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RESEARCH ARTICLE

Morpho-anatomical investigation and metabolic profiling of *Gastrochilus calceolaris* (Buch.-Ham. ex Sm.) D.Don: A critically endangered epiphytic orchid

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Abstract

Gastrochilus calceolaris (Buch.-Ham. ex Sm.) D.Don, commonly known as the shoe-shaped belly-lip orchid, is a rare monopodial epiphytic orchid of high medicinal value. The present study provides the first integrated morpho-anatomical and metabolic profiling of this critically endangered species. Morphological and anatomical observations revealed distinct xerophytic adaptations, including a thick cuticle, well-developed velamen and prominent vascular bundles, which enable drought tolerance. Preliminary phytochemical screening of the methanolic leaf extract confirmed the presence of diverse secondary metabolites, including alkaloids, flavonoids, tannins and phenols. Gas Chromatography-Mass Spectrometry (GC-MS) identified 43 phytoconstituents, of which major compounds such as 3-Heptyn-1-ol and 3-heptyn-1-ol are known for their antioxidant, antifungal and anti-inflammatory activities. These findings highlight the pharmacological and conservation significance of *G. calceolaris*, providing a baseline for future bioprospecting and *in vitro* conservation strategies.

Keywords: anatomy; *Gastrochilus calceolaris*; GC-MS; secondary metabolites

Introduction

Medicinal plants have served as a vital source of therapeutic compounds since ancient times. Their secondary metabolites, such as flavonoids, saponins, alkaloids, terpenoids, steroids, etc., play key roles in protecting plants from environmental stress and are extensively utilized in modern drug development. Globally, these secondary metabolites form the basis of major pharmaceutical, cosmetic and nutraceutical products due to their diverse pharmacological activities. (1, 2).

The family Orchidaceae, one of the largest among the flowering plants, includes approximately 28000 species within 763 genera (3). Members of this family exhibit remarkable ecological and morphological diversity occurring as epiphytes, terrestrials, or lithophytes (4). The anatomical features correlate with ecological adaptations and can serve as an important marker for distinguishing and authenticating species within a genus (5). Epiphytic orchids in particular show xeromorphic adaptations such as thick cuticle, well-developed velamen and reduced stomata to withstand intermittent water availability (6-8).

Gastrochilus calceolaris (Buch.-Ham. ex Sm.) D.Don is a small, pendulous, monopodial epiphytic orchid renowned for its attractive yellowish-green flowers with purple specks. It commonly grows on the tree trunks of *Ficus glomerata* and *Mangifera indica* across the Eastern Himalayas, extending through India, Nepal, Bhutan, China, Thailand, Vietnam and Malaysia (9, 10). Despite its horticultural and medicinal values, *G. calceolaris* faces severe threats from habitat destruction and overcollection.

Metabolic profiling is a widely used technique to identify and analyze various bioactive compounds present within plant species. Previously, Gas Chromatography-Mass Spectrometry (GC-MS) of *Dendrobium amoenum* and *Dendrobium anceps* has revealed a variety of phytoconstituents with antioxidant, antimicrobial and anticancer potential (11, 12). As there are no previous studies, to the best of our knowledge, investigating the bioactive compounds in *G. calceolaris,* the present study aims to explore its morpho-anatomical features and metabolic profile.

Materials and Methods

Collection of plant material

The plant samples were collected from Sakoh, Kunal Pathri near Dharamshala, District Kangra, Himachal Pradesh, India (Latitude 32.175036°, Longitude 76.310357°). The collection was carried out during the flowering season (March-April 2024). The collected plants were authenticated at the Herbarium, Department of Botany, Panjab University, Chandigarh and recorded under accession number 22974. Fresh leaves and root tissues were used for morpho-anatomical and metabolic investigation. All experiments were performed in triplicate to ensure reproducibility.

Morpho-anatomical investigation

Morphological studies

The morphological study of *G. calceolaris* was conducted through detailed field observations and examination of freshly collected specimens from their natural habitat. Morphological assessments were performed on vegetative and floral parts, including leaves, stems and flowers. Key parameters such as length, breadth, shape, size and color were recorded following standard orchid terminology (13). Measurements were taken in triplicate and representative photographs were captured to document diagnostic features.

