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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS FROM STEM, ROOT AND CALLUS CULTURES OF *CARALLUMA STALAGMIFERA* C.E.C. FISCH

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Keywords:

Caralluma stalagmifera, Stem, Root, Callus cultures, GC–MS analysis, Bioactive compounds

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ABSTRACT: The present study investigates the GC–MS profiling of stem, root, and *in-vitro*–derived callus cultures of *Caralluma stalagmifera* C.E.C. Fisch., an Apocynaceae succulent endemic to Southern India, commonly known as the "dark purple *Caralluma*," and traditionally valued for its anti-obesity, antimicrobial, antidiabetic, antioxidant, and anti-inflammatory properties. The major bioactive constituents reported from the genus are pregnane glycosides and aglycone steroids**.** Methanolic extracts of stem and root from field-grown plants and *in-vitro* callus cultures were prepared and each extract was analysed using GC–MS to identify its phytochemical constituents. Compounds were identified based on retention times and mass spectral data, and comparative profiling was performed to assess variations in chemical composition among the stem, root and *in-vitro* callus culture extracts. The stem extract exhibited the highest phytochemical diversity with 27 compounds, including 10 major constituents. The root extract contained 17 compounds, of which 6 were major bioactive molecules. The callus extract comprised 20 compounds, including 10 major constituents, demonstrating its ability to synthesize diverse secondary metabolites under controlled *in-vitro* conditions. Overall, the study provides a scientific basis for the phytochemical characterization of *C. stalagmifera* and highlights the potential of its stem, root, and callus tissues as viable sources of pharmacologically important compounds for natural drug discovery and biotechnological applications.

INTRODUCTION: *Caralluma stalagmifera* C.E.C. Fisch. is a rare, endemic, perennial succulent of the family Apocynaceae, occurring in the rocky regions of southern India. It forms dense clumps of slender, quadrangular stems with small sessile leaves. The species bears dark purple, star-shaped flowers with a fleshy corolla whose downward-hanging clavate hairs inspired the epithet *stalagmifera* [1]. Taxonomically, *Caralluma* belongs to the tribe Ceropegieae within the subfamily Asclepiadoideae [2, 3].

Across India, *Caralluma* species play a significant role in ethnomedicinal and ethnoveterinary practices, being used traditionally to treat fever, diabetes, intestinal worms, and inflammatory conditions [4, 5]. Tender stems and roots are also consumed as famine food due to their nutritional richness [6, 7]. Phytochemical studies reveal that the

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genus is abundant in pregnane glycosides, steroids, saponins, triterpenes, alkaloids and flavonoids supporting diverse pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory and anti-diabetic activities. Additionally, some species act as natural appetite suppressants and enhance physical endurance [8, 9, 10, 11].

Despite its significance, *C. stalagmifera* remains underexplored. Therefore, the present study aims to analyze its bioactive compounds using GC–MS to provide scientific validation for its traditional uses and support future pharmacognostic and drug-development research.

MATERIALS & METHODS:

Plant Collection: The *C. stalagmifera* plants were collected from Medikonda village from Jogulamba Gadwal District, the southern part of Telangana state in India during the month of June-July in the year 2021. The plant was authenticated by the Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana, India. (BSI/DRC/2025-26/Tech/identification/151).

Callus Induction: Callus was initiated from the nodal explants of *C. stalgmifera* on MS medium in combination with 2.4-D (2, 4-Dichlorophenoxy acetic acid). The nodal explants were treated with Tween 20 (detergent) for 10 minutes and repeatedly washed with running tap water followed by sterile water. Then the explants were rinsed with 70% alcohol for 1 minute followed by washing with distilled water.

The explants were then sterilized with 0.1 % mercuric chloride ($HgCl_2$) for 3 minutes under aseptic conditions followed by 3-4 washes with sterilized water to remove traces of Mercuric chloride. The explants were inoculated onto MS media under aseptic conditions with various concentrations of 2,4-D (0.5-4.0 mg/l) and incubated in culture room with 25 ± 2°C temperature and 16 hours photoperiod. For each experiment 3-4 replicates were maintained. The response of the explants towards callusing was recorded. Callus was induced from nodal explants within 10 days of inoculation.

Extraction: Stem and roots were collected from the field grown plants of *C. stalagmifera*. The plant parts were thoroughly cleaned and cut into pieces, oven dried and powdered by mechanical grinder. The 50 grams powder sample was extracted with methanol by Soxhlet apparatus at 60-80 °C for analysis of presence of different phytochemicals.

