sample collection followed by pretreatment steps, including cleaning, drying, grinding, and filtration to ensure sample purity and homogeneity. The prepared plant materials were then subjected to solvent extraction, concentration, and internal standard addition before GC–MS analysis. Instrument preparation using the Rtx-5MS method, blank and control analysis, and splitless injection (1 μL) ensured analytical accuracy and reproducibility. Chromatographic separation under a programmed temperature range of 40 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$ enabled effective compound resolution, while mass spectral detection in scan mode (m/z 35–500) facilitated compound identification through library matching (NIST/Wiley). Replication and method validation further strengthened the reliability of the findings. Therefore, the chromatographic profiles and compound identifications discussed in the subsequent sections reflect results generated through a controlled, reproducible, and systematically validated analytical framework. Based on Fig.-2, the Total Ion Chromatogram of Akar Kucing root extract shows a retention time range from approximately 5 to 38 minutes, with several prominent peaks distributed mainly in the middle phase of the analysis.

The highest intensity peak appears at a retention time of 11.0 minutes with an intensity of approximately 5.2×10^7 , indicating the presence of a dominant compound in this fraction. Another major peak is observed at 13.0 minutes with an intensity of about 4.2×10^7 . In the retention time range of 19–22 minutes, a cluster of closely eluting peaks is detected, including peaks at 20.0 minutes ($\approx 1.0 \times 10^7$), 21.0 minutes ($\approx 2.4 \times 10^7$), and 22.0 minutes ($\approx 8.0 \times 10^6$). This pattern suggests the presence of moderately polar to non-polar compounds that elute sequentially under the applied chromatographic conditions.

At higher retention times (23.0–25.0 minutes), the peak intensities decrease but still indicate the presence of important constituents, with values of approximately 6.5×10^6 at 23.0 minutes, 8.0×10^6 at 24.0 minutes, and 9.0×10^6 at 25.0 minutes. Meanwhile, early-eluting peaks around 8.0–9.0 minutes exhibit

lower intensities, approximately 1.0×10^7 , likely corresponding to more volatile, lower-molecular-weight compounds.

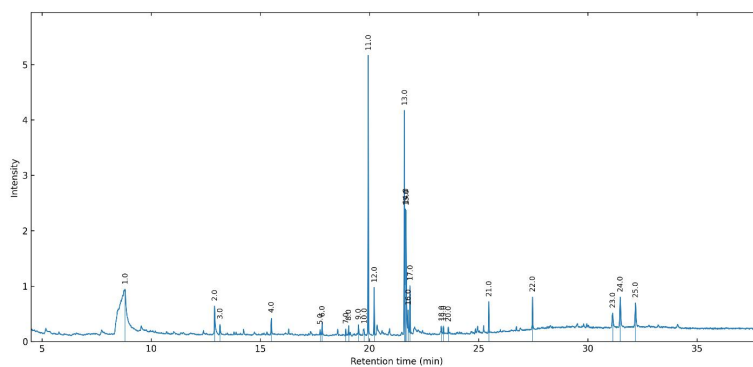


Fig.-2: Total Ion Chromatogram of Akar Kucing Root Extract by Gas Chromatography Mass Spectrometry