Anatomical study

For anatomical investigations, healthy leaf and root samples of *G. calceolaris* were collected from naturally growing individuals. The samples were washed thoroughly to remove surface debris and sectioned manually using a sharp razor blade. Thin transverse sections (TS) were stained with safranin to enhance the visibility of cellular structures, mounted on glass slides using glycerine and observed under a binocular light microscope (Model CH20iLED, Olympus, Japan). Photomicrographs (40X) were captured to document and analyze the anatomical features qualitatively. 10-15 manually sectioned leaf and root segments were analyzed.

Preparation of plant extract

The leaves and roots of *G. calceolaris* were first washed thoroughly to remove any dirt or debris. After washing, the plant material was weighed and left to dry naturally in the shade at room temperature to preserve its chemical constituents. Once completely dried, the leaves and roots were ground separately into a fine powder using a mechanical grinder.

To prepare the methanolic extract, 10 g of each powdered sample (leaves and roots) were placed in a Soxhlet apparatus and extracted with methanol at 60°C for about 6 hr. This process allowed the active compounds to be thoroughly extracted from the plant material. The obtained extracts were then concentrated using a rotary evaporator to remove the solvent and yield a thick, crude extract. These extracts were stored in clean, airtight containers at 4 °C until further analysis.

Qualitative test for the phytochemical analysis

Prepared plant extracts were investigated for several qualitative tests using standard procedures (Table 1) such as alkaloids, proteins, carbohydrates, flavonoids, phenolics, etc. Each test was conducted in triplicate and the presence of various secondary metabolites was indicated by characteristic colour changes or precipitate formation.

Gas chromatography-mass spectroscopic analysis of *G. calceolaris*

To determine the existence of active constituents and the chemical composition of *G. calceolaris* extract were analyzed using gas chromatography and mass spectrometry (GC-MS). GC-MS analysis of the whole plant extract of *G. calceolaris* was characterized using the equipment Shimadzu QP 2010 Ultra GC-MS. An injection volume of 1 µL was employed through splitless mode at 280 °C injection temperature and 200 °C ion source temperature. Three replicates were used in the study. The oven was programmed to 70 °C for a duration of 5 min, after which the temperature was to rise by 5 °C min^{-1} until it attained 310 °C for 10 min. The identification of constituents was done by the National Institute of Standards and Technology Library (NIST20) as well as comparison of their retention indices. The constituents were characterized after comparison with those accessible within the computer library (NIST) attached to the GC-MS instrument and the gathered results have been tabulated (Table 2). Peaks identified in the GC-MS chromatogram serve as an indicative of the presence of secondary metabolites.

Table 1. Tests for qualitative phytochemical analysis

Phytochemical class	Name of the tests	References
Alkaloids	Dragendroff`s test	(2,14)
	Wagner`s test	(2,14)
	Picric acid test	(14)
	Iodine test	(14)
Carbohydrates	Benedict`s test	(14)
	Fehling`s test	(14)
	Resorcinol test	(14)
	Starch test	(14)
Proteins	Million test	(14)
	Ninhydrin test	(14)
	Xanthoproteic test	(14)
Flavonoids	Lead acetate test	(2,14)
	Ferric chloride test	(2,14)
	Conc. H_2SO_4 test	(2,14)
	Shinoda`s test/ Mg-hydrochloride reduction test	(2,14)
Phenolic	Lead acetate test	(2,14)
	Iodine test	(2,14)
	Ferric chloride test	(2,14)
Tannins	10 %NaOH test	(2,14)
Phylobatannins	HCL test	(2,14)
Saponins	Foam Test	(2,14)
Steroids	Liebermann-Burchard test	(2)
Phytosterols	Salkowski Test	(2,14)
Terpenoids	Sulphuric acid test	(2,14)
Quinones	Sulphuric acid test	(2,14)
	Alcoholic KOH test	(2,14)
Anthroquinones	Ammonium hydroxide test	(2,14)
Coumarin	NaOH test	(2,14)
Fats and oils	Spot/stain test	(14)
Lignin	Labat test	(14)