Preparation of Callus Extract: Two month old callus was collected, dried and extracted with Methanol in Soxhlet apparatus. GC-MS analysis of methanol extract of in *vitro* callus was performed to know the important chemical compounds present in the callus.

GCMS Analysis: GC–MS analysis of the three methanolic extracts was carried out using a Clarus 680 Gas Chromatography–Mass Spectrometer. A fused silica column (Elite-5 MS: 5v% diphenyl, 95v% dimethyl polysiloxane; 30 mm × 0.25 mm × 0.25 µm film thickness and ID250 µm df) was used for separation, with helium as the carrier gas at a constant flow rate of 1 mL/min. A 5 µL aliquot of each sample extract was injected into the instrument.

During the GC run, the oven temperature was maintained at 60°C with a 2-minute hold, 300°C at the rate of 10°C for 1-minute followed by 6 min at 300°C while the injector temperature was set at 260°C. The mass detector conditions were: transfer line and ion source temperatures were both maintained at 240°C. Mass spectra were recorded at an ionization energy of 70 eV, with a scan interval of 0.2 seconds.

RESULTS AND DISCUSSION: The GC–MS chromatographic spectra obtained for all three extracts revealed that *Caralluma stalagmifera* is abundantly rich in bioactive compounds. Each chromatogram exhibited distinct peaks corresponding to the retention times of compounds eluted through the column, with the peak intensities representing their relative abundance in the extract. The methanolic extracts of the stem, root, and in vitro callus tissues demonstrated a diverse chemical profile, indicating the presence of numerous volatile and semi-volatile bioactive constituents. These findings highlight the complex phytochemical composition of *C. stalagmifera* and confirm its potential as a valuable source of pharmacologically active metabolites.

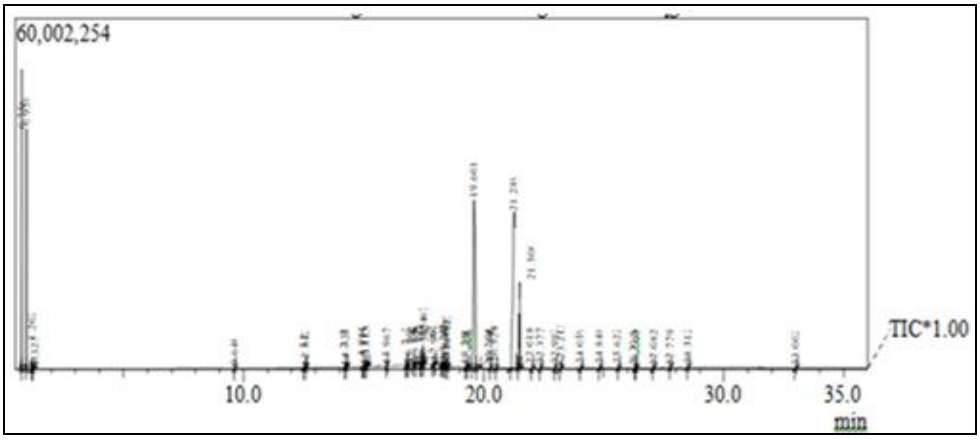
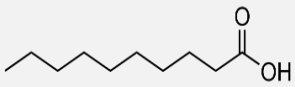
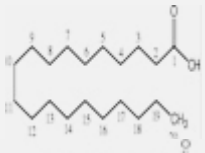
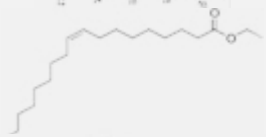
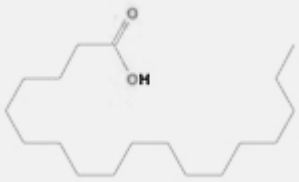
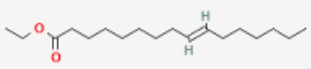
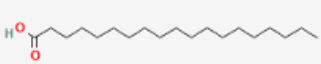


