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ORIGINAL ARTICLE

Comparative HPLC Analysis of Bioactive Compounds from Stem, Root and Callus of *Caralluma stalagmifera* C.E.C. Fisch

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Abstract

Objectives: This study aims to conduct HPLC profiling of the stem, root and callus extracts of *C. stalagmifera* in order to identify, evaluate and compare their bioactive phytoconstituents, particularly pregnane glycosides, across both natural and *in vitro* derived plant callus. **Method:** In this study, the stem, root and callus tissues of *C. stalagmifera* were collected, dried, powdered, and extracted using suitable solvents. The extracts were then analyzed using High Performance Liquid Chromatography (HPLC). A standard reference compound (epoxy- polyoxypregnane glycosides) was used for comparison. The chromatographic profiles obtained from the stem, root and callus extracts were examined and compared based on their retention times and peak intensities to identify common and unique bioactive phytoconstituents and to evaluate similarities between natural and *in vitro* samples. **Findings:** The HPLC analysis revealed distinct chromatographic profiles for the stem, root and callus extracts of *C. stalagmifera*, confirming the presence of bioactive pregnane glycosides across all samples. This was evidenced by a prominent peak in the standard compound at a retention time of 3.521 minutes, which closely matched the major peaks observed in the stem (3.562 min) and root (3.540 min) extracts. Furthermore, variations in peak intensity and the presence of minor peaks indicated differential metabolite accumulation influenced by tissue type and physiological state. These results confirm that tissue culture techniques have potential for biotechnological applications and providing a sustainable alternative to wild plant harvesting. **Novelty:** This is one of the first comparative HPLC studies on *C. stalagmifera,* simultaneously analyzing stem, root and callus extracts. It demonstrates that *in vitro* callus cultures can biosynthesize bioactive pregnane glycosides, similar to those found in natural tissues. The study provides a unique phytochemical fingerprint of *Caralluma stalagmifera* which is useful for species authentication and standardization.

Keywords: *Caralluma stalagmifera*; Callus; HPLC analysis; Bioactive Compounds; Pregnane glycosides

1 Introduction

High-Performance Liquid Chromatography (HPLC) has emerged as one of the most used and adaptable method in modern analytical chemistry. HPLC offers exceptional resolution, sensitivity and reproducibility, making it indispensable for the separation, identification and quantification of natural and synthetic bioactive molecules [1]. The method is commonly employed for assessing drug purity, validating formulations and ensuring the quality control of herbal and pharmaceutical preparations. Fundamentally, HPLC operates as a liquid–phase separation system, where analytes are distributed between a mobile phase (liquid solvent) and a stationary phase (solid or liquid) and separation occurs based on the compounds' polarity, molecular weight and chemical affinity for the stationary medium [2].

The application of HPLC in phytochemical analysis has gained significant importance due to the increasing global interest in natural products as sources of therapeutic agents. Medicinal plants synthesize a vast array of secondary metabolites, including alkaloids, flavonoids, terpenoids, glycosides, saponins and phenolic compounds, which contribute to their pharmacological efficacy. These bioactive components have antioxidant, antimicrobial, anti-inflammatory and antidiabetic effects, which makes them useful for both traditional and modern medicine. The renewed interest in herbal medicine stems from its safety profile, minimal side effects and cost-effectiveness.

The genus *Caralluma* (family: Apocynaceae, subfamily: Asclepiadoideae) represents a group of perennial stem-succulent plants distributed mainly across arid and semi-arid regions of Asia, Africa and Europe [3] and the Indian subcontinent. In India, about thirteen species and five varieties have been documented, including *C. fimbriata*, *C. adscendens*,*C. tuberculata* and *C. stalagmifera* [4]. Traditionally, these plants have been consumed as famine food and used in folk medicine to manage diabetes, obesity, fever and infections [5]. *Caralluma europaea* (Guss.) is used in popular Moroccan phytomedicine to treat several illnesses including inflammation, hepatotoxicity and cancer [6]. The pharmacological potential of *Caralluma* species is largely attributed to the presence of pregnane glycosides, triterpenes, steroids, polyphenolic compounds and oleic acid [7], [8], [9], [10], [11], due to their abundance of phytochemicals with antioxidant and ant diabetic qualities, as well as variations in their ant diabetic, antimicrobial, antioxidant and free radical scavenging capabilities [12], both the wet and dry extracts of *C. fimbriata* have the potential to play a significant, beneficial role in providing a healthy and balanced diet [13], [14].