Results

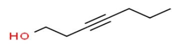
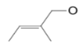
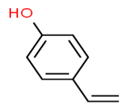
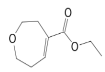
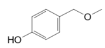
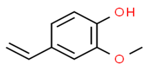
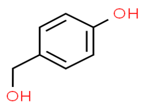
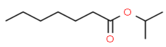
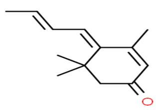
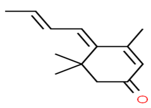
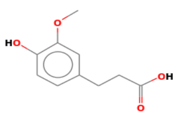
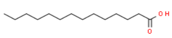
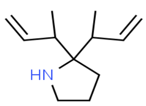
Morphology

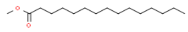
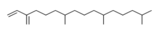
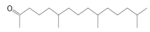
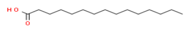
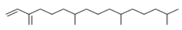
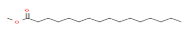
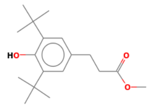
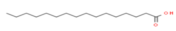
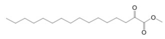
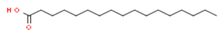
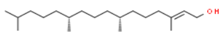
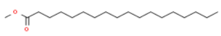
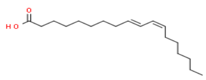
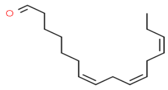
G. calceolaris is an epiphytic species with a slender stem bearing 8-9 distichously arranged, fleshy leaves that are oblong-lanceolate to linear-lanceolate. Inflorescences are lateral, short and bear 4-10 crowded flowers, faintly fragrant and typically green with reddish-brown blotches. The labellum is distinctly divided into a cup shaped hypochile and a broader epichile with white-papillose surface and purplish-red blotches along the margins. The column is short and carries 2 ovoid-elliptic pollinia with a linear stipe and oblong-elliptic viscidium. The detailed morphological characters are mentioned in Table 3 and the digital diagrams in Fig. 1.

Anatomy

The TS of leaf of the *G. calceolaris* showed the hexagonal-shaped epidermis covered with a thick layer of cuticle; mesophyll cells were filled with green pigment called chloroplast. Vascular bundles were packed within the bundle sheath layer and differentiated the phloem and xylem tissues (Fig. 2). The TS of the

Table 2. Phytoconstituents identified in methanolic leaves extract of *G. calceolaris*

Peak No.	Compound name	Retention time	Peak area %	Molecular weight (g mol^{-1})	Molecular formula	Chemical structure
1.	3-Heptyn-1-ol	10.518	31.07	112.17	$C_7H_{12}O$	
2.	2-Buten-1-ol, 2-methyl	14.327	5.94	86.13	$C_5H_{10}O$	
3.	4-Vinylphenol	15.608	0.49	120.15	C_8H_8O	
4.	4-Oxepincarboxylic acid, 2,3,6,7-tetra	17.677	7.63	170.21	$C_9H_{14}O_3$	
5.	Phenol,4-(methoxymethyl)-	18.047	3.23	138.16	$C_8H_{10}O_2$	
6.	2-Methoxy-4-vinylphenol	18.304	1.24	150.170	$C_9H_{10}O_2$	
7.	Benzenemethanol, 4-hydroxy-	19.683	0.32	124.14	$C_7H_8O_2$	
8.	Heptanoic acid, 1-methylethyl ester	19.825	1.34	172.26	$C_{10}H_{20}O_2$	
9.	Megastigmatrienone	25.600	0.16	190.280	$C_{13}H_{18}O$	
10.	Megastigmatrienone	26.754	0.23	190.280	$C_{13}H_{18}O$	
11.	beta-(4-Hydroxy-3-methoxyphenyl)pr	29.483	0.82	196.20	$C_{10}H_{12}O_4$	
12.	Tetradecanoic acid	29.940	0.36	228.370	$C_{14}H_{28}O_2$	
13.	2,3-Bis(1-methylallyl) pyrrolidine	30.653	0.91	179.30	$C_{12}H_{21}N$	
14.	Octadecanal	31.184	0.25	268.500	$C_{18}H_{36}O$	