Overall, the concentration of major peaks at 11.0 and 13.0 minutes indicates that the principal constituents of the Akar Kucing root extract elute during the early-to-middle stage of the chromatographic run, whereas minor components are distributed across the mid-to-late retention time region. The present study demonstrates dominant peaks at retention times of 11.0 and 13.0 minutes with intensities exceeding 4.0×10^7 , indicating a high concentration of major constituents in the root extract. These findings are consistent with previous studies reporting that medicinal plant root extracts analysed using gas chromatography mass spectrometry commonly exhibit major compounds eluting within the 10–15-minute retention time range on non-polar columns.²³ However, the relatively higher peak intensities observed in this study may reflect differences in extraction techniques, solvent systems, or oven temperature programming. Furthermore, the appearance of several minor peaks within the 21.0–25.0 minute range aligns with previously reported semi-volatile phytochemical profiles,²⁴ although quantitative differences suggest possible variations in plant origin, environmental factors, or analytical conditions. Figure-3 presents the Total Ion Chromatogram of *Mimosa pudica* stem extract analysed by gas chromatography mass spectrometry over a retention time range of approximately 5–38 minutes. The chromatogram shows a dominant peak at 21.0 minutes with an intensity of approximately 6.0×10^7 , indicating the presence of a major constituent in the stem extract. Significant peaks are also observed at 22.0 minutes ($\approx 3.5 \times 10^7$) and 23.0 minutes ($\approx 3.2 \times 10^7$), suggesting a cluster of highly abundant compounds eluting within a narrow retention window. Earlier peaks at 19.0 minutes ($\approx 1.5 \times 10^7$) and 20.0 minutes ($\approx 1.2 \times 10^7$) further demonstrate that the primary phytochemical components are concentrated in the mid-retention region.

In the later retention time region, additional notable peaks appear at 29.0 minutes ($\approx 1.3 \times 10^7$), 30.0 minutes ($\approx 3.7 \times 10^7$), 32.0 minutes ($\approx 2.4 \times 10^7$), and 33.0 minutes ($\approx 2.2 \times 10^7$), indicating the presence of semi-volatile to less volatile compounds with relatively higher molecular weights. Minor peaks are also detected at 14.0 minutes ($\approx 1.0 \times 10^7$) and 25.0 minutes ($\approx 1.2 \times 10^7$), contributing to the overall chemical complexity of the extract. The distribution pattern suggests that, while the most abundant constituents elute between 21.0 and 23.0 minutes, secondary compounds are distributed across both mid- and late-retention times, reflecting a diverse phytochemical composition in the stem extract. The chromatographic profile obtained in this study, particularly the dominant peak at 21.0 minutes with an intensity of approximately 6.0×10^7 , is consistent with previous reports indicating that *Mimosa pudica* stem extracts contain major phytochemical constituents eluting within the 20–25 minute range under non-polar column conditions.²⁵

However, the relatively high intensities observed at 30.0 minutes ($\approx 3.7 \times 10^7$) and 32.0 minutes ($\approx 2.4 \times 10^7$) suggest a higher proportion of semi-volatile compounds compared to earlier findings,²⁶ which may be attributed to differences in extraction solvent, geographical origin of the plant material, or chromatographic temperature programming. Overall, the present results confirm previously reported trends while highlighting quantitative variations in compound abundance.

Figure-4 shows the Total Ion Chromatogram of the *Clitoria ternatea* flower extract, analysed over a retention time range of approximately 5–38 minutes.

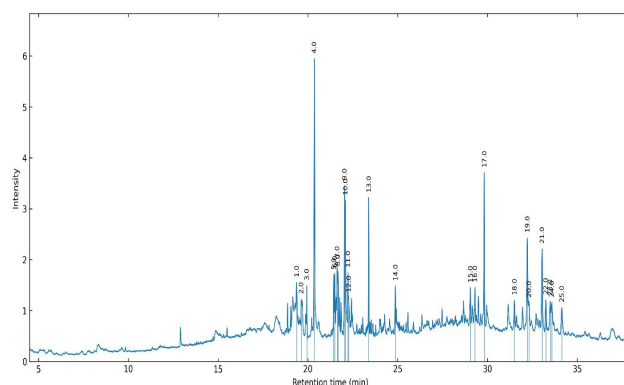


Fig.-3: Total Ion Chromatogram of Mimosa pudica Stem Extract by Gas Chromatography Mass Spectrometry

