



RESEARCH ARTICLE

Evaluation of bioactive compounds in the methanolic extract of butterfly pea plant (*Clitoria ternatea* L.) using gas chromatography-mass spectroscopy

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Abstract

Clitoria ternatea L. (Fabaceae) is a medicinal plant valued for its diverse phytochemical constituents; however, systematic studies on the white-flowered variety (*C. ternatea* var. *pilosula*) remain limited. The present study provides a comparative, part-wise gas chromatography-mass spectrometry (GC-MS) profiling of methanolic extracts from the leaf, stem, root, seed and flower of the white-flowered variety, addressing an important research gap. Methanolic extracts were prepared by maceration and analysed using a Shimadzu QP-2010 gas chromatography-mass spectrometry system equipped with an RTX-5MS capillary column. Compound identification was achieved through spectral matching with NIST and Wiley libraries and retention index comparison. Gas chromatography-mass spectrometry analysis revealed distinct phytochemical compositions among plant parts, including fatty acids, long-chain hydrocarbons, terpenoid alcohols, esters and phenolic heterocycles. Leaves exhibited the highest chemical diversity with hexacontane (19.96 %), pentadecane, 2,6,10,13-tetramethyl (7.44 %) and isoamyl acetate (5.82 %) as major constituents. Flowers displayed a unique profile dominated by oxygenated and heterocyclic compounds, particularly 3-furanmethanol (47.16 %), along with glycerin and pyranone derivatives. Roots, stems and seeds were mainly characterised by fatty acids and alkanes. Several identified compounds possess previously reported antioxidant, antimicrobial and anti-inflammatory activities, indicating potential biological relevance. This study highlights marked part-specific phytochemical variation in *C. ternatea* var. *pilosula* and provides a chemical basis for future pharmacological and chemotaxonomic investigations.

Keywords: bioactive compounds; butterfly pea plant; *Clitoria ternatea*; gas chromatography-mass spectrometry; methanolic extract

Introduction

Medicinal plants remain a major source of bioactive compounds that play a vital role in human health and disease management. *Clitoria ternatea* L., commonly known as butterfly pea or blue pea, belongs to the family Fabaceae and is widely distributed across India, China, the Philippines and Madagascar, thriving in humid tropical lowland regions under both wild and cultivated conditions (1, 2). Two principal varieties of *C. ternatea*, the blue-flowered (*C. ternatea* var. *ternatea*) and white-flowered (*C. ternatea* var. *pilosula*) forms are commonly reported. In India, the plant is popularly known as “Aparajita” or “Shankhapushpi” due to the conch shell-like appearance of its flowers (3, 4). Like many medicinal plants, *C. ternatea* is rich in secondary metabolites that are responsible for its diverse pharmacological activities. Traditionally, it has been used to enhance cognitive function and to treat ailments such as fever, inflammation, pain and diabetes (5). Modern scientific studies have validated several of these traditional claims, reporting antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective properties and metabolite profiling of the plant (6, 7). Gas chromatography-mass spectrometry (GC-MS) is a powerful analytical technique widely employed for the identification

of volatile and semi-volatile phytoconstituents in plant extracts. By combining the high separation efficiency of gas chromatography with the sensitive and selective detection of mass spectrometry, GC-MS enables reliable compound identification through spectral library matching (8, 9). Consequently, GC-MS has become an essential tool in herbal medicine research for phytochemical characterisation.

Although extensive studies have been conducted on *C. ternatea*, most research has predominantly focused on the blue-flowered variety, while systematic phytochemical investigations of the white-flowered variety remain limited. Moreover, comparative analyses encompassing different plant parts of *C. ternatea* var. *pilosula* are scarce, representing a significant research gap. The white variety is of particular scientific interest due to its distinct morphological features and potential differences in phytochemical composition, which may influence its pharmacological efficacy. Therefore, the present study aims to identify and characterise the volatile phytoconstituents present in the methanolic extract of *C. ternatea* var. *pilosula* using GC-MS analysis, thereby contributing to a better understanding of its chemical profile and supporting its traditional medicinal significance.

Materials and Methods

Plant materials

Fresh and healthy plant material of *C. ternatea* var. *pilosula* (white-flowered variety) was collected from Kolhapur district, Maharashtra, India, at approximately 7:00 am local time (Fig. 1). The plant was taxonomically authenticated using regional floras (10).

The collected plant material was thoroughly washed with running tap water followed by distilled water to remove adhering dust and impurities. The samples were shade-dried at room temperature until constant weight was achieved and then coarsely powdered using a mechanical grinder.