Caralluma stalagmifera C.E.C. Fisch., known commonly as the *Dark Purple Caralluma*, belong to the family Apocynancae, is an endemic and less-explored medicinal plant of peninsular India. It is characterized by its small, fleshy, quadrangular stems and distinctive dark purple star- shaped flowers [15]. Ethnobotanical reports indicate its traditional use as a food supplement and therapeutic agent. However, comprehensive phytochemical profiling of this species remains limited. Given its close taxonomic relationship with other bioactive *Caralluma* species, there is substantial interest in exploring its secondary metabolite composition. *C. stalagmifera* has been successfully demonstrated to regenerate *in vitro* through leaf callus tissue (LCT) culture and *ex vitro* rooting [16]

The present study focuses on HPLC profiling of stem, root and callus extracts of *C. stalagmifera* to identify and evaluate their bioactive phytoconstituents. By comparing chromatographic patterns across different plant parts and tissue-culture-derived callus, this research aims to contribute to the standardization of the phytochemical fingerprint of *Caralluma stalagmifera*.

2 Methodology

2.1 Collection of Plants

The *C. stalagmifera* plants have been collected from a rural area of Medikonda village in Jogulamba Gadwal District, Telangana state, India, in the months of June and July in the year 2021. The plant has been authenticated by Botanical Survey of India, Deccan Regional Center, Hyderabad, Telangana.

2.2 Drying & extraction

The collected C. *stalagmifera* plants were cleaned and cut into pieces. Later, the pieces are allowed to dry in a hot air oven at 40^0 C to remove the moisture. Then the dried herb stem and root is powdered by a mechanical grinder. The powder is sieved twice to get the fine powder. The extraction process is carried using methonol solvent by Soxhlet apparatus.

2.3 Preparation of callus extraction

Callus was initiated from the nodal explants of *C. stalgmifera* on MS medium in combination with 2.4-D(2,4-Dichlorophenoxy acetic acid). Callus was induced from nodal explants within 10 days of inoculation. A two month old callus was collected, dried and extracted in the Soxhlet apparatus using Methanol solvent.

2.4 HPLC Analysis

HPLC was carried out for the purpose of determining glycosides. The column used is C18 equipped with, a pump (LC-10AT VP1), SIL-6A automatic injector and detector (SPD-10AVP) set at 370 nm. The sample extract was injected into the loop, and the temperature was maintained at 40 °C and the mobile phase consists of 50 ml of methanol, 1ml of water and 50ml of phosphoric acid (100:100:1) with the flow rate of about 1.5 mL/ min. The glycosides were expressed as mg/g of fresh weight.

2.5 Reagents and plant materials

Standard of Epoxy–polyoxy pregnane glycoside (Purity 90.0%, w/w by HPLC) has been purchased from Sigma-Aldrich (St. Louis, MO, USA).Solvent used is of HPLC grade and was purchased from SD Fine Chemicals, Mumbai, India. The stem and roots of *Caralluma stalagmifera* were obtained from field grown plants and callus from *in vitro* cultures. The injection volume was kept 5.0 μL. The analysis was carried out using C18 Column and (50 mm $\times$ 4.6 mm I.D., 5 μm, Waters, USA).Extract was prepared by macerating 20.0 g of dried powder of *C. stalagmifera* 24 hours with 200 ml Methanol. The procedure was repeated three times to ensure complete extraction. The gradient system that gave satisfactory results is given below Mobile Phase: Solvent-A: 0.1% trifluoro acetic acid (TFA); Solvent-B: 0.1% TFA: Acetonitrile (50:50, v/v). Detection was carried out at 255 nm. Column oven was equilibrated at 25°C. Mobile phase flow rate was maintained at 1.0 ml min^{-1} The standard solution was prepared by dissolving 10.0 mg of standard Epoxy–polyoxy pregnane glycoside in 100 ml of methanol to get a solution containing 100 μg ml^{-1}. The percentage of pregnane glycosides was calculated from the peak area obtained and compared with the standard glycosides concentration.