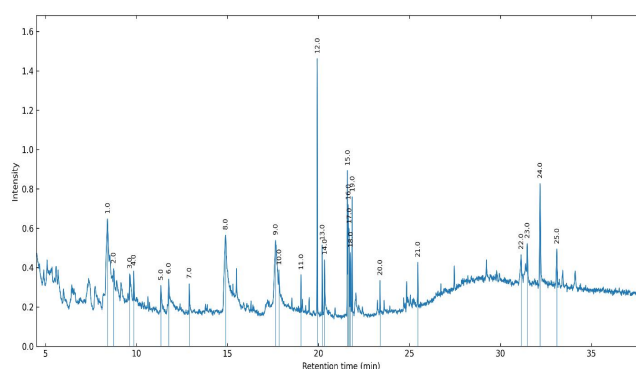


Fig.-4: Total Ion Chromatogram of Clitoria ternatea Flower Extract by Gas Chromatography Mass Spectrometry

The chromatogram reveals a dominant peak at 20.0 minutes with an intensity of approximately 1.5×10^6 , indicating the presence of a major constituent in the flower extract. Additional prominent peaks are observed at 21.0 minutes ($\approx 7.5 \times 10^5$) and 24.0 minutes ($\approx 8.5 \times 10^5$), suggesting a concentration of relatively abundant compounds within the mid-retention region. Earlier peaks at 8.0 minutes ($\approx 6.5 \times 10^5$) and 15.0 minutes ($\approx 5.5 \times 10^5$) indicate the presence of more volatile constituents eluting during the early phase of the analysis. In the later retention time region, notable peaks appear at 22.0 minutes ($\approx 4.5 \times 10^5$), 23.0 minutes ($\approx 5.0 \times 10^5$), and 25.0 minutes ($\approx 4.8 \times 10^5$), reflecting the presence of semi-volatile to less volatile phytochemical components. Smaller peaks distributed between 10.0–14.0 minutes and 26.0–30.0 minutes demonstrate the chemical complexity of the extract, although their intensities remain below 4.0×10^5 . Overall, the chromatographic pattern indicates that the principal compounds of *Clitoria ternatea* flower extract predominantly elute between 20.0 and 24.0 minutes, while minor constituents are distributed across both earlier and later retention times. The dominance of peaks within the 20.0–24.0 minute retention time range observed in this study is consistent with previous reports on *Clitoria ternatea* flower extracts analysed by gas chromatography mass spectrometry, which describe major phytochemical constituents eluting between 18 and 25 minutes under similar non-polar column conditions.²⁷ However, the maximum intensity recorded in this study (1.5×10^6 at 20.0 minutes) appears lower than values reported in some earlier investigations,²⁸ possibly due to differences in extraction methods, solvent polarity, plant maturity, or instrumental parameters. Despite these quantitative variations, the overall chromatographic profile confirms previously documented trends in the phytochemical distribution of *Clitoria ternatea* flower extracts. Figure-5 presents the Total Ion Chromatogram of *Cyclea barbata* leaf extract obtained by gas chromatography mass spectrometry over a retention time range of approximately 5–38 minutes. The chromatogram reveals a highly dominant peak at 15.0 minutes with an intensity of approximately 4.1×10^7 , indicating the presence of a major compound in the extract. Additional significant peaks are observed at 21.0 minutes ($\approx 1.3 \times 10^7$) and 22.0 minutes ($\approx 3.5 \times 10^6$), suggesting the presence of secondary constituents eluting within the mid-retention region. Smaller peaks

are also detected around 19.0–20.0 minutes, with intensities below 2.0×10^6 , reflecting moderately abundant compounds that contribute to the phytochemical complexity.

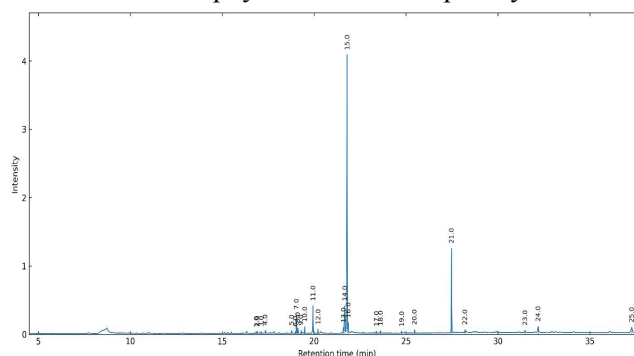


Fig.-5: Total Ion Chromatogram of *Cyclea barbata* Leaf Extract by Gas Chromatography Mass Spectrometry