15.	Pentadecanoic acid, methyl ester	31.327	0.13	256.420	$C_{16}H_{32}O_2$	
16.	Neophytadiene	31.590	1.42	278.500	$C_{20}H_{38}$	
17.	2-Pentadecanone, 6,10,14-trimethyl	31.707	0.17	268.5	$C_{18}H_{36}O$	
18.	Pentadecanoic acid	32.140	0.76	242.400	$C_{15}H_{30}O_2$	
19.	Neophytadiene	32.520	0.57	278.500	$C_{20}H_{38}$	
20.	Hexadecanoic acid, methyl ester	33.492	1.80	270.500	$C_{17}H_{34}O_2$	
21.	Benzenepropanoic acid, 3,5 -bis(1,1-dimethylethyl)-4-hydroxy-,ethylester	33.586	0.20	292.4131	$C_{18}H_{28}O_3$	
22.	n-Hexadecanoic acid	34.365	7.11	256.420	$C_{16}H_{32}O_2$	
23.	Hexadecanoic acid, 2-oxo-, methyl ester	34.874	0.21	284.4	$C_{17}H_{32}O_3$	
24.	Heptadecanoic acid, methyl ester	35.556	0.23	284.500	$C_{18}H_{36}O_2$	
25.	Heptadecanoic acid	36.304	0.80	270.500	$C_{17}H_{34}O_2$	
26.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	36.888	3.40	294.472	$C_{19}H_{34}O_2$	
27.	11,14,17-Eicosatrienoic acid, methyl ester	37.010	4.28	320.509	$C_{21}H_{36}O_2$	
28.	Phytol	37.215	1.46	296.500	$C_{20}H_{40}O$	
29.	Methyl stearate	37.530	0.27	298.500	$C_{19}H_{38}O_2$	
30.	10E,12Z-Octadecadienoic acid	37.770	4.93	280.400	$C_{18}H_{32}O_2$	
31.	cis,cis,cis-7,10,13-Hexadecatrienal	37.895	6.80	234.38	$C_{16}H_{26}O$	

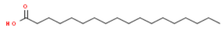
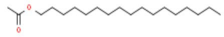
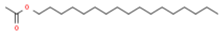
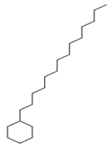
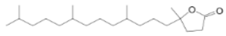
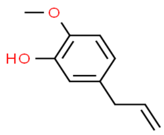
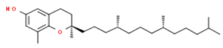
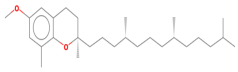
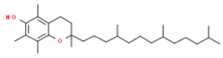
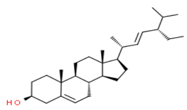
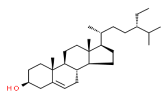
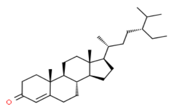
32.	Octadecanoic acid	38.281	0.98	284.500	$C_{18}H_{36}O_2$	
33.	Heptadecyl acetate	40.555	0.25	298.500	$C_{19}H_{38}O_2$	
34.	Eicosanoic acid, methyl ester	41.225	0.06	326.600	$C_{21}H_{42}O_2$	
35.	Tetradecyl cyclohexane	41.502	0.32	280.500	$C_{20}H_{40}$	
36.	4,8,12,16-Tetramethylheptadecan-4-ol	41.615	0.14	324.500	$C_{21}H_{40}O_2$	
37.	3-Allyl-6-methoxyphenol	50.489	1.16	164.20	$C_{10}H_{12}O_2$	
38.	Delta-Tocopherol	50.957	0.42	402.700	$C_{27}H_{46}O_2$	
39.	(R)-6-Methoxy-2,8-dimethyl -2-(4R,8R	52.200	2.50	416.679	$C_{28}H_{48}O_2$	
40.	DL-alpha-Tocopherol	53.504	1.13	430.700	$C_{29}H_{50}O_2$	
41.	Stigmasterol	55.547	0.66	412.7	$C_{29}H_{48}O$	
42.	gamma-Sitosterol	56.626	2.56	414.700	$C_{29}H_{50}O$	
43.	Stigmast-4-en-3-one	59.197	1.30	412.700	$C_{29}H_{48}O$	