Preparation of plant extract

Different plant parts leaf, stem, root, flower and seed were separated and processed individually. Approximately 10 g of dried powder from each plant part was weighed accurately and transferred into separate clean conical flasks. Each sample was extracted using analytical-grade methanol (Merck $\geq 99.8\%$) at a plant material to solvent ratio of 1:10 (w/v).

Methanol was selected as the extraction solvent due to its high polarity, efficiency in extracting a wide range of bioactive phytochemicals and compatibility with GC-MS analysis. The mixtures were kept for 24 hr at room temperature with intermittent shaking to ensure efficient extraction. The extracts were filtered through Whatman No. 41 filter paper and the filtrates were concentrated to dryness under reduced pressure. The dried residues were weighed and reconstituted in a known volume of methanol to obtain the final extract solutions, which were stored at 4 °C until GC-MS analysis (11, 12).

Gas chromatography-mass spectrometry analysis of the methanolic extracts was carried out using a Shimadzu QP-2010 gas chromatography-mass spectrometry system equipped with a non-polar RTX-5MS capillary column (60 m). High-purity helium was used as the carrier gas. The oven temperature program was set with an initial temperature of 40 °C, held for 3 min, followed by a gradual increase to a final temperature of 280 °C at a rate of 10 °C per min. A sample volume of 2 μ L was injected in split less mode.

Mass spectra were recorded over a scan range of 35–650 amu using electron impact ionization at 70 eV. The total run time for each analysis was approximately 45 min. The relative percentage composition of each detected compound was calculated by comparing its peak area with the total ion chromatogram (TIC) area. The mass spectra and chromatographic data were processed using dedicated GC-MS analysis software.

Identification of compounds was performed by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) mass spectral library, which contains over 62000 reference spectra. The molecular weight, probable structure and chemical identity of each compound were determined based on spectral matching. Further prediction of biological relevance was supported using Dr. Duke's Phytochemical and Ethnobotanical Database.

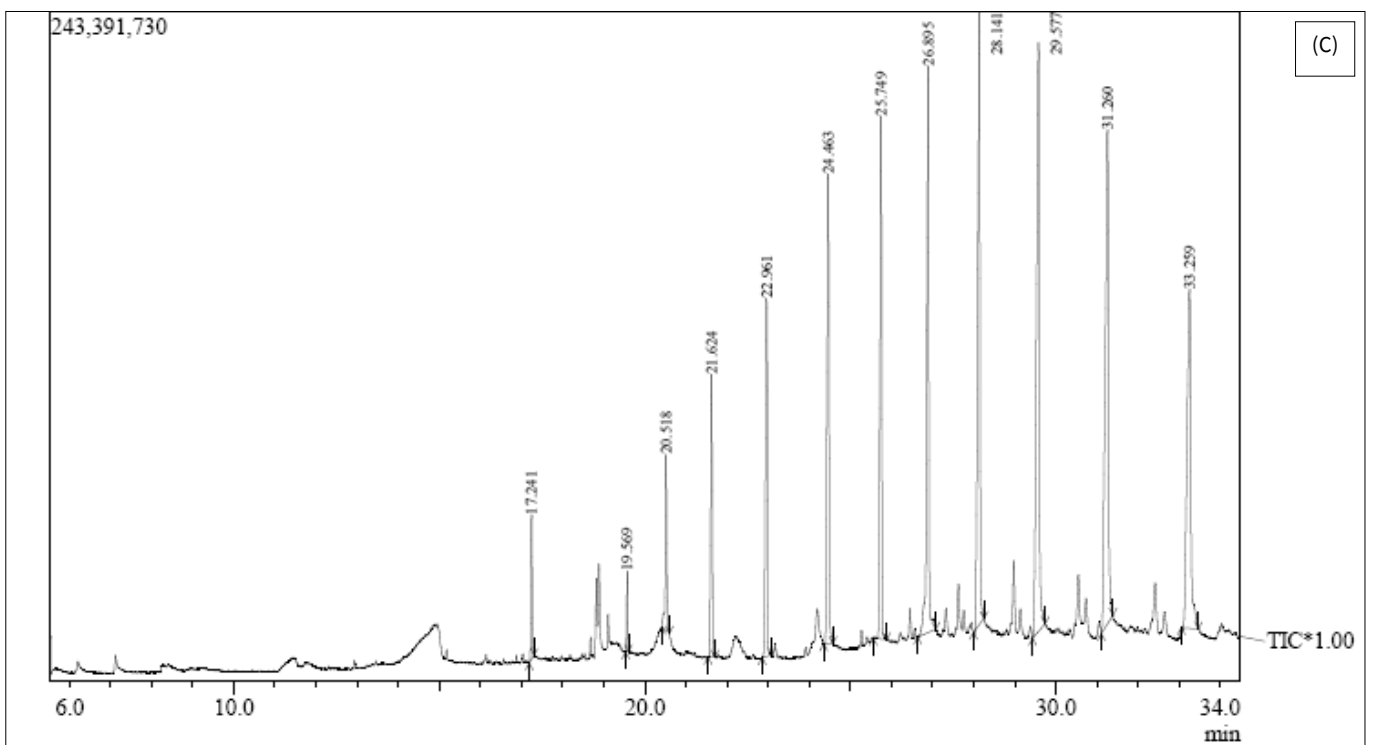
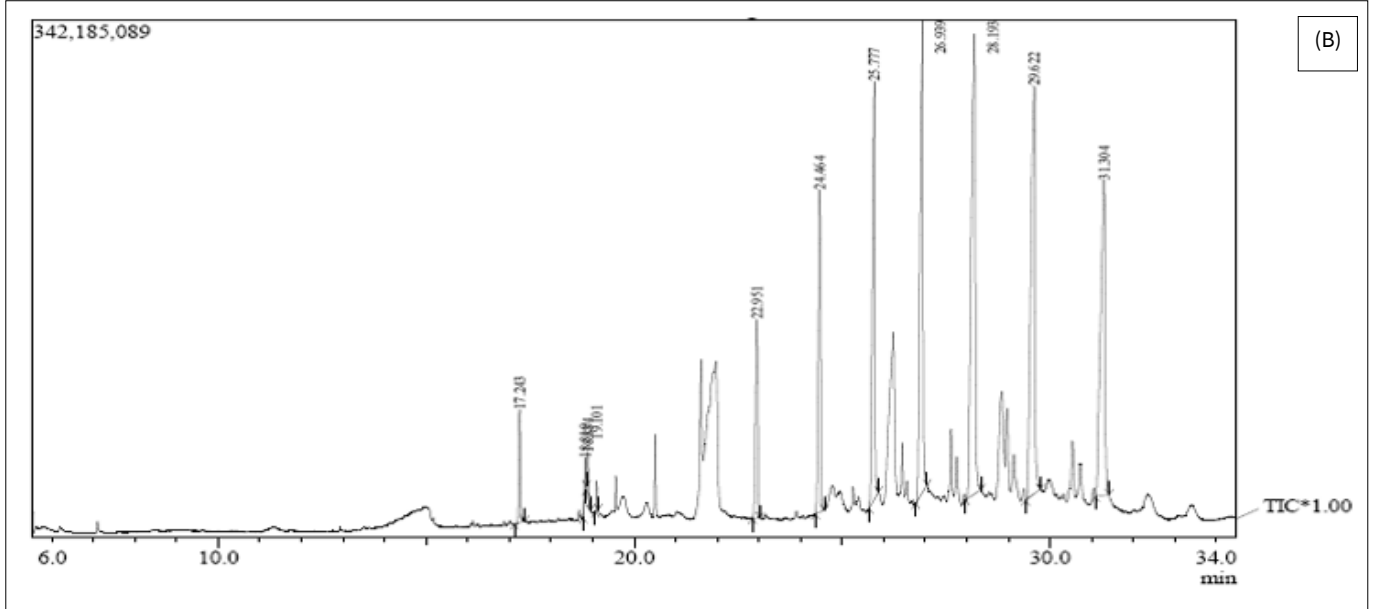