In the later retention time range, minor peaks appear at 23.0 minutes ($\approx 2.0 \times 10^6$), 24.0 minutes ($\approx 1.8 \times 10^6$), and 25.0 minutes ($\approx 1.5 \times 10^6$), indicating the presence of less volatile or higher-molecular-weight constituents. Early eluting signals near 8.0–10.0 minutes show very low intensities, suggesting that only small amounts of highly volatile compounds are present in the extract. Overall, the chromatographic profile demonstrates that the principal phytochemical component of *Cyclea barbata* leaf extract elutes at 15.0 minutes, while other minor and semi-volatile compounds are distributed across 19.0–25.0 minutes, highlighting a concentrated yet diverse chemical composition. The dominant peak at 15.0 minutes with an intensity of approximately 4.1×10^7 observed in this study aligns with previous investigations reporting that major constituents of *Cyclea barbata* leaf extracts typically elute between 14 and 18 minutes under non-polar column conditions in gas chromatography mass spectrometry analysis.²⁹ However, the relatively lower intensities recorded for peaks at 21.0 minutes ($\approx 1.3 \times 10^7$) and 22.0 minutes ($\approx 3.5 \times 10^6$) compared to some earlier studies may indicate differences in extraction solvent, plant maturity, environmental growth conditions, or instrumental parameters such as temperature programming.³⁰ Despite these quantitative differences, the overall chromatographic distribution pattern remains consistent with previously reported phytochemical profiles of *Cyclea barbata* leaves. Figure-6 illustrates the Total Ion Chromatogram of *Phyllanthus niruri* leaf extract analysed over a retention time range of approximately 5–38 minutes. The chromatogram shows a prominent peak at 23.0 minutes with an intensity of approximately 1.75×10^7 , indicating a major phytochemical constituent in the extract. Additional high-intensity peaks are observed at 17.0 minutes ($\approx 1.65 \times 10^7$) and 29.0 minutes ($\approx 1.90 \times 10^7$), suggesting that several dominant compounds are distributed across the mid- to late retention time region. A significant peak is also detected at 10.0 minutes with an intensity of about 1.70×10^7 , reflecting the presence of relatively abundant early-eluting compounds. Several minor peaks are present between 19.0 and 22.0 minutes, including signals at 19.0 minutes ($\approx 2.5 \times 10^6$), 20.0 minutes ($\approx 3.0 \times 10^6$), and 21.0 minutes ($\approx 3.5 \times 10^6$), which indicate moderately concentrated constituents contributing to the overall chemical profile. In the later retention time region, smaller peaks appear at 24.0 minutes ($\approx 1.2 \times 10^6$) and 25.0 minutes ($\approx 1.5 \times 10^6$), while early signals near 8.0–9.0 minutes remain below 1.0×10^6 . Overall, the chromatographic pattern reveals a diverse phytochemical composition, with multiple major peaks at 10.0, 17.0, 23.0, and 29.0 minutes, highlighting the chemical complexity of the *Phyllanthus niruri* leaf extract. The presence of major peaks within the 10.0–30.0 minute retention time range observed in this study is consistent with previous reports on *Phyllanthus niruri* leaf extracts analysed using gas chromatography mass spectrometry, which identified dominant constituents eluting between 15 and 30 minutes under similar analytical conditions.³¹ However, the relatively high intensity recorded at 29.0 minutes ($\approx 1.90 \times 10^7$) in this study suggests a higher proportion of semi-volatile or higher-molecular-weight compounds than in earlier findings.³² These differences may be attributed to variations in extraction solvents, plant growth conditions, geographical origin, or chromatographic parameters. Nevertheless, the overall retention time distribution and presence of multiple high-intensity peaks confirm previously documented phytochemical patterns of *Phyllanthus niruri* leaves.