FIG. 1: GC-MS CHROMATOGRAM OF STEM EXTRACT OF *CARALLUMA STALAGMIFERA*

TABLE 1: COMPOUNDS IDENTIFIED IN GC-MS CHROMATOGRAM OF STEM EXTRACT OF *CARALLUMA STALAGMIFERA*

S. no.	R. Time (min)	Area %	Height	Name of Biological active compound	Molecular structure	Biological activity
1	9.64	0.02	58211	Octanoic acid, ethyl ester		Anti microbial activity Antifungal [12,13,14]
2	12.522	0.02	57914	1-Tridecanol		Antibacterial and antifungal [15,16]
3	12.612	0.03	65259	Decanoic acid, ethyl ester		Antibacterial activity, antiviral, anti-inflammatory [17,18]
4	19.281	0.02	39777	Eicosenoic acid, methyl ester (C		Platelet and endothelial function [19,20]
5	21.289	44.38	26468083	Ethyl Oleate		Antimicrobial and antioxidant properties [21,22]
6	21.5	5.82	14738174	Octadecanoic acid, ethyl ester		Antiviral, antibacterial and antioxidant activities [23, 24]
7	22.018	0.04	91619	Ethyl linoleate		Anti-inflammatory, antioxidant, and antimicrobial properties [25, 26]
8	22.377	0.03	81315	Eicosane (CAS) n-Eicosane		Anti fungal compound [27, 28,29]
9	22.992	0.16	429798	Ethyl 9-hexadecenoate		Antioxidant, antimicrobial, and anti-inflammatory activities [30,31]
10	23.213	0.56	1289100	Nonadecanoic acid, ethyl ester		Anti-cancer and cytotoxic effects [32, 33]

*****RT-Retention Time

GC–MS Analysis of Stem Extract: The GC–MS analysis of the stem extract of *Caralluma stalagmifera* revealed the presence of 27 compounds, among which 10 major bioactive constituents were identified with distinct biological functions **Fig. 1** and **Table 1.** The analysis revealed

the presence of several bioactive fatty acid esters in the extract. Minor constituents such as Octanoic acid ethyl ester and Decanoic acid ethyl ester exhibited antimicrobial, antibacterial, antiviral, and anti-inflammatory activities. The dominant compound was Ethyl oleate accounting for 44.38% of the total peak area, and is known for its strong antimicrobial and antioxidant properties. Other notable compounds, including Octadecanoic acid ethyl ester, Ethyl linoleate and Ethyl hexadecenoate, contributed antibacterial, antiviral, anti-inflammatory and antioxidant activities. Nonadecanoic acid ethyl ester, detected in low abundance, is reported to possess anticancer and cytotoxic effects. Additionally, eicosane and eicosenoic acid methyl ester further enhanced the extract's antifungal and endothelial-modulating potential.

These findings align with earlier reports on the antimicrobial efficacy of *Caralluma* species and scientifically validate the traditional and ethnomedicinal uses of this species and suggest that its stem extract could serve as a promising source of naturally derived therapeutic agents

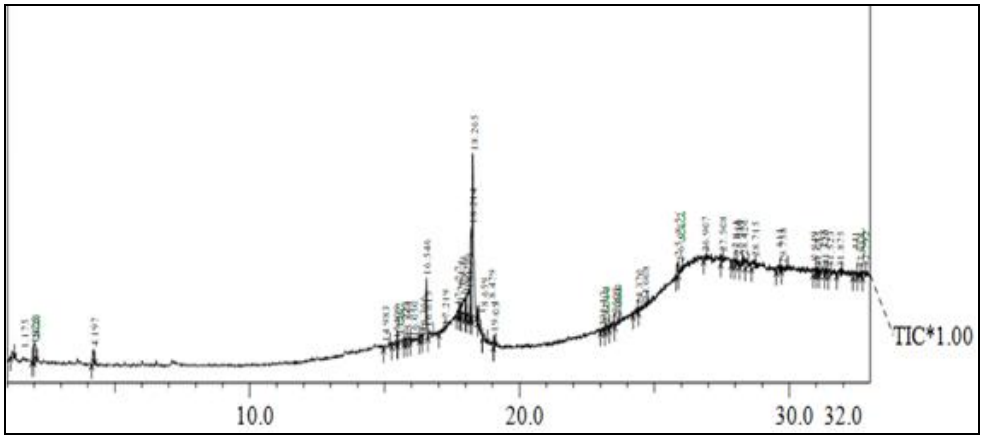
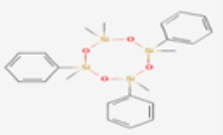
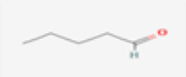
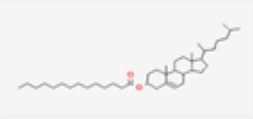
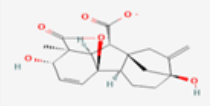