Table 3. Morphological description of *G. calceolaris*

Features	Description
Habit	Epiphytic; plant length ranges from 10-30 cm (root to stem)
Stem	Length: 12 cm; diameter: 0.4-1.5 cm; internode enclosed by sheathing leaf bases
Leaves	Number: 8-9; shape: oblong-lanceolate to narrowly oblong or linear-lanceolate; size: 6-30 × 0.8-3.0 cm; arrangement: distichous; texture: fleshy; structure: carinate or nearly flat; base: articulate; apex: acute to unevenly bifid
Inflorescence	Type: lateral; number: 1-4; length: 3.5-5.0 cm; shape: corymbose to umbellate; flowers per inflorescence: 4-10
Floral bracts	Shape: broadly ovate-triangular; size: 4-5×3-3.5 mm; apex: acute
Flowers	Size: 1.5-3.0 cm long × 1.3-2.0 cm wide; arrangement: crowded; fragrance: faint
Coloration	Sepals and petals: soft green with reddish-brown blotches (variable); hypochile: light greenish yellow (base) to yellowish-white (mouth), purplish-red spots; epichile: white with yellow subtriangular disc blotched with purplish-red
Pedicel and ovary	Length: 1.2-2.8 cm
Sepals	Shape: obovate-oblong to obovate-lanceolate; Size: 5.5-12.0 × 3.5-7.0 mm; Apex: obtuse; arrangement: spreading; laterals: slightly narrower and shorter
Petals	Shape: obliquely oblong-spathulate to oblong-obovate or elliptic-obovate; size: 5.5-11.0 × 3-6 mm; apex: obtuse
Labellum	Attachment: to the lower half of the column
Hypochile	Shape: cup-shaped; size: 6-7 × 5-6 mm; features: may have a truncate upper margin and vertical front edges, or lack vertical edges
Epichile	Shape: suborbicular to subreniform or subovate; size: 3.2-4.5 × 6.0-8.5 mm; surface: white-papillose adaxially (except central yellow cushion); cushion minutely hairy or rough, with a basal cavity; margins: lacerate-fringed to erose
Column	Length: 2.5-4.0 mm
Anthers	Size: 1.5-2.0 × 2.0-2.4 mm
Pollinia	Number: 2; shape: ovoid-elliptic; size 0.7-1.1 × 0.4-0.8 mm
Stipe	Shape: linear; length: 1.6-2.5 mm; texture: hyaline
Viscidium	Shape: oblong-elliptic; size: 0.5-0.8 × 0.4-0.6 mm