3 Results and Discussion

The percentage of pregnane glycosides was calculated from the peak area obtained from the various samples and compared with the standard glycosides concentration. High-Performance Liquid Chromatography (HPLC) analysis was performed to identify and compare the phytochemical profiles of the methanolic extracts obtained from the stem, root and callus of *C. stalagmifera*. Standard samples of pregnane glycosides were used as reference compounds to compare retention behavior and peak characteristics, since pregnane derivatives are among the principal secondary metabolites reported in the *Caralluma* genus. The chromatographic patterns obtained for each extract are presented in Figures 1- 4 and the detailed parameters are summarized in **Table-1.**

3.1 Stem Extract

The chromatogram of the stem methanolic extract exhibited two major peaks at retention times (RT) of 3.521 min and 5.074 min. The first peak was dominant, accounting for 84.01% of the total area while the second peak represented 15.99%. The concentration of pregnane glycosides was 81μg/ml. The higher peak intensity and area percentage suggest that the stem contains major bioactive compounds. The elevated concentration indicates that the stem tissues are metabolically more active and serve as a major site of accumulation for secondary metabolites responsible for the plant's reported medicinal properties. The observed chromatographic profile is consistent with earlier findings in *Caralluma tuberculata*, *Caralluma quadrangula* and *Caralluma europaea* where pregnane and flavonoid glycosides were reported as dominant constituents in stem tissues [17]. Study confirms that *caralluma* species contains abundance of bioactive metabolites with strong antioxidant and therapeutic potentials [18], [19]. These results confirm the pharmacological potential of *C. stalagmifera* stem extract, as pregnane glycosides are known to possess anti-obesity, anti-inflammatory and antimicrobial activities [20], [21].

3.2 Root Extract

The HPLC chromatogram of the root extract revealed two peaks at 3.540 min and 4.586 min. Among them, the peak at 4.586 min was predominant, constituting 78.48% of the total area, whereas the earlier peak represented 21.54%. The total concentration of the pregnane glycoside was 26 μg/ml, which is notably lower than that of the stem extract. This suggests that although the root also contains bioactive components, their overall abundance is reduced. Roots generally function as storage and transport organs, which might explain the lower metabolic turnover and compound accumulation compared to aerial parts. Nonetheless, the presence of distinct peaks indicates the occurrence of characteristic secondary metabolites, possibly in modified forms. Roots typically accumulate secondary metabolites such as terpenoids, alkaloids and phenolics that play a crucial role in plant defense against soil pathogens and abiotic stress. The HPLC peaks observed in *C. stalagmifera* roots suggest the occurrence of similar compounds, as reported in other *Caralluma* species like *C. quadrangular, C. umbellata, C. attenuata* and where root

extracts exhibited distinctive preliminary phytochemical analysis bioactive compounds like glycoside and triterpenoid profiles. The presence of these bioactive components in root implies its potential therapeutic properties, particularly antioxidant and antimicrobial activities [21], [22].

3.3 Callus Extract

The *in vitro* derived callus extract displayed two well resolved peaks at 3.506 min and 4.433 min, representing 44.90% and 55.10% of the total area respectively. The total concentration of pregnane glycosides was 49 μg/ml. The balanced peak distribution suggests that the callus tissue synthesized multiple phytochemical compounds in considerable amounts. This reflects that callus cultures, under optimized culture conditions, are capable of maintaining and even enhancing the biosynthetic potential of the parent plant. Preliminary phytochemical analysis findings have been reported in *C. tuberculata*, where callus and cell suspension cultures successfully synthesized pregnane type glycosides, at lower concentrations [23]. The presence of the compounds *in vitro* callus cultures highlights the potential of plant tissue culture as a sustainable alternative for secondary metabolite production, minimizing the need for large-scale wild harvesting of endangered medicinal plants.

Table 1. HPLC analysis of stem, root and callus extracts of *Caralluma stalagmifera*

Sample	Retention time (RT)	Area	Percentage of Area	Height	Pregnane glycosides (μg/ml)
1.Stem extract	3.521	50147	84.01	8730	81 μg /ml
	5.074	9543	15.99	776	
2.Root extract	3.540	27596	21.54	4280	26 μg /ml
	4.586	100509	78.48	1978	
3.Callus extract	3.506	31879	44.90	4494	49 μg /ml
	4.433	39122	55.10	1523	

Figure 1- 4: HPLC analysis of Stem, Root and Callus extract of *Caralluma stalagmifera*.

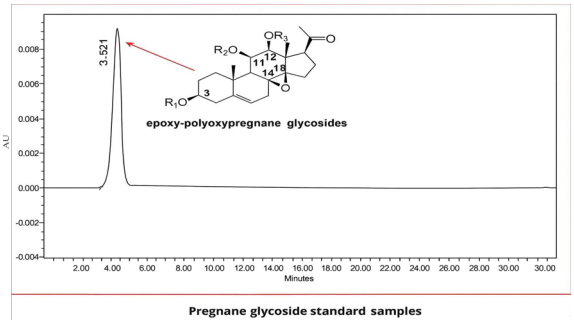
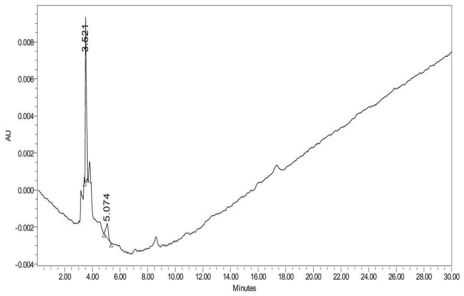


Fig 1. Glycoside standard

3.4 Comparative Analysis

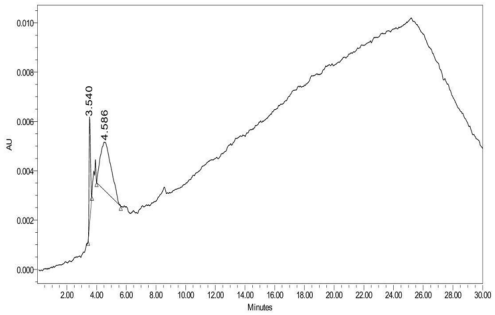
A comparative analysis of the three chromatograms indicates clear differences in quantitative aspects of phytochemical content. The stem extract exhibited the highest concentration and a dominant single peak, highlighting its rich accumulation of specific secondary metabolites. The root extract showed comparatively lower concentration and fewer dominant peaks, reflecting limited biosynthetic activity. The callus extract, however, displayed a more evenly distributed and moderately high concentration profile, suggesting that *in vitro* cultured tissues can effectively mimic the metabolic capacity of natural plant organs.

The variation in retention times and peak intensities among stem, root and callus samples signifies tissue-specific differences in metabolite synthesis and storage. The presence of characteristic peaks similar to the pregnane glycoside standards suggests that these compounds are widely distributed across different plant parts, though in varying concentrations. These findings corroborate earlier reports on *Caralluma* species, where pregnane glycosides were identified as the primary bioactive constituents contributing to their pharmacological effect.



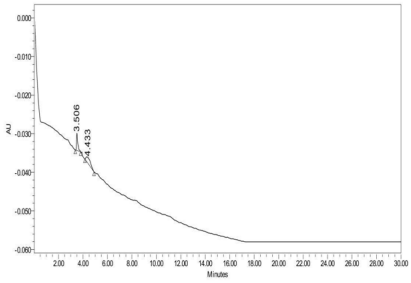
	RT	Area	% Area	Height
1	3.521	50147	84.01	8730
2	5.074	9543	15.99	776

Fig 2. HPLC analysis of Stem extract



	RT	Area	% Area	Height
1	3.540	27596	21.54	4280
2	4.586	100509	78.46	1978

Fig 3. HPLC analysis of Root extract



	RT	Area	% Area	Height
1	3.506	31879	44.90	4494
2	4.433	39122	55.10	1523

Fig 4. HPLC analysis of Callus extract

4 Conclusion

Comparative HPLC analysis of different plant parts of *Caralluma stalagmifera* demonstrated that the stem and root extracts exhibited major peaks at retention times closely matching that of the standard (3.562 and 3.540 minutes, respectively), confirming the presence of pregnane glycoside constituents. The callus extract also showed peaks near these retention times, suggesting successful *in vitro* biosynthesis of secondary metabolites similar to those found in natural tissues. The observed variations in peak intensity and additional minor peaks among the three extracts indicate differential metabolite accumulation influenced by tissue type and physiological state. The similarity in retention behavior between the standard and the *in vitro* derived callus extract confirms that tissue culture techniques can effectively sustain or enhance the production of pharmacologically valuable compounds. Therefore, this study highlights that the callus culture of *Caralluma stalagmifera* can serve as a potential alternative source for the production of bioactive pregnane glycosides, supporting its biotechnological and medicinal significance.

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