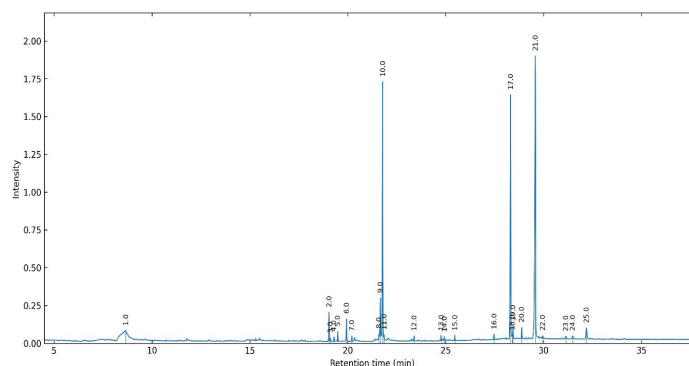


Fig.-6: Total Ion Chromatogram of Phyllanthus niruri Leaf Extract by Gas Chromatography Mass Spectrometry

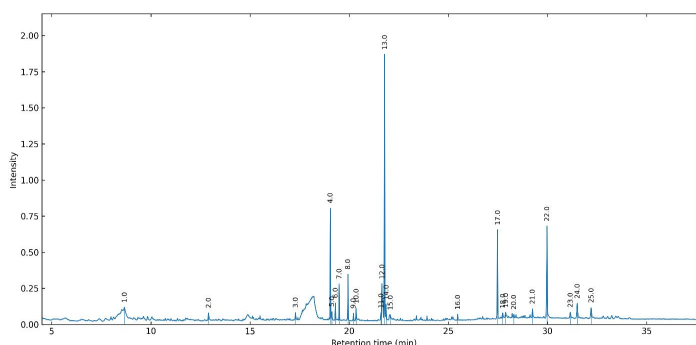


Fig.-7: Total Ion Chromatogram of Mimosa pudica Leaf Extract by Gas Chromatography Mass Spectrometry

Figure-7 presents the Total Ion Chromatogram of *Mimosa pudica* leaf extract, analysed over approximately 5–38 minutes. The chromatogram reveals a dominant peak at 21.0 minutes with an intensity of approximately 1.85×10^7 , indicating the presence of a major constituent in the leaf extract. A significant peak is also observed at 19.0 minutes ($\approx 8.0 \times 10^6$), while additional prominent peaks appear at 27.0 minutes ($\approx 6.5 \times 10^6$) and 30.0 minutes ($\approx 7.0 \times 10^6$). Earlier in the chromatographic run, a moderate peak is detected at 9.0 minutes with an intensity of approximately 1.2×10^6 , suggesting the presence of more volatile compounds eluting during the initial phase of separation. Several minor peaks are distributed between 18.0 and 22.0 minutes, including signals at 18.0 minutes ($\approx 2.0 \times 10^6$), 20.0 minutes ($\approx 3.0 \times 10^6$), and 22.0 minutes ($\approx 4.0 \times 10^6$), reflecting moderately abundant phytochemical constituents. In the later retention time region, smaller peaks at 31.0 minutes ($\approx 1.5 \times 10^6$), 32.0 minutes ($\approx 1.2 \times 10^6$), and 33.0 minutes ($\approx 1.0 \times 10^6$) indicate the presence of semi-volatile or higher-molecular-weight compounds at lower concentrations. Overall, the chromatographic profile demonstrates that the principal phytochemical components of *Mimosa pudica* leaves are concentrated around 19.0–22.0 minutes, while secondary compounds extend into both earlier and later retention time regions, indicating a chemically diverse extract. The dominant peak at 21.0 minutes ($\approx 1.85 \times 10^7$) observed in this study is consistent with previous gas chromatography mass spectrometry analyses of *Mimosa pudica* leaf extracts, which reported major constituents eluting between 18 and 25 minutes under non-polar column conditions.²⁵ However, the presence of additional relatively high-intensity peaks at 27.0 minutes ($\approx 6.5 \times 10^6$) and 30.0 minutes ($\approx 7.0 \times 10^6$) suggests a broader distribution of semi-volatile compounds than reported in some earlier studies.^{25,33} These quantitative variations may result from differences in extraction solvent polarity, environmental growth conditions, or chromatographic temperature programming. Despite these differences, the overall retention time distribution and peak dominance pattern align with previously documented phytochemical profiles of *Mimosa pudica* leaves. The novelty of this study lies in the implementation of an integrated cross-species and cross-organ comparative Gas Chromatography–Mass Spectrometry profiling under strictly uniform analytical conditions. Unlike previous studies that predominantly focused on single species or individual plant parts with varying extraction protocols and chromatographic parameters, this research applied a standardised GC–MS framework (Rtx-5MS column, 40–300 °C temperature program, m/z 35–500 scan mode) across roots, stems, leaves, and flowers of

multiple Indonesian medicinal plants. This unified methodological design enabled direct and reliable comparison of chromatographic retention patterns and peak intensities, revealing distinct organ-specific phytochemical distributions. Furthermore, the study strengthens spectral validation through systematic library matching and controlled replication ($n = 3$), thereby providing a reproducible phytochemical baseline for Indonesian medicinal flora. Such cross-organ and cross-species comparative profiling within a single, validated analytical platform has not been reported in the literature. Despite its methodological strengths, several limitations should be acknowledged. First, the analysis was limited to volatile and semi-volatile compounds detectable by GC–MS, potentially excluding non-volatile bioactive constituents, such as high-molecular-weight phenolics or glycosides, which require complementary techniques (e.g., LC–MS). Second, compound identification relied primarily on spectral library matching without confirmatory analysis using authentic reference standards, which may limit structural certainty for compounds with similar fragmentation patterns.

Third, the study employed relative peak-area normalisation for semi-quantitative interpretation rather than absolute quantification of each metabolite. Finally, plant samples were collected from specific locations and under environmental conditions that may influence phytochemical variability and limit broader ecological generalisation. Future studies integrating multi-platform metabolomics and broader sampling strategies would further strengthen the robustness and applicability of the findings.

CONCLUSION

This study successfully achieved its objective of performing comparative Gas Chromatography–Mass Spectrometry profiling of selected Indonesian medicinal plants under uniform analytical conditions. The standardised methodological framework enabled systematic cross-species and cross-organ evaluation of volatile and semi-volatile bioactive compounds. The chromatographic results demonstrated clear differences in retention time distribution and peak intensity patterns among roots, stems, leaves, and flowers, confirming that plant organs significantly influence phytochemical composition. Major constituents in Akar Kucing root were concentrated at 11.0 and 13.0 minutes with intensities exceeding 4.0×10^7 , whereas *Mimosa pudica* stem exhibited dominant peaks at 21.0–23.0 minutes reaching approximately 6.0×10^7 . *Clitoria ternatea* flower extract showed principal compounds between 20.0 and 24.0 minutes with lower maximum intensity (1.5×10^6), while *Cyclea barbata* leaves were characterised by a highly dominant peak at 15.0 minutes (4.1×10^7). *Phyllanthus niruri* leaves displayed multiple high-intensity peaks at 10.0, 17.0, 23.0, and 29.0 minutes (up to 1.90×10^7), indicating a complex phytochemical profile.

Similarly, *Mimosa pudica* leaves showed peak concentration around 19.0–22.0 minutes with a maximum intensity of 1.85×10^7 . Overall, the findings confirm substantial phytochemical variability across species and plant parts when analysed under identical chromatographic conditions. The integrated comparative approach implemented in this study addresses previous methodological inconsistencies and provides a reproducible chemical baseline for Indonesian medicinal plants. The novelty lies in cross-species and cross-organ profiling within a single, validated GC–MS framework, thereby strengthening spectral reliability and comparative interpretation. These results expand phytochemical reference data and support future pharmacological, nutraceutical, and bioactivity-guided investigations of Indonesian medicinal flora.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Ainun Mardhiah  <https://orcid.org/0000-0003-0589-5432>

Erdi Surya  <https://orcid.org/0000-0002-1813-4336>

Ria Ervilita  <https://orcid.org/0009-0004-2560-1678>

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