

Original Research Article

Phytochemical characterization and HPLC analysis of *caralluma fimbriata* wall: An integrated investigation of bioactive secondary metabolites

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Citation D. Avasthi, K. Pawar, Phytochemical characterization and HPLC analysis of *caralluma fimbriata* wall: An integrated investigation of bioactive secondary metabolites. *J. Pharm. Sci. Comput. Chem.* **2026**, 2(1), 45-51.

 <https://doi.org/10.48309/jpscc.2026.579020.1040>

**Article info:****Submitted:** 2026-01-15**Revised:** 2026-02-22**Accepted:** 2026-02-24**ID:** JPSCC-2603-1040**Article Podcast:** [Click here](#)**Keywords:**

Caralluma fimbriata, Phytochemical screening, HPLC analysis, Secondary metabolites, Ethnomedicine, Bioactive compounds

ABSTRACT

Background and objective: *Caralluma fimbriata* Wall, a succulent medicinal plant of the Apocynaceae family, has been traditionally utilized in various therapeutic applications including metabolic disorders, appetite regulation, and diabetes management. Despite its ethnomedicinal importance, comprehensive analytical characterization of its bioactive constituents remains limited. This investigation aimed to conduct systematic phytochemical screening and chromatographic profiling of *C. fimbriata* extracts to identify and characterize the major secondary metabolites responsible for its therapeutic activities.

Methods: Fresh plant specimens were collected, authenticated, and processed for extraction using methanol and aqueous solvents. Qualitative phytochemical screening was performed using standard chemical tests. High-performance liquid chromatography (HPLC) analysis was conducted using a C18 column with acetonitrile-water mobile phase system and multi-wavelength detection at 210, 230, 254, and 280 nm.

Results: Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and glycosides with differential solvent-dependent extraction patterns. HPLC chromatographic analysis demonstrated distinct retention profiles with major peaks identified at 5.557, 7.923, 12.047, 15.047, 16.736, 20.802, 23.183, 24.731, 33.760, 38.118, and 43.925 min, indicating the presence of diverse bioactive compounds with varying polarities.

Conclusion: The comprehensive analytical profile confirms the chemical diversity of *C. fimbriata*, providing scientific validation for its traditional therapeutic applications. The identified secondary metabolites, particularly pregnane glycosides, flavonoids, and phenolic compounds, may contribute to the plant's reported anti-obesity, antidiabetic, and cardioprotective effects. These findings establish a foundation for standardization, quality control, and further pharmacological investigations of this medicinally important species.

Introduction

The therapeutic utilization of medicinal plants represents one of humanity's oldest approaches to healthcare management, with plant-based remedies forming the foundation of traditional medicine systems worldwide. Contemporary

pharmaceutical research continues to recognize the significant contribution of natural products in drug discovery, with approximately 40% of currently marketed drugs having natural origins or being derived from natural product scaffolds [1]. Among the diverse array of medicinal plants, succulent species have attracted particular attention due to their unique adaptations to

harsh environmental conditions, which often correlate with the production of specialized secondary metabolites possessing biological activity [2].

C. fimbriata Wall, belonging to the family Apocynaceae (formerly Asclepiadaceae), represents a medicinally significant succulent plant species indigenous to the Indian subcontinent. This xerophytic herb has been extensively utilized in traditional Ayurvedic and folk medicine systems, particularly among tribal communities, for the management of various ailments including diabetes, obesity, cardiovascular disorders, and gastrointestinal conditions [3,4]. The species is characterized by its distinctive fringed corolla lobes, succulent stems, and remarkable ability to survive in arid environments with minimal water availability. Ethnobotanical investigations have documented the traditional consumption of *C. fimbriata* as both a medicinal agent and famine food, with communities utilizing fresh stems, decoctions, and powdered preparations for appetite suppression, blood glucose regulation, and general health maintenance [5]. Modern pharmacological studies have provided scientific validation for several traditional uses, demonstrating significant anti-obesity, hypoglycemic, cardioprotective, and antioxidant activities in experimental models [6,7].

The therapeutic properties of medicinal plants are primarily attributed to their secondary metabolite content, which includes diverse chemical classes, such as alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, and glycosides [8]. These bioactive constituents often exhibit synergistic interactions, contributing to the multi-target therapeutic effects observed in plant-based medicines. Members of the genus *Caralluma* are particularly noted for their content of pregnane glycosides, steroidal compounds that have been associated with appetite regulation and metabolic modulation [9,10].

Despite the documented ethnomedicinal significance and demonstrated biological activities of *C. fimbriata*, comprehensive analytical characterization of its phytochemical constituents remains incomplete. Previous investigations have provided limited insights into the chemical profile of the species, with

most studies focusing on crude extracts rather than detailed compositional analysis. Furthermore, standardized analytical protocols for quality assessment and authentication of *C. fimbriata* preparations are currently lacking, hindering the development of standardized herbal products [11].

HPLC has emerged as a powerful analytical technique for the separation, identification, and quantification of plant secondary metabolites. The technique offers advantages including high resolution, reproducibility, and the ability to analyze complex mixtures of bioactive compounds [12]. HPLC fingerprinting has become an essential tool for quality control, standardization, and authentication of herbal medicines, providing characteristic chromatographic profiles that can serve as chemical signatures for specific plant species [13].

The present investigation was designed to address the existing knowledge gaps through a comprehensive analytical approach combining qualitative phytochemical screening with HPLC profiling of *C. fimbriata* extracts. The primary objectives include: (1) systematic identification of major secondary metabolite classes present in the plant, (2) comparative evaluation of extraction efficiency using different solvent systems, (3) development of characteristic HPLC fingerprints for quality assessment, and (4) correlation of analytical findings with traditional therapeutic applications.

Materials and Methods

Plant material collection and authentication

Fresh specimens of *C. fimbriata* Wall were collected during the pre-flowering stage from Shrigonda tehsil, Ahmednagar district, Maharashtra, India (GPS coordinates: 18°73'18.734451" N, 74°79' E) at an altitude of approximately 561 meters above sea level. Collection was performed during optimal morning hours (08:00-10:00 a.m.) under clear atmospheric conditions to minimize variability associated with diurnal environmental fluctuations. Multiple mature plants were sampled from the same geographical location to ensure representativeness (Figure 1).



Figure 1. *C. fimbriata* Wall in natural habitat

Taxonomic authentication was performed by the Botanical Survey of India, Pune, with voucher specimen (Authentication No. JVD182) deposited for permanent reference. The collection site represents a typical semi-arid environment characterized by black cotton soil derived from basaltic formations, with average annual rainfall of 448.6 mm and temperature ranging from 18.5 °C to 33 °C.

Sample preparation and extraction

Collected plant material was thoroughly cleaned under running tap water followed by distilled water to remove surface contaminants. Cleaned samples were shade-dried at ambient temperature (25-30 °C) for 15-20 days in a well-ventilated environment to preserve thermolabile compounds. The dried material was mechanically pulverized and passed through a 40-mesh sieve to achieve uniform particle size distribution. Powdered samples were stored in airtight containers under cool, dry conditions until analysis.

Two extraction protocols were employed to maximize recovery of compounds with varying polarities. Methanolic extraction was performed using 100 g of plant powder with methanol (1:10 w/v) by cold maceration for 72 h with intermittent agitation. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40-45 °C. For aqueous extraction, plant powder (100 g) was subjected to hot water extraction (1:10 w/v) at 60-70 °C for 3 h, filtered, and freeze-dried to obtain powdered extract.

Qualitative phytochemical screening

Comprehensive phytochemical screening was conducted following established protocols to detect major classes of secondary metabolites. Stock solutions of both extracts were prepared at 100 mg/mL concentration in appropriate solvents. Alkaloid detection was carried out using Dragendorff's, Mayer's, and Wagner's reagents with characteristic precipitate formation indicating positive results. Flavonoids were identified through Shinoda test, alkaline reagent test, and lead acetate test based on color development patterns.

Phenolic compounds and tannins were detected using ferric chloride, lead acetate, and gelatin tests with color changes or precipitation confirming presence. Saponin content was evaluated through foam test involving vigorous shaking with water and observation of persistent froth formation. Terpenoids were identified using Salkowski test with reddish-brown interface coloration indicating positive response. Steroid detection employed Liebermann-Burchard reaction, while glycosides were identified through Keller-Killiani, Legal's, and Borntrager's tests.

HPLC instrumental analysis

High-performance liquid chromatographic analysis was conducted to develop characteristic fingerprint profiles of *C. fimbriata* extracts. The HPLC system was equipped with a reversed-phase C18 analytical column for optimal separation of both polar and moderately polar compounds. Mobile phase composition consisted of acetonitrile (50%) and water

containing 0.3% acetic acid (50%) to enhance peak resolution and maintain appropriate pH conditions.