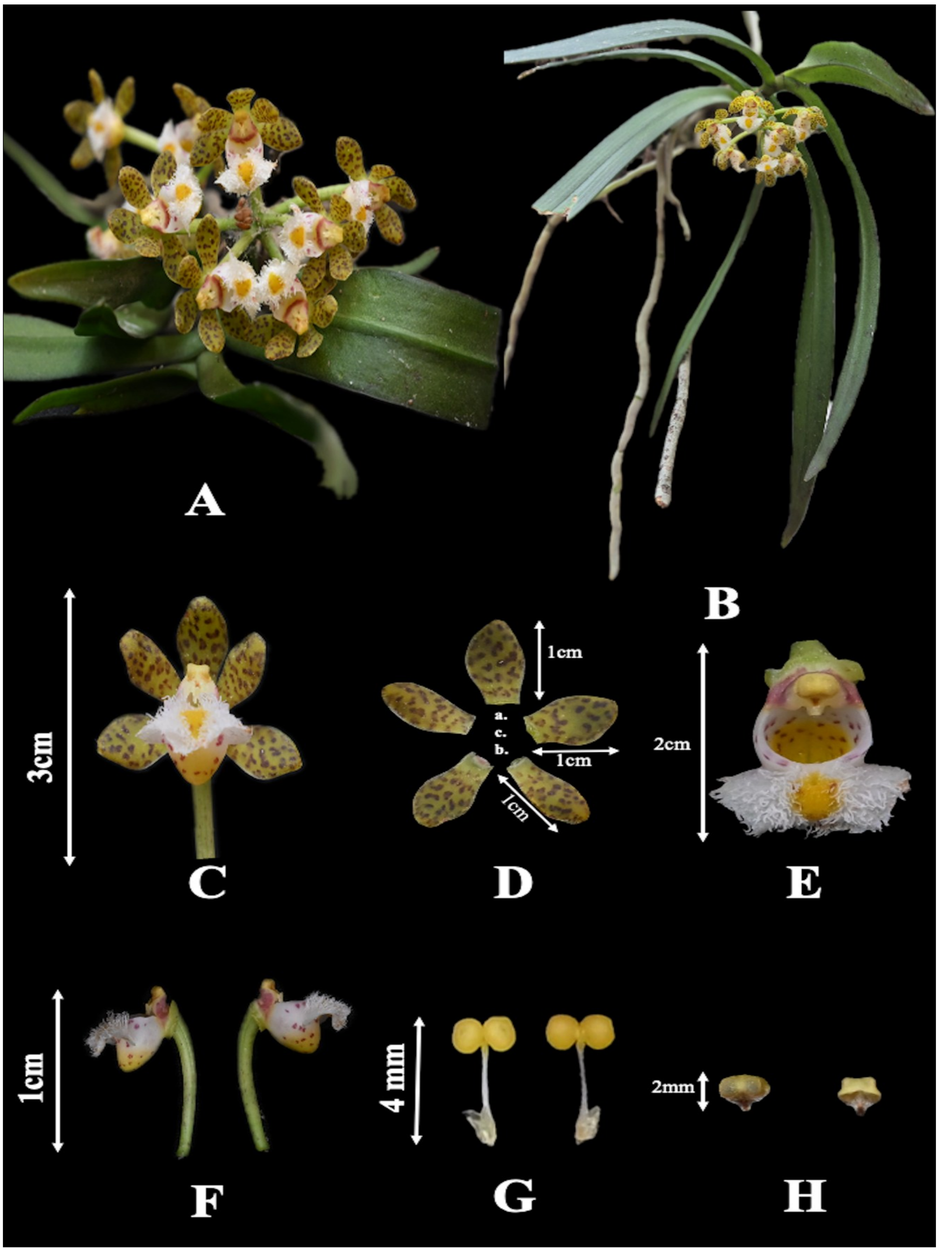


Fig. 1. A. Front view of inflorescence b. whole plant with young inflorescence; c. front view of flower; d. floral perigone showing: a. dorsal sepal, b. lateral sepal, c. petals; e. lip; f. pedicellate ovary, column and lip with mentum; g. pollinia; h. anther cap.

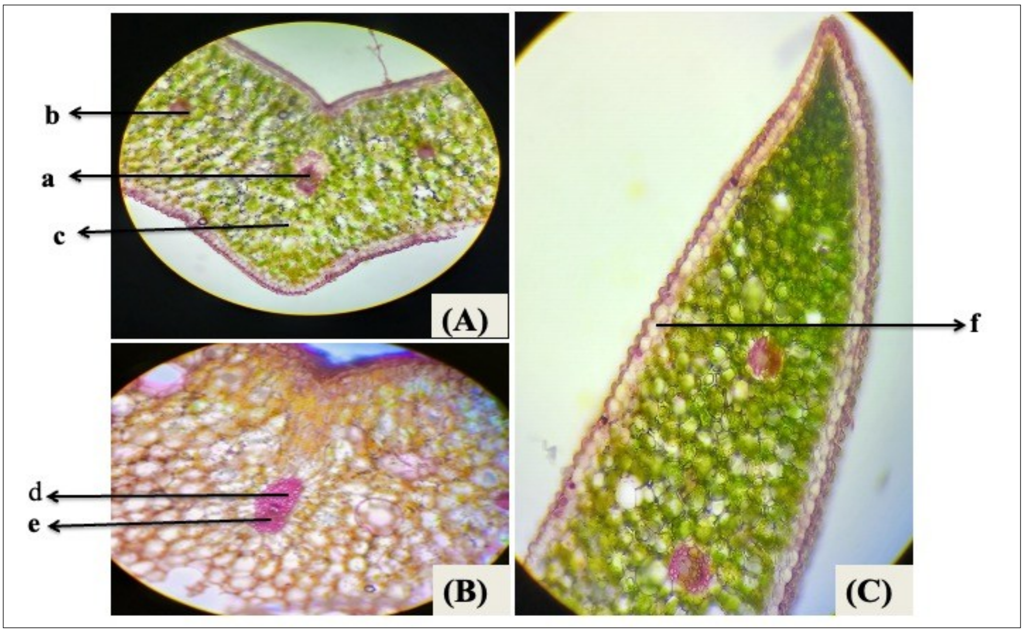


Fig. 2. Transverse section of *G. calceolaris* leaf- (A): shows mesophyll with choloroplasts in green color (c), mid rib vascular bundles (a), side vein vascular bundles (b); (B): shows vascular bundles xylem tissues (d) and phloem tissues (e); (C): shows the epidermis covered with thick layer of cