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**Research Article**

# ANTI-INFLAMMATORY POTENTIAL EVALUATION OF *CLITORIA TERNATEA* THROUGH METABOLITE PROFILING BASED ON INDIAN TRADITIONAL KNOWLEDGE

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## ABSTRACT

Indian Traditional Knowledge is a storehouse of medicinal plants which can be used to treat various acute and chronic diseases. *Clitoria ternatea* L. (Fabaceae) the butterfly pea, a well-known medicinal plant in Ayurvedic medicine falls under the category of Medhya Rasayana due to its cognitive-enhancing, antioxidant and neuroprotective properties. The current study systematically analyses the phytochemical constituents of an acidified hydroalcoholic extract of *Clitoria ternatea* flowers by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Dried flower samples were extracted and the extract was analysed using a mass spectrometer in positive and negative ion modes. The metabolite profile was identified which allowed the identification of 60 metabolites, including flavonoids, isoflavones, phenolic acids, lignans, and polyunsaturated fatty acids. The metabolite profile was dominated by stable C-glycosyl flavonoids isovitexin, isoorientin, isoflavones (genistein), and phenolic acids including sinapic acid. These compounds have established therapeutic effects as they are anti-inflammatory, antioxidant, antidiabetic, antimicrobial, anticancer and neuroprotective in nature. Anthocyanin content of *Clitoria ternatea* flowers is well documented but there is a lack of comprehensive metabolomic analysis of the hydroalcoholic extracts which are much closer to traditional tinctures. The present study determines the metabolic profiling of *Clitoria ternatea* flowers and provides strong phytochemical evidence supporting the pharmacological relevance of hydroalcoholic formulations. The results strengthen the potential of the flower as a source of functional compounds which can help reduce inflammation and act as a nutraceutical and a phytopharmaceutical.

**Keywords:** *Clitoria ternatea*, LC-ESI-MS/MS, Hydroalcoholic extraction, Flavonoids, Metabolomics.

## INTRODUCTION

Indian Traditional Knowledge system including Ayurveda has helped to manage inflammation through natural methods with the help of plants like turmeric, ginger, aparajita, neem etc. along with many other herbs have been proven to have properties effective to deal with physical issues of pain, inflammation, swelling, odema etc (Devi *et al.,* 2003). *Clitoria ternatea* L., (Aparajita) family Fabaceae, is a perennial herbaceous climber widely distributed in tropical and subtropical climates. Historically, this plant has held a prestigious place in traditional healing systems, especially Ayurveda, where it is classified as `Medhya Rasayana`, a rejuvenating agent for memory and cognition (Mukherjee *et al.,* 2008). This plant is considered in traditional Ayurveda as well as modern pharmacology for its anti- inflammatory, analgesic and antioxidant properties. Ayurvedic texts like Sushruta Samhita describe this plant as an agent which can decrease swelling and inflammation (Swathi *et al.,* 2020). The therapeutic activity of *Clitoria ternatea* is inherent in its complex phytochemistry. Modern pharmacological studies confirm these traditional claims and demonstrate that the plant is an anti-inflammatory, nootropic, anxiolytic and anticonvulsant agent (Oguis *et al.,* 2019). The blue pigmentation of the flowers is attributed to ternatins, a unique group of anthocyanin pigments (Netravati *et al.,* 2022). Anthocyanins are the largest group of water-soluble pigments belonging to flavonoids, a subclass of the polyphenol family, contributing to the attractive orange,

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red, purple, violet, and blue colours of fruits, vegetables, and flowers (Gamage *et al.,* 2021).

However, in addition to anthocyanins, the plant also synthesizes other metabolites, including flavonol glycosides, steroids, alkaloids and triterpenoids (Mukherjee *et al.,* 2008). These bioactive compounds have pharmacologic activity including antioxidant, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, neuroprotective, and anticancer. Glycosides are effective for cardiac function and cholesterol regulation. Phytosterols lower LDL cholesterol and have beneficial effects for cardiovascular health (Kumar *et al.,* 2023; Sparg *et al.,* 2004). Alkaloids are known for their analgesic, antimicrobial, antimalarial, and anticancer properties. Triterpenoids have significant antioxidant and anti-inflammatory properties and can be potentially used in disease prevention and management of diabetes, liver disorders, and cancer (Siddique & Saleem, 2011).