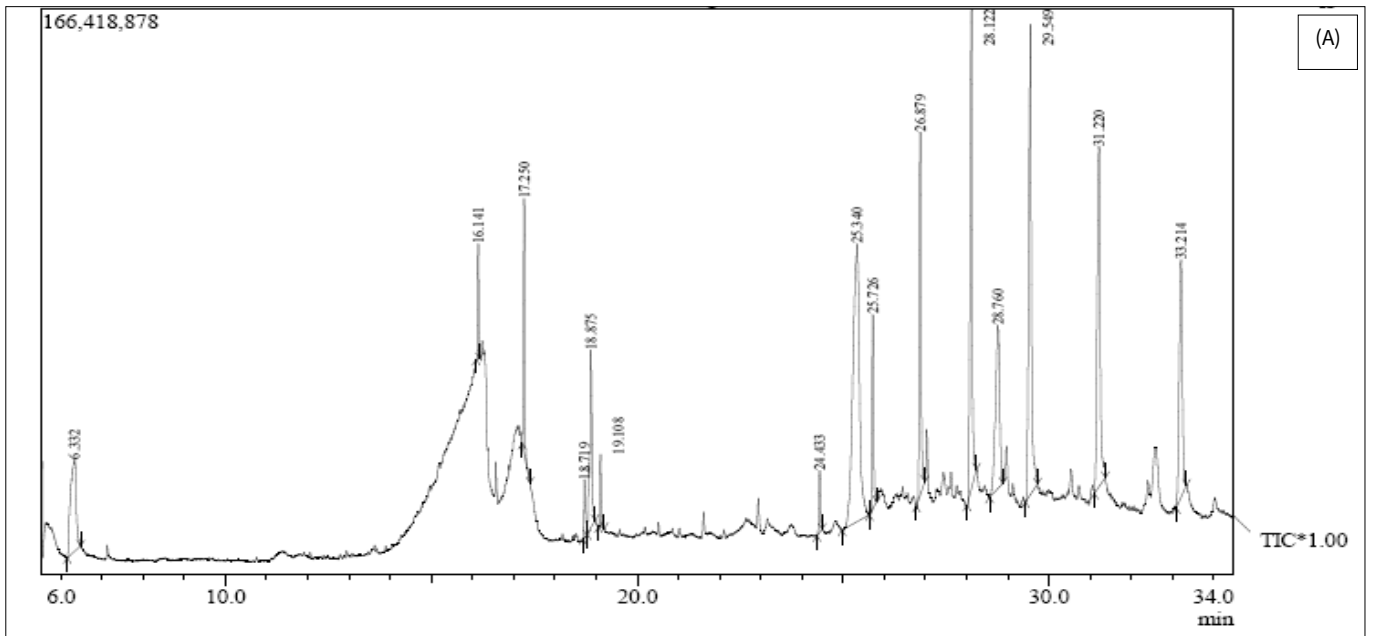
Results

The phytochemical investigation of the leaf, stem, root, flower and seed of *C. ternatea* (white flower plant) revealed that each plant part possesses a distinct chemical composition. Gas chromatography-mass spectrometry analysis generated well-resolved chromatograms, indicating the presence of a wide range of bioactive constituents. The detected compounds belonged to diverse chemical classes, including fatty acids, carbohydrates, aromatic compounds, organic acids, amino acids, ketones, flavonoids, phenols, alcohols, esters, aldehydes, terpenoids, amides, amines and ascorbic acid.

Distinct chromatographic profiles were observed for each plant organ, reflecting variations in their metabolic composition and relative abundance of phytochemicals. Individual peaks in the chromatograms corresponded to specific compounds, providing qualitative and semi-quantitative information on the chemical constituents present. The GC-MS chromatograms of *C. ternatea* extracts are illustrated in Fig. 2, highlighting the differences in phytochemical composition among the various plant parts.



Fig. 1. Habitat of white flower variety of *Clitoria ternatea*.



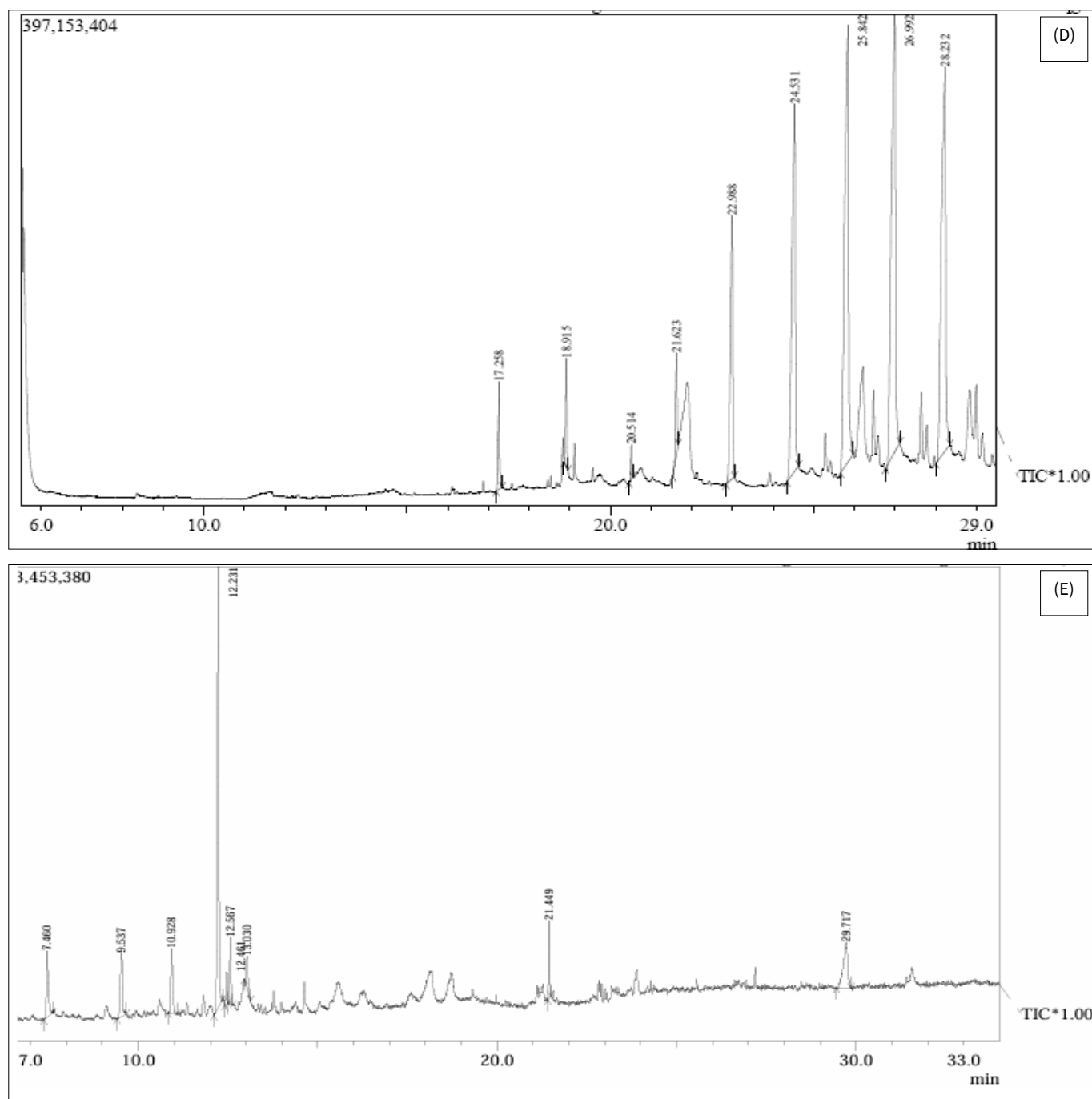


Fig. 2. Gas chromatography-mass spectrometry chromatograms of methanolic extracts of *Clitoria ternatea* (white-flowered variety). (A) Leaves extract showing dominance of long-chain hydrocarbons and fatty acids such as hexadecanoic acid and pristane; (B) Stem extract characterised mainly by saturated and unsaturated fatty acids; (C) Root extract enriched with aliphatic hydrocarbons; (D) Seed extract containing fatty acids and alkanes; (E) Flower extract dominated by oxygenated compounds including 3-furanmethanol, glycerol and heterocyclic compounds.

Gas chromatography-mass spectrometry analysis of the methanolic extracts of different plant parts of *C. ternatea* (white-flowered variety) revealed the presence of a diverse range of bioactive compounds with varying chemical compositions and relative abundances. The number and type of compounds detected differed among the leaf, stem, root, seed and flower, indicating part-specific metabolic diversity.

The leaf extract exhibited the highest number of identified constituents, dominated by long-chain hydrocarbons, fatty acids and terpenoid compounds. Major constituents included hexadecanoic acid, octadecanoic acid, phytol, pristane (2,6,10,13-tetramethylpentadecane), hexacontane and isoamyl acetate, suggesting a rich profile of lipid-derived and terpenoid metabolites. These compounds contributed significantly to the overall peak area, indicating their abundance in leaf tissue.

The stem extract was characterised mainly by saturated and unsaturated fatty acids, with hexadecanoic acid, octadecanoic acid and linoleic acid derivatives being predominant. Hydrocarbon components such as pristane were also detected, although in comparatively lower concentrations. The chromatographic pattern of the stem suggested a simpler chemical composition compared to the leaf.

In the root extract, fewer compounds were detected, with dominance of long-chain hydrocarbons and fatty acids. Hexadecanoic acid, tetracosane, pristane and hexacontane were the principal constituents, indicating the prevalence of non-polar compounds in underground tissues. The relatively high abundance of hydrocarbons may reflect structural or protective roles in the root.

The seed extract contained a moderate number of constituents, primarily fatty acids and alkanes. Hexadecanoic acid and 9-octadecenoic acid were among the major components, along with heneicosane and pristane. The chemical profile of the seed suggests a lipid-rich composition, consistent with its physiological role in energy storage.