FIG. 2: GC-MS CHROMATOGRAM OF ROOT EXTRACT OF *CARALLUMA STALAGMIFERA*

TABLE 2: COMPOUNDS IDENTIFIED IN GC-MS CHROMATOGRAM OF ROOT EXTRACT OF *CARALLUMA STALAGMIFERA*

S. no.	R. Time (min)	Area %	Height	Name of Biological active compound	Molecular structure	Biological activity
1	4.197	1.68	114987	Cyclotetrasiloxan ester.		Antioxidant, Antibacterial, and Antiproliferative Activities [34,35]
2	16.366	0.85	40794	Valeraldehyde, 2,2-dimethyl		Flavouring agent [36]
3	18.265	20.55	1209899	9- Octadecenoic acid (Z)- (CAS)		Antioxidant, anti-inflammatory and antimicrobial properties [13,37]
4	25.857	1.43	90188	Cholest-5-en-3-ol (3.beta.)-, 9-oc		Plays a vital role in cell membrane structure, hormone synthesis, and is a precursor for bile acids [38]
5	26.017	0.99	46675	3-Isopropoxy-1,1,1,5,5,5-hexam		Anti-bacterial and wound healing properties [39]
6	31.326	0.73	43959	Gibb-3-ene-1ethyl esters		Promotes plant growth and elongation [40,41]

RT-Retention Time

GC–MS Analysis of Root Extract: The GC–MS analysis of the root extract of *Caralluma stalagmifera* revealed the presence of 17 compounds, among which six major bioactive constituents were identified, each exhibiting distinct biological functions **Fig. 2** and **Table 2.** GC–MS analysis of the root extract revealed a distinct phytochemical profile dominated by 9-octadecenoic acid (Z)-, which constituted 20.55% of the total peak area and is known for its antioxidant, anti-inflammatory, and antimicrobial properties. Other identified constituents included Cyclotetrasiloxane ester, exhibiting antioxidant, antibacterial and antiproliferative activities; Cholest-5-en-3-ol (3β), which plays a key role in membrane stability and hormone biosynthesis; and 3-isopropoxy-1,1,1,5,5,5-hexamethyl-, associated with antibacterial and wound-healing effects. Additionally, Gibb-3-ene-1 ethyl ester, a plant growth–promoting compound, highlights the root's involvement in hormonal regulation and developmental physiology [42]. Overall, the root extract demonstrated both pharmacological and plant growth–promoting potential, supporting the ethnopharmacological relevance of this species and its promise as a natural source of therapeutic agents.

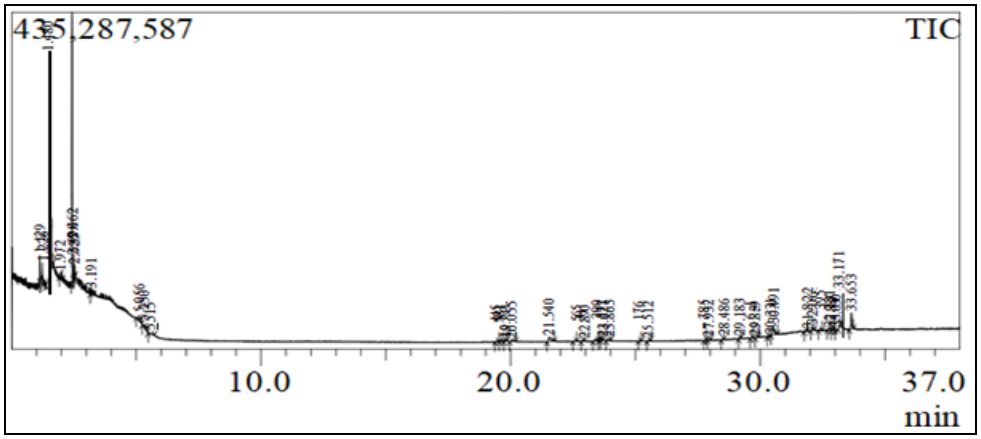
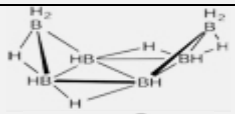
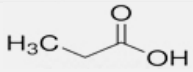
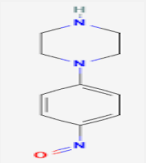
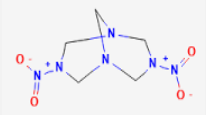
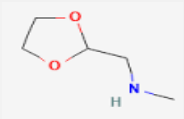
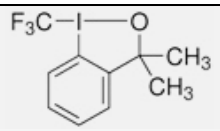
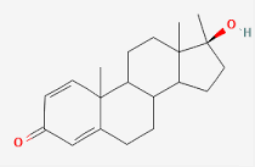
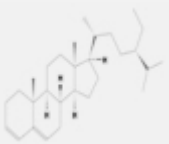
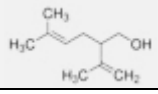


FIG. 3: GC-MS CHROMATOGRAM OF CALLUS EXTRACTS OF *C. STALAGMIFERA*

TABLE 3: COMPOUNDS IDENTIFIED IN GC-MS CHROMATOGRAM OF CALLUS EXTRACTS OF *C. STALAGMIFERA*

S. no.	R. Time (min)	Area %	Height	Name of Biological active compound	Molecular structure	Biological activity
1	1.48	68.17	292533695	Tetrahydro-4H-pyran- 4ol		Antimicrobial activity [36]
2	1.972	0.51	7414787	Propanoic acid, ethyl ester		Antimicrobial, antioxidant, and metabolic effects [13,24]
3	2.462	7.58	45210657	Urea, N-nitroso-N-phenyl- compounds		Potential carcinogenicity and antimicrobial effects [43,44]
4	5.056	1.09	1823011	N,N-Dinitro-1,3,5,7-tetrazabicyclo[3,3,1]nonane		Biological active compound antimicrobial and cytotoxic potential [34]
5	21.54	1.53	5874664	Nonadecanoic acid, ethyl ester		Antiproliferative effects against cancer cells [32,33]
6	27.932	0.03	705684	2-Methylaminomethyl-1,3-dioxolane		Antimicrobial and antifungal effects [16,28]

7	28.486	0.06	991440	3,3dimethylbutylbis(trifluoromethyl)borane methylglycine		Anti cancer activity against HepG2 and HeLa cells [45]
8	32.731	0.06	1395786	Androsta-1,4-dien-3-one, 17-hydroxy-17-methyl-, (17.alpha.)-		Estrogen-dependent breast cancer [46]
9	33.171	5.14	48249299	Stigmastan-3,5-dien		Antiinflammatory, anti-diabetic, and potential anti-viral properties [38,47]
10	33.653	2.59	20885175	Kauren-18-ol, acetate, (4.beta.)-		Biological activity of the red alga Laurencia Brandenii [48,49]

GC–MS Analysis of Callus Extract: The GC–MS analysis of the callus extract of *Caralluma stalagmifera* revealed the presence of 20 compounds, among which 10 major bioactive constituents were identified, each exhibiting distinct pharmacological properties **Fig. 3** and **Table 3.**

GC–MS analysis of the callus extract of *Caralluma stalagmifera* revealed a complex phytochemical profile dominated by Tetrahydro-4H-pyran-4-ol (68.17%), a compound with strong antimicrobial activity. Other identified constituents included Propanoic acid ethyl ester and urea, N-nitroso-N-phenyl compound, which contribute antioxidant, antimicrobial, and metabolic functions, along with Nonadecanoic acid ethyl ester, known for antiproliferative and defensive roles. Several minor compounds with antifungal, antimicrobial, cytotoxic and hormone related anticancer potential, including steroidal and terpenoid derivatives, were also detected [50, 51, 52, 53].

The presence of pharmacologically significant compounds in the callus extract demonstrates that *C. stalagmifera* can serve as a sustainable *in-vitro* source of bioactive metabolites, reducing the dependence on wild populations and supporting conservation efforts. Overall, the integrated GC–MS data indicate that *Caralluma stalagmifera* is a phytochemically rich species possessing multifunctional bioactive compounds with antimicrobial, antioxidant, anti-inflammatory, anticancer and growth-promoting activities. The results scientifically substantiate its traditional medicinal applications and highlight the potential of biotechnological approaches such as callus culture for the enhanced production of valuable secondary metabolites with medicinal importance.

CONCLUSION: The present study demonstrated that the extracts of the stem, root and *in-vitro* callus tissues of *Caralluma stalagmifera* contain a wide spectrum of bioactive phytoconstituents, as revealed by GC–MS analysis. The detection of pharmacologically active metabolites across all three extracts confirms the therapeutic potential of *C. stalagmifera* and supports its traditional ethnomedicinal applications. Overall, the findings establish *Caralluma stalagmifera* as a promising source for natural drug discovery and biotechnological applications, providing new avenues for the development of plant-derived antimicrobial, antioxidant, anti-inflammatory and anticancer agents.

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CONFLICTS OF INTEREST Nil

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