These compounds are obtained through several traditional as well as modern methods of extraction where extraction is defined as the process of removing or recovering from the raw material. A traditional solvent-based method that combines the advantages of aqueous-organic solvents is hydroalcoholic (or alcoholic-aqueous) extraction. It is utilized for recovering both polar and nonpolar compounds. There are other non-conventional more sophisticated technologies but hydroalcoholic (or alcoholic-aqueous) extraction is widely used as it is simpler, has reproducibility and is low in cost (Goti & Dasgupta, 2023). Liquid chromatography combined with tandem mass spectrometry (LC-MS-TMS) is the gold standard for plant profiling due to its high sensitivity and ability to characterise compounds in complex biological matrices. Previous studies have often focused on specific classes of compounds or used techniques with lower resolution (Oniszczuk *et al.,* 2016). It has been observed that there is a specific literature gap for the simultaneous profiling of positive and negative ionising metabolite-responsive metabolites in acidified hydroalcoholic extracts stabilising sensitive phenols. So, the aim of the study was to assess the extraction efficiency of the acidified hydrocarbon solvent system and to provide a comprehensive phytochemical profile of *Clitoria ternatea* by LC-MS. This research provides a detailed chemical record necessary to standardize pharmaceuticals based on *C. ternatea* and the results will help to understand this profiling as well as identify its potential as a potent anti-inflammatory agent.

## MATERIALS AND METHODS

### Plant Material and Hydroalcoholic Extraction

Fresh flowers of the species *Clitoria ternatea* were collected and identified for any physical damage. The flowers were dried in the shade completely to avoid photolytic degradation of the sensitive phytochemicals. The morphological integrity of samples was checked and then they were ground to a fine powder. 10 gm of powdered Aprajita Flower (*Clitoria ternatea*) was taken and mixed with 100 ml of solvent Mixture (80% Methanol, 1% HCL in distilled water). Sample mixture was incubated on a rocker shaker for 24 hours following which the extract was filtered through Whittman Filter Paper 1 to remove plant debris. The filtrate was subsequently dried in a hot air oven at 40°C to obtain a concentrated solid residue. The final extracts were weighed to calculate percentage yield and were collected in micro centrifuge tubes at 4°C until analysis.

### LC-MS Analysis

### Chemicals and Reagents

Solvents and reagents used for chromatographic separation and mass spectrometric analysis included: methanol, acetonitrile, ammonium formate buffer and formic acid. High purity nitrogen gas was used as a nebuliser and drying gas during ionisation.

### Preparation of Plant Extract for LC-MS Analysis

Accurately weighed 10 mg of dried methanolic extract of *Clitoria ternatea* was taken in a sterile Eppendorf tube and dissolved in 1 ml of HPLC grade methanol till complete dissolution of phytochemicals. It was then filtered using a 0.22 mm nylon membrane filter to remove particles and prevent potential clogging of the column. The extract was loaded in the LC-MS vials of the samples. The prepared samples were then injected into the LC-MS at 2 ml for analysis.

### Instrumentation

LC-MS analysis was carried out using Waters Alliance e2695 liquid chromatographic system coupled to the triple quadrupole XEVO-TQD mass spectrometer prepared with an electrospray ionisation source (ESI).

### Mass Spectrometric Conditions

The mass spectrometer was operated in both positive and negative ionization modes to enable detection of a wide range of phytochemical classes.

### Data Acquisition and Processing

Data acquisition and processing were performed using MassLynx software. Compound identification was based on precursor molecular ions (m/z), retention times, and fragmentation patterns compared to online spectral databases (ReSpect: RIKEN Expert System for Pattern Recognition) and internal libraries, and were reported on the basis of Base Peak Intensity (BPI) and hit scores. Data acquired in full scan mode was used to generate chromatograms on the basis of baseline peak intensity. Retention time, molecular ion peaks, and fragmentation patterns were recorded for each compound detected.

### Identification of Phytochemical Constituents

The phytochemical components were identified by comparing the observed mass spectra with available online

spectral databases and references in the literature. The compounds were identified based on: Mass-to-charge ratio (m/z), Retention time, Ionization pattern ([M+H] + or [M−H] − and Base peak intensity. The use of positive and negative ionization methods provided additional coverage of the compound and enhanced the accuracy of identification.