Sample preparation involved dissolving 1 mg/mL of extract in the mobile phase followed by membrane filtration. Injection volume was set to 20 μ L with column temperature maintained at 25 °C to ensure reproducible chromatographic conditions. Multi-wavelength detection was employed at 210, 230, 254, and 280 nm to maximize compound detection across different chromophore systems. Flow rate was optimized to provide adequate resolution while maintaining reasonable analysis time.

Results and Discussion

Qualitative phytochemical analysis

Systematic phytochemical screening revealed the presence of diverse secondary metabolite classes in *C. fimbriata* extracts, with notable differences in extraction efficiency between methanol and aqueous solvents. The comprehensive analysis confirmed the chemical complexity of the species, supporting its traditional therapeutic applications through multiple bioactive constituents.

Phytochemical analysis results

- Alkaloids: Strongly positive in methanolic extract, negative in aqueous extract
- Flavonoids: Positive in methanolic extract, weakly positive in aqueous extract
- Phenols and Tannins: Strongly positive in methanolic extract, positive in aqueous extract
- Saponins: Strongly positive in both extracts
- Terpenoids: Strongly positive in methanolic extract, negative in aqueous extract
- Steroids: Positive in methanolic extract, negative in aqueous extract
- Glycosides: Positive in both extracts
- Carbohydrates: Positive in both extracts

The phytochemical analysis demonstrated significant solvent-dependent variations in metabolite extraction. Methanolic extraction proved superior for recovering lipophilic and moderately polar compounds including alkaloids, terpenoids, and steroids, which were either absent or present in trace amounts in aqueous extracts. This pattern reflects the

fundamental principle of solubility, where compounds with similar polarity characteristics are preferentially extracted by corresponding solvents.

The exclusive presence of alkaloids in methanolic extracts is particularly significant, as these nitrogen-containing compounds are often associated with pharmacological activities including appetite suppression and metabolic regulation. The detection of terpenoids and steroids primarily in methanol aligns with the known occurrence of pregnane glycosides in *Caralluma* species, compounds that have been linked to anti-obesity effects through mechanisms involving adipogenesis inhibition and lipid metabolism modulation.

Saponins demonstrated equivalent extraction in both solvents, reflecting their amphiphilic nature with both hydrophilic sugar residues and lipophilic aglycone portions. These compounds contribute to cholesterol-lowering and cardioprotective effects, supporting traditional applications in cardiovascular health. The presence of flavonoids and phenolic compounds in both extracts, with higher concentrations in methanol, indicates the occurrence of both aglycones and glycosylated forms, contributing to the antioxidant and anti-inflammatory properties of the plant.

HPLC chromatographic analysis

High-performance liquid chromatographic analysis of *C. fimbriata* extract revealed a complex chemical profile with multiple well-resolved peaks across the chromatographic run. The optimized analytical conditions using acetonitrile-water (50:50) mobile phase with 0.3% acetic acid provided excellent separation efficiency and peak resolution suitable for fingerprint development.

The chromatographic profile demonstrated distinct retention patterns with major peaks eluting at 5.557, 7.923, 12.047, 15.047, 16.736, 20.802, 21.776, 23.183, 24.731, 33.760, 38.118, and 43.925 min. This retention pattern indicates the presence of compounds with varying polarities, from highly polar early-eluting compounds to more lipophilic late-eluting constituents (Figure 2).

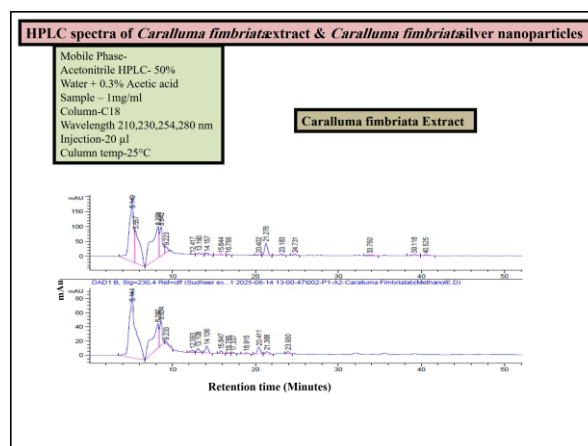


Figure 2. HPLC of *C. fimbriata* Wall extract

Multi-wavelength detection at 210, 230, 254, and 280 nm revealed differential UV absorption patterns characteristic of various chromophore systems. Early eluting peaks (5-15 min) likely represent highly polar compounds such as organic acids, amino acids, and polar glycosides. Mid-range retention times (15-25 min) are consistent with flavonoid glycosides and phenolic acids, while late-eluting peaks (>30 min) suggest the presence of less polar compounds, including flavonoid aglycones, terpenoids, and steroidal derivatives.

The peak at retention time 5.557 min, showing strong absorption at multiple wavelengths, likely corresponds to polar organic acids or amino acid derivatives. Peaks in the 12-16 minute range exhibit UV absorption patterns characteristic of flavonoid compounds, potentially representing quercetin, kaempferol, or their glycosylated derivatives commonly found in medicinal plants.

The significant peaks observed at 23.183 and 24.731 min may represent pregnane glycosides or related steroidal compounds, which are characteristic constituents of *Caralluma* species and have been associated with appetite-suppressing and metabolic regulatory activities. The late-eluting peaks at 38.118 and 43.925 min suggest the presence of highly lipophilic compounds, possibly including sterols, triterpenes, or steroidal aglycones.

Comparison with silver nanoparticles synthesized using *C. fimbriata* extract revealed distinct chromatographic differences, with the plant extract showing significantly more

complex peak patterns. This complexity underscores the chemical diversity of the natural extract and supports the multi-target therapeutic approach characteristic of traditional herbal medicines.

Correlation with therapeutic applications

The comprehensive analytical profile obtained in this investigation provides substantial scientific support for the traditional therapeutic applications of *C. fimbriata*. The presence of alkaloids, particularly those concentrated in methanolic extracts, correlates well with the plant's traditional use as an appetite suppressant. These compounds likely interact with neurotransmitter systems involved in satiety regulation, including serotonin and dopamine pathways.

The identification of flavonoids, phenolic compounds, and saponins supports the antidiabetic applications of the plant. These compound classes are known to enhance insulin sensitivity, inhibit carbohydrate-digesting enzymes, and protect pancreatic β -cells from oxidative damage. The HPLC peaks corresponding to these compounds provide a chemical basis for the reported hypoglycemic effects observed in experimental studies.

The detection of saponins and phenolic compounds through both phytochemical screening and chromatographic analysis supports the cardioprotective traditional uses. These compounds contribute to lipid profile improvement, endothelial function enhancement, and reduction of cardiovascular

risk factors. The multi-compound nature of these effects explains the broad-spectrum cardiovascular benefits associated with traditional *C. fimbriata* preparations.

The strong antioxidant activity traditionally attributed to the plant is well-supported by the presence of multiple antioxidant compound classes including flavonoids, phenolic acids, and tannins. The HPLC peaks in the mid-retention range likely represent these antioxidant constituents, which contribute to cellular protection against oxidative stress and chronic disease prevention.

Conclusion

This comprehensive investigation successfully characterized the phytochemical composition and chromatographic profile of *C. fimbriata* Wall, providing valuable scientific insights into the chemical basis of its traditional therapeutic applications. The systematic analysis revealed significant chemical diversity with multiple classes of bioactive secondary metabolites including alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, steroids, and glycosides. The solvent-dependent extraction patterns demonstrated the importance of appropriate extraction methodology for maximizing recovery of target compounds. Methanolic extraction proved superior for alkaloids, terpenoids, and steroids, while aqueous extraction was effective for saponins and polar glycosides. This information is crucial for optimizing extraction protocols in pharmaceutical and nutraceutical applications. HPLC analysis established characteristic fingerprint profiles that can serve as quality control markers for authentication and standardization of *C. fimbriata* preparations. The identification of specific retention patterns and UV absorption characteristics provides a foundation for developing analytical standards and ensuring product consistency in commercial formulations.

The strong correlation between analytical findings and traditional therapeutic uses validates the ethnomedicinal knowledge associated with this species. The presence of compounds known to influence appetite regulation, glucose metabolism, cardiovascular

function, and oxidative stress provides mechanistic support for the documented biological activities. Future research directions should focus on isolation and structural characterization of individual bioactive compounds, particularly those corresponding to major HPLC peaks. Quantitative analysis, bioactivity-guided fractionation, and detailed pharmacological evaluation will further enhance understanding of the therapeutic potential of *C. fimbriata*. The findings of this investigation contribute significantly to the scientific knowledge base supporting the development of evidence-based herbal medicines derived from *C. fimbriata*. The established analytical protocols and chemical profiles will facilitate quality control, standardization, and regulatory compliance for botanical products derived from this medicinally important species.

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Graphical Abstract