The flower extract displayed a distinct chromatographic profile dominated by oxygenated compounds, including alcohols, ketones, esters and heterocyclic compounds. Notably, 3-furanmethanol showed the highest relative peak area, followed by glycerol, methyl 1-methylcyclopropyl ketone and phenolic heterocycles such as 4H-pyran-4-one derivatives. Fatty acids and steroidal compounds were also detected in lower proportions. This unique composition highlights the metabolic specialisation of floral tissues.

The identified compounds belong to several biologically relevant chemical classes, including fatty acids, esters, alcohols, terpenoids, hydrocarbons, phenolic compounds and heterocycles. Many of these compounds have been previously reported to exhibit antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, hypocholesterolemic and anticancer activities, supporting the traditional medicinal use of *C. ternatea*. Overall, the GC-MS analysis demonstrates significant variation in phytochemical composition among different plant parts, emphasising the importance of part-specific investigations in medicinal plant research.

Discussion

Gas chromatography-mass spectrometry analysis of the methanolic extracts of different plant parts of *C. ternatea* (white flower variety) revealed considerable variation in phytochemical composition, as presented in Table 1. The diversity and distribution of compounds across leaves, stem, root, seed and flower indicate part-specific metabolic specialisation and differential accumulation of secondary metabolites.

The leaf extract exhibited the highest number of compounds, dominated by long-chain hydrocarbons and fatty acids. Among these, hexacontane recorded the highest peak area (19.96 %), followed by pentadecane, 2,6,10,13-tetramethyl (7.44 %) and isoamyl acetate (5.82 %). These findings are broadly consistent with earlier GC-MS investigations on *C. ternatea*. For instance, 10 compounds reported in the ethyl acetate extract of white-flowered leaves including fatty acids, alcohols, sulfur-containing esters and ascorbic acid derivatives (13). The predominance of hydrocarbons such as hexacosane, tetracosane and hexacontane suggests their role in protective functions, including antimicrobial defense and stress tolerance. Fatty acids such as n-hexadecanoic acid, octadecanoic acid and polyunsaturated alcohols were also detected, which are known to contribute to antioxidant and anti-inflammatory activities (Table 2). Comparative analysis with the blue-flowered variety of *C. ternatea* further supports varietal and solvent-dependent chemical diversity. Eight compounds reported in the methanolic

Table 1. Bioactive compounds identified in the methanolic extract of *Clitoria ternatea* (White flower variety)

Sl. no.	Plant part used	Retention time	Compound name	Molecular formula	Molecular weight	Peak area (%)
1	Leaves (10)	6.332	1-Butanol, 3-methyl-, acetate (Isoamyl acetate)	C ₇ H ₁₄ O ₂	130	5.82
		16.141	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.15
		17.250	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	3.63
		18.719	Phytol	C ₂₀ H ₄₀ O	296	0.83
		18.875	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)	C ₁₈ H ₃₂ O	264	4.34
		19.108	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	0.83
		24.433	Hexacosane	C ₂₆ H ₅₄	366	1.04
		25.340	Hexacontane	C ₆₀ H ₁₂₂	842	19.96
		25.726	Tetracosane	C ₂₄ H ₅₀	338	3.33
		26.879	Pentadecane,2,6,10,13-tetramethyl	C ₁₉ H ₄₀	268	7.44
		17.243	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	1.76
		18.819	9,12-Octadecadienoic acid (Z, Z)	C ₁₈ H ₃₂ O ₂	280	0.48
		18.884	9-Octadecenoic acid (E)	C ₁₈ H ₃₄ O ₂	282	0.62
		19.101	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	0.33
2	Stem (5)	22.951	Pentadecane,2,6,10,13-tetramethyl	C ₁₉ H ₄₀	268	4.45
		17.241	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	1.60
		19.569	Tetracosane	C ₂₄ H ₅₀	338	0.69
		21.624	Pentadecane,2,6,10,13-tetramethyl	C ₁₉ H ₄₀	268	4.03
3	Root (4)	33.259	Hexacontane	C ₆₀ H ₁₂₂	842	12.47
		17.258	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	2.04
		18.915	9-Octadecenoic acid(E)	C ₁₈ H ₃₄ O ₂	282	1.73
		20.514	Heneicosane	C ₂₁ H ₄₄	296	0.52
4	Seed (4)	21.623	Pentadecane,2,6,10,13-tetramethyl	C ₁₉ H ₄₀	268	2.17
		7.458	Glycerin	C ₃ H ₈ O ₃	92	8.01
		9.533	Methyl 1-methylcyclopropyl ketone	C ₆ H ₁₀ O	98	9.21
		10.925	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144	7.88
		12.233	3-Furanmethanol	C ₅ H ₆ O ₂	98	47.16
		12.458	Propanoic acid, 2-oxo-, methyl ester	C ₄ H ₆ O ₃	102	2.69
		12.567	Aziridine, 1-(2-buten-1-yl)-, (E)-	C ₆ H ₁₁ N	97	5.40
		13.033	1-Butanol, 2-ethyl-	C ₆ H ₁₄ O	102	2.66
		21.450	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	4.23
		29.717	Cholestane, 4,5-epoxy-, (4. alpha.,5. alpha.)-	C ₂₇ H ₄₆ O	386	12.76