## RESULTS AND DISCUSSION

The Hydro-alcoholic extraction from *Clitoria ternatea* flowers (80% Methanol, 1% HCl in distilled water) yielded 1285 mg of dried extract from 10,000 mg of raw powder equivalent to a 12.85% w-w extraction yield. This likely facilitates the effective extraction of anthocyanins and phenols by preserving their structural stability. The metabolic profile obtained by LC-MS was observed to be complex. 60 different peaks were identified, out of which 36 were positive ionization compounds and 24 were negative ionization compounds. These compounds exhibited multiple derivative and isomeric forms, indicating important biochemical diversity. The metabolites found belonged to many chemical classes, including flavonoids, phenolic acid, alkaloids, lignans and fatty acids. In positive ionisation mode, 36 phytochemicals were identified in the extract of *Clitoria ternatea*. The predominant compounds identified were flavonoids, isoflavones, phenols, alkaloids and quinones. Major compounds detected included sinapic acid, seneciphylline, kaempferol-3-O-galactoside derivatives, genistein, isovitexin, isoorientin, casticin, daphnoretin and phylloquinone. Several metabolites were detected in the form of structurally related isomers and glycosylated derivatives, particularly among flavonoid compounds, indicating considerable chemical diversity within the extract. The retention time of these compounds ranged from 1.41 - 34.34 minutes, indicating that both polar and non-polar components were present in the sample.

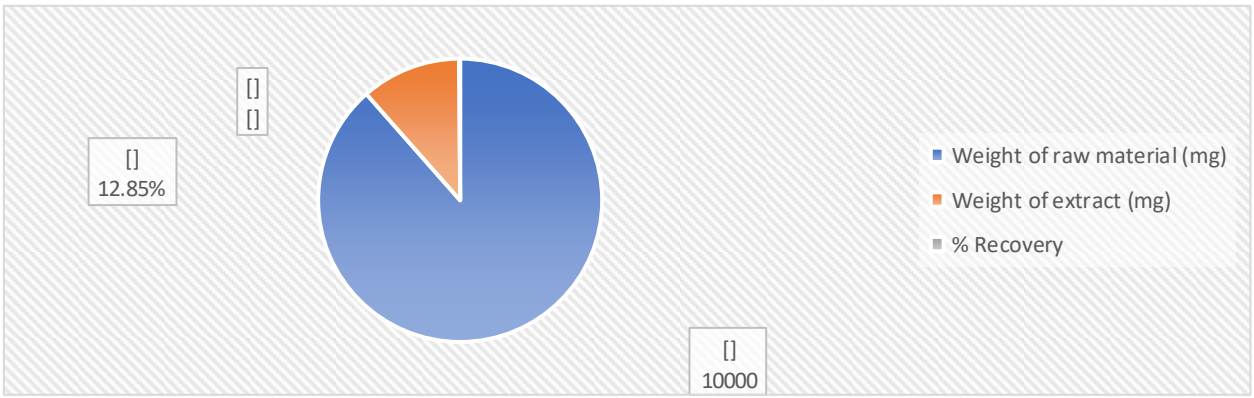
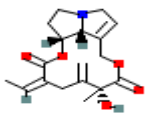
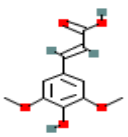
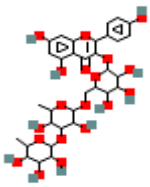
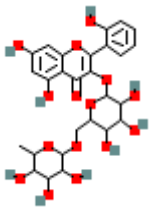
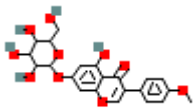
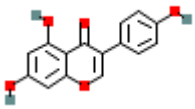
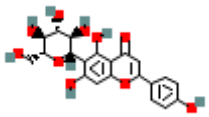
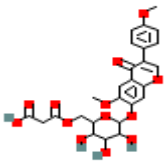
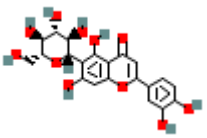
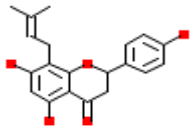
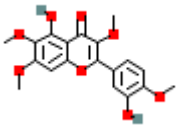
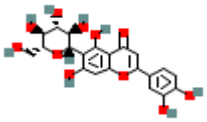
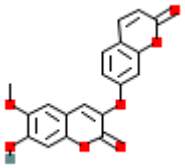
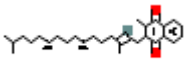
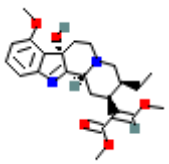



**Figure 1.** Pie chart representing the weight of raw material, extract yield, and percentage recovery of *Clitoria ternatea* flower extract.

**Table 1.** Identification details, Retention time, Molecular formula, Ion type ([M+H+] [C] and [R] and the score) of Phytochemicals in Positive Ionization Mode Profiling.

| Compound Name | Formula | Chemical structure | Activity | Reference |
|---|---|---|---|---|
| **Seneciphylline** | C18H23NO5 |  | Hepatotoxic<br>Genotoxic effect | (Pubchem) |
| **Sinapic acid** | C11H12O5 |  | Anti-inflammatory<br>Antioxidant<br>Antimicrobial<br>Anticancer<br>Antianxiety | (Chen, 2015) |

| | | | | |
|---|---|---|---|---|
| **Kaempferol-3-O-galactoside-6''-rhamnoside-3'''-rha** | C33H40O19 |  | Anti-inflammatory<br>Antioxidant<br>Cardioprotective<br>Neuroprotective<br>Hepatoprotective<br>Anti-diabetic | (Alrumaihi *et al.,* 2024) |
| **Datiscetin-3-O-rutinoside** | C27H30O15 |  | Anti-inflammatory<br>Anti-oxidant | (Shehata *et al.,* 2021) |
| **Biochanin-7-O-glucoside** | C22H22O10 |  | Anti- oxidant<br>Anti-inflammatory | (*Biochanin A-7-O-glucoside \| 5928-26-7 \| FB65335*, n.d.),<br>(Kole *et al.,* 2010) |
| **Genistein** | C15H10O5 |  | Anti- oxidant<br>Anti-inflammatory | (Ji *et al.,* 2012)<br>(Y. X. Goh *et al.,* 2022) |
| **Isovitexin** | C21H20O10 |  | Anti- oxidant<br>Anti-inflammatory | (Lv *et al.,* 2015),<br>(Zhang *et al.,* 2021) |
| **Flavone base + 1O, 2MeO, O-MalonylHex** | C26H26O13 |  | Anti-inflammatory | (Jaworska *et al.,* 2025) |
| **Isoorientin** | C21H20O11 |  | Anti- oxidant<br>Anti-inflammatory<br>Anti-cancer<br>Treat obesity and Insulin resistance | (Mazibuko-Mbeje *et al.,* 2020)<br>(Laurindo *et al.,* 2024) |

| | | | | |
|---|---|---|---|---|
| **Flavanone base + 3O, 1Prenyl** | C20H20O5 |  | Anti-inflammatory | (*High Purity Licoflavanone \| CAS:119240-82-3 Guaranteed by HPLC,NMR,MS for Research Use*, n.d.) |
| **Casticin** | C19H18O8 |  | Anti-inflammatory | (Chan *et al.,* 2018) |
| **Luteolin-6-C-glucoside** | C21H20O11 |  | Anti-inflammatory | (*Luteolin-6-C-glucoside \| 4261-42-1 \| ML65431 \| BioSynth*, n.d.) |
| **Daphnoretin** | C19H12O7 |  | Anti inflammatory<br><br>Anti-cancer | (Wu *et al.,* 2024)<br><br>(Jiang *et al.,* 2014) |
| **Phylloquinone** | C31H46O2 |  | Anti inflammatory | (Xie *et al.,* 2024) |
| **7-Hydroxymitragynine** | C23H30N2O5 |  | Analgesic Effects | (Kruegel *et al.,* 2019) |

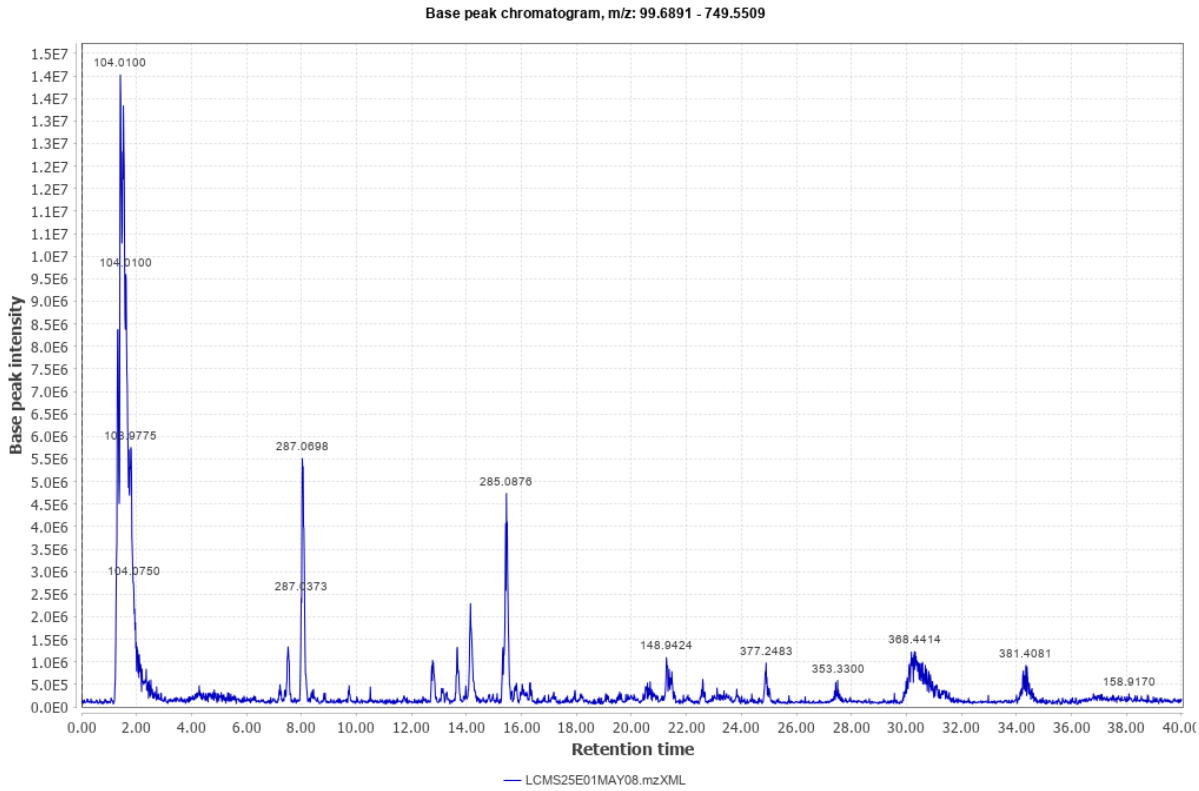
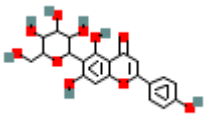
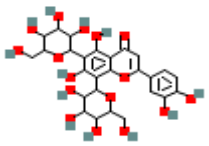
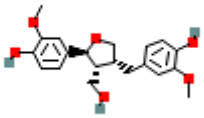
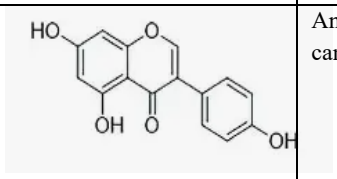
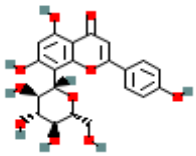
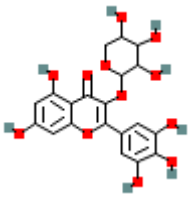
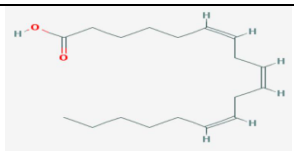
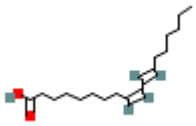
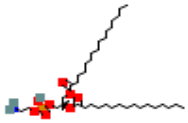
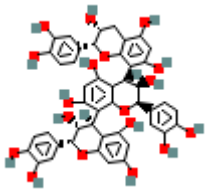
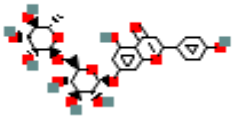
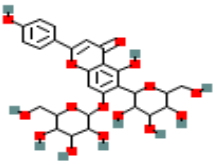



**Figure 2.** Chromatogram For Positive Ionization Mode.

**Table 2.** Identification details, Retention time, Molecular formula, Ion type ([M-H-] [C] and [R] and the score) of Phytochemicals in Negative Ionization Mode Profiling.

| **Compound Name** | **Formula** | **Chemical structure** | **Function** | **References** |
|---|---|---|---|---|
| Isovitexin(4) | C21H20O10 |  | Anti-oxidant<br><br>Anti-inflammatory | Lv *et al.,* 2015),<br><br>(Zhang *et al.,* 2021) |
| Luteolin 6-C-glucoside 8-C-arabinoside | C27H30O16 |  | Anti-inflammatory | (*Luteolin 6-C-glucoside 8-C-arabinoside\|C27H30 O16*, n.d.)<br><br>(Lin *et al.,* 2008) |
| isosakuranetin-7-O-rutinoside | C28H34O14 |  | Anti-inflammatory | (PubChem)<br><br>(Ali *et al.,* 2019) |

| | | | | |
|---|---|---|---|---|
| Lariciresinol | C20H24O6 |  | Anti inflammatory | (PubChem)<br>(Henna *et al.,* 2025) |
| Hex-hexA Apigenin (or Galangin or Genistein) | C27H28O16 |  | Anti cancerous | (Ji *et al.,* 2022) |
| Apigenin-8-C-glucoside | C21H20O10 |  | Anti-inflammatory Antioxidant | (PubChem)<br>(Allemailem *et al.,* 2024) |
| Myricetin-3-O-xyloside | C20H18O12 |  | Antioxidant, Anti-inflammatory Antifibrotic | (Domitrović *et al.,* 2015) |
| y-Linolenic acid | C18H30O2 |  | Anti-inflammatory | (Kapoor & Huang, 2006) |
| 9Z, 11E-Linoleic acid | C18H32O2 |  | Anti-inflammatory | (Kapoor & Huang, 2006) |
| 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine | C37H74NO8P |  | Creating artificial model membranes and lipid monolayers | (PubChem) |
| 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol | C39H75O8P |  | Use as Clinical & Forensic Biomarker | (PubChem) |

| | | | | |
|---|---|---|---|---|
| Procyanidin C1 | C45H38O18 |  | Anti inflammatory<br>Anti-oxidant | (Dasiman *et al.,* 2021) |
| Isorhoifolin | C27H30O14 |  | Anti inflammatory | (Pubcehm)<br>(Peng *et al.,* 2020) |
| Apigenin-6-C-glucoside-7-O-glucoside | C27H30O15 |  | Anti inflammatory<br>Anti-oxidant | (Pubcehm)<br>(Wang *et al.,* 2020) |

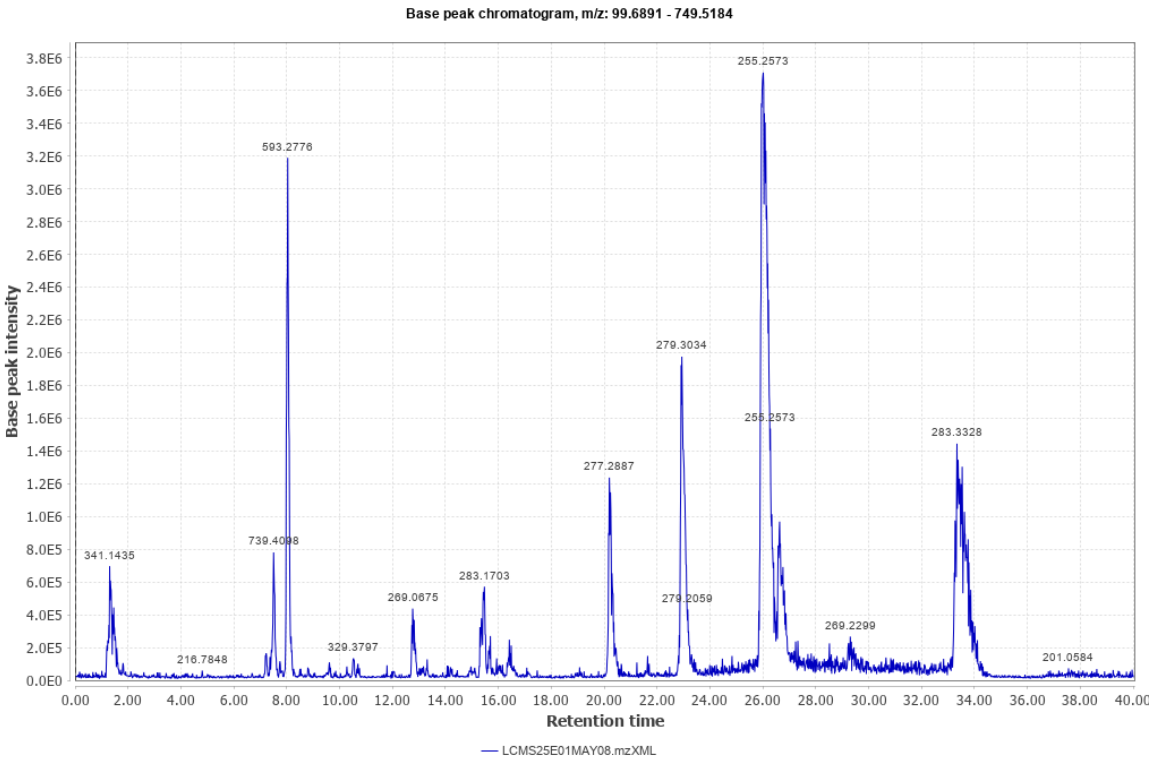



**Figure 2.** Chromatogram For Negative Ionization Mode.

In the negative ionization mode, 24 phytochemicals were identified in the extract of *Clitoria ternatea*. Complex phytochemical profile was obtained containing metabolites from diverse chemical classes, including predominantly flavonoid glycosides (isovitexin, luteolin C-glycoside derivatives), lignans (lariciresinol), fatty acids and phospholipids (linoleic acid; linolenic acid; procyanidin C1 and phosphatidylethanolamine derivatives) and negatively ionized compounds for acidic and phenolic compounds. Several compounds were detected in the form of structurally related isomers and derivative forms, indicating considerable chemical diversity within the extract. The identified metabolites showed a broad range of retention times from 1.31 - 33.34 minutes, suggesting the presence of both polar and non-polar constituents in the sample. In the present study, the phytochemical investigation of the hydroalcoholic extract of *Clitoria ternatea* flowers was carried out using LC-ESI-MS/MS. This study helps to fill a

gap in metabolomic studies of traditionally relevant extraction systems (i.e., aqueous or methanolic extracts) which have been the main focus of previous studies. The extraction yield of 12.85% (w/w) obtained in this study reflects efficient recovery of bioactive compounds. The use of an acidified solvent system (80% methanol with 1% HCl) may have stabilized anthocyanins and other phenolic compounds that are prone to degradation under neutral or alkaline conditions, which is in line with previous reports which state that acidic conditions are important for flavonoid stability and efficient extraction (Gamage *et al.,* 2021). A detailed metabolite profile with 60 compounds was identified in positive ionization mode and negative ionization mode. There was a marked abundance of flavonoids, including isovitexin, isoorientin, genistein, and kaempferol derivatives in the extract. These compounds emphasize the anti-inflammatory and antioxidant potential of this plant as supported by earlier studies. Previous studies corroborate and have identified flavonoids as major contributors to the pharmacological activities of *Clitoria ternatea*. They substantiate the anti-inflammatory, neuroprotective and anxiolytic effects of this plant (Mukherjee *et al.,* 2008; Oguis *et al.,* 2019). Dual ionization modes enabled the detection of a broad spectrum of chemical classes, including phenolic acids, lignans, fatty acids, alkaloids, and phospholipids, in agreement with the well-documented utility of LC-MS/MS for plant metabolomics (Oniszczuk *et al.,* 2016). The detection of biologically active compounds like sinapic acid, procyanidin C1 and linoleic acid reinforces the poly pharmacological potential of the plant and can be established by comparative assessment with previous work (Kaneria *et al.,* 2012). Studies that were primarily focused on anthocyanins further emphasize the significance of the present study by expanding the metabolite coverage to a diverse set of secondary metabolites (Netravati *et al.,* 2022). Kaempferol and quercetin have been shown to attenuate oxidative stress and inflammation, which promotes cardiovascular and metabolic health, while genistein-like compounds display phytoestrogenic and anticancer activities that may aid bone and hormonal health (Multisona *et al.,* 2023). The blue color of butterfly pea flowers is attributed to the presence of anthocyanins, specifically ternatins, which exhibit potent antioxidant activity and protect cells from oxidative damage and support cognitive function (Gamage *et al.,* 2021). Moreover, *Clitoria ternatea* exhibits antimicrobial and antidiabetic activities, which further demonstrate its potential as a therapeutic agent for the prevention of chronic diseases and as a health supplement (Jeyaraj *et al.,* 2022).