leaf extract of the blue variety with cis-9,10-epoxyoctadecan-1-ol contributing the highest peak area (50.66 %) (14). In contrast, the present investigation identified 10 compounds in the methanolic extract of white-flowered leaves, with hexacontane showing the maximum peak area (19.96 %), suggesting potential differences in biosynthetic pathways or metabolite accumulation between varieties.

The stem extract showed comparatively fewer compounds, primarily fatty acids and their derivatives. Pentadecane, 2,6,10,13-tetramethyl exhibited the highest peak area (4.45 %), while unsaturated fatty acids such as 9,12-octadecadienoic acid and 9-octadecenoic acid were present in minor quantities. These compounds are reported to possess anti-inflammatory and hepatoprotective properties, supporting the medicinal relevance of the stem extract. Root extracts were characterised by the presence of n-hexadecanoic acid, tetracosane, pentadecane derivatives and a notable proportion of hexacontane (12.47 %). Similar observations were reported earlier, where n-hexadecanoic acid was the dominant constituent in the ethanolic root extract of *C. ternatea* (15). Palmitic acid (n-hexadecanoic acid) has been reported to exhibit moderate cholinesterase inhibitory activity, highlighting its potential neuroprotective relevance (16, 17). Palmitic acid (n-hexadecanoic acid) has been widely reported for its antioxidant, antimicrobial and enzyme inhibitory properties, while hexacontane has gained attention for its antimicrobial potential and possible role in inhibiting bacterial efflux pumps. The occurrence of these compounds supports the traditional use of *C. ternatea* roots in medicinal preparations.

Seed extracts contained moderate levels of fatty acids and hydrocarbons, with n-hexadecanoic acid and 9-octadecenoic acid being the major bioactive constituents. These fatty acids are associated with hypocholesterolemic and antimicrobial effects, indicating possible nutritional and therapeutic value of seeds. The floral extract exhibited a distinct phytochemical profile compared to vegetative parts. The most abundant compound was 3-furanmethanol (47.16 %), followed by cholestane, 4,5-epoxy- (12.76 %), methyl 1-methylcyclopropyl ketone (9.21 %), glycerin (8.01 %) and 4H-pyran-4-one derivatives (7.88 %). The presence of 4H-pyran-4-one, a phenolic heterocycle related to flavonoid biosynthesis, highlights the antioxidant and antimicrobial potential of flowers. Aziridine derivatives detected exclusively in flowers are notable due to their alkylating properties and reported antibacterial and cytotoxic activities. These findings suggest that

floral extracts may possess unique pharmacological properties not observed in other plant parts. Several phytoconstituents detected in the present study were also reported in the blue-flowered variety, including n-hexadecanoic acid, 1-butanol-3-methyl acetate, 9,12,15-octadecatrien-1-ol (Z, Z, Z), pentadecane (2,6,10,13-tetramethyl), octadecenoic acid, tetracosane, hexacosane, hexacontane and linoleic acid derivatives (18). Additionally, ethanolic extracts of aerial parts of *C. ternatea* were reported to contain compounds such as n-hexadecanoic acid, phytol and 9,12-octadecadienoic acid methyl ester, several of which were also identified in the present methanolic extracts (19).

Phytol, a diterpene alcohol and precursor of vitamins E and K₁, was consistently detected across different plant parts, reinforcing its widespread occurrence in medicinal plants. Phytol has been reported to exhibit antibacterial activity through membrane disruption, along with anti-inflammatory and anticancer effects, thereby supporting the traditional medicinal applications of *C. ternatea*. Comparable bioactive compounds identified in other medicinal plants, such as *Cassia italica*, further corroborate the pharmacological relevance of fatty acids and diterpenes detected in this study.

Earlier phytochemical investigations have reported the presence of sterols, flavonoid glycosides, anthocyanins, phenolic acids and triterpenoids in different parts of *C. ternatea* (20). There are 7 compounds identified in the ethanolic aerial extract using GC-MS, with n-hexadecanoic acid and 1-butanol-3-methyl acetate as major constituents (21). The detection of tetracosane and hexacosane in both leaves and roots in the present study further supports their potential antimicrobial roles.

Hexacontane, identified as a major constituent in the present investigation, has been reported as a potential inhibitor of the AcrB efflux pump protein, contributing to antimicrobial resistance modulation (22). Its antimicrobial efficacy against various bacterial strains has been documented. Additionally, aziridine derivatives detected exclusively in floral extracts are known alkylating agents with reported antibacterial and cytotoxic activities.

Table 2 summarises the biological activities of selected major compounds identified in the methanolic extracts. Fatty acids such as hexadecanoic acid, octadecanoic acid and linoleic acid derivatives are well documented for their antioxidant, anti-inflammatory, hypocholesterolemic and antimicrobial effects.

Table 2. Activity of some of the bioactive compounds identified in the methanolic extract of *Clitoria ternatea* (white flower variety)

Sl. no.	Compound name	Compound nature	** Biological activity
1	Isoamyl acetate	Aliphatic ester	Antimicrobial, fragrance agent (23)
2	3-Furanmethanol	Heterocyclic alcohol	Antioxidant, flavour compound (24)
3	Hexadecanoic acid	Saturated fatty acid	Antioxidant, anti-inflammatory, hypocholesterolemic, antimicrobial (25)
4	Octadecanoic acid	Saturated Fatty acid	Hypocholesterolemic, lubricant, cosmetic ingredient (26)
5	9,12-Octadecadienoic acid (Z, Z)	Polyunsaturated fatty acid	Anti-inflammatory, hepatoprotective, antiarthritic, anticancer (27)
6	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)	Polyunsaturated fatty alcohol	Antimicrobial activity (28)
7	Phytol or 3,7,11,15-Tetramethyl- 2-hexadecen-1-ol	Diterpene alcohol	Antioxidant, antimicrobial, anti-inflammatory, anticancer, hepatoprotective (29)
8	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	Phenolic heterocycle	Antioxidant, antimicrobial, anti-inflammatory (30)
9	Tetracosane	Long-chain alkane	Antibacterial, anti-inflammatory, antioxidant (31)

** Activity sources: Dr. Duke's Phytochemical and Ethnobotanical Database and published literature (32).

Phytol and its isomer 3,7,11,15-tetramethyl-2-hexadecen-1-ol, detected in leaf extracts, are diterpene alcohols known for broad-spectrum bioactivities, including antimicrobial, anticancer and hepatoprotective actions. The identification of tetracosane and other long-chain alkanes further supports the antibacterial and antioxidant potential of *C. ternatea* extracts.

Overall, the GC-MS-based phytochemical profiling demonstrates that methanolic extracts of *Clitoria ternatea* (white flower variety) are rich in biologically active compounds with diverse pharmacological properties. The variation in compound distribution among different plant parts suggests that each part may serve as a valuable source of specific bioactivities. These findings provide strong scientific validation for the traditional medicinal use of *C. ternatea* and support its potential application in the development of plant-based therapeutic agents.

Conclusion

This study presents a comparative GC-MS analysis of methanolic extracts from different plant parts of *C. ternatea* (white-flowered variety), demonstrating clear part-specific variation in phytochemical composition. Leaves showed the greatest chemical diversity, dominated by fatty acids, long-chain hydrocarbons and terpenoid compounds, whereas stems and roots exhibited simpler profiles mainly composed of fatty acids and alkanes. Seeds were characterised by lipid-associated constituents, while flowers displayed a distinct abundance of oxygenated and heterocyclic compounds, indicating metabolic specialisation.

The novelty of this work lies in the systematic, part-wise profiling of the white-flowered variety using methanolic extracts, revealing both shared and unique metabolites across plant organs. Compounds such as hexadecanoic acid, phytol, long-chain alkanes and phenolic heterocycles were recurrently detected, suggesting potential biological relevance based on previous literature.

However, the biological activities inferred from these compounds should be considered indicative rather than definitive, as this study is limited to qualitative and semi-quantitative GC-MS analysis. Non-volatile and highly polar metabolites may not be fully represented. Future studies involving targeted compound isolation, bioactivity-guided assays and complementary analytical techniques are necessary to validate the pharmacological potential of *C. ternatea*.

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Authors' contributions

SSK carried out the experimentation and manuscript writing. VDJ contributed by assisting with the identification of the plant species. Both authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflicts of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used AI and ChatGPT, developed by OpenAI in order to improve the clarity, grammar and readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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