The pharmacologically significant compounds detected such as genistein (anti-inflammatory), isoorientin (anti-diabetic and anti-obesity), and daphnoretin (anticancer activity), provide scientific evidence for the traditional use of *Clitoria ternatea* in Ayurveda and support the therapeutic significance of the plant, as well as its potential for nutraceutical and phytopharmaceutical development. The study successfully combines traditional knowledge with contemporary analytical methods to establish a comprehensive metabolomic profile for *Clitoria ternatea*, which advances phytochemical understanding and supports the standardization and scientific validation of herbal formulations (Maia *et al.,* 2025). Thus, *Clitoria ternatea* has significant potential for application in functional foods, nutraceuticals, and pharmaceutical research.

## CONCLUSION

*Clitoria ternatea* is a versatile medicinal plant with a long-standing of traditional use and increasing scientific interest. There is mounting evidence that the flower is a treasure trove of phytochemicals with antioxidant, antihyperglycemic, and neuroprotective properties, and that anthocyanins and flavonol glycosides are the active constituents of the flower. The extraction procedure is an important factor in determining the phytochemical content and hence the biological activity. In this current study, detailed LC-ESI-MS/MS analysis of the hydroalcoholic extract of *Clitoria ternatea* flowers allowed the identification of 60 metabolites. Several compounds were detected in the form of structurally related isomers and derivative forms, particularly among flavonoid glycosides, indicating substantial structural diversity within the extract. The broad retention time distribution of the detected metabolites suggested the presence of compounds with varying polarities, reflecting the rich phytochemical composition. The metabolite profile showed the dominance of stable C-glycosyl flavonoids, isoflavones like genistein, and phenolic acids like sinapic acid, thus justifying that the flowers have a rich source of functionally important secondary metabolites. The hydroalcoholic extraction method resulted in 12.85%, thus justifying its use for optimal phytochemical extraction. Taken together, the current data offers a strong phytochemical rationale for the traditional use of the plant as a nootropic and antioxidant agent. The study provides a detailed qualitative phytochemical analysis, yet the LC-ESI-MS/MS analysis was basically qualitative, and there was no absolute quantification of the identified metabolites. The phytochemical analysis indicates the possible antioxidant and neuroprotective activities, there were no in vitro, cell-based, or in vivo bioactivity assays to directly link the identified metabolites to their activities. Isolation of major bioactive compounds through quantitative analysis can help in comparison studies between the anthocyanin fraction and the flavanol fraction to understand the structure-activity relationship and identify the major bioactive compounds that contribute to particular biological activities.

Future In vitro and in vivo studies, to validate antioxidant, neuroprotective, and antihyperglycemic activities, will help to confirm the biological relevance of the identified metabolites. Additionally, pharmacokinetic, bioavailability, and metabolism studies would be valuable for understanding the systemic disposition of the *Clitoria ternatea* phytochemicals. These studies would help to enhance the translational application of the flower extract as a functional food ingredient or phytopharmaceutical. In summary, the current phytochemical analysis provides a pivotal reference for future bioactivity-guided studies.

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**CONFLICT OF INTERESTS**

The authors declare no conflict of interest

**ETHICS APPROVAL**

Not applicable

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**AI TOOL DECLARATION**

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

**DATA AVAILABILITY**

Data will be available on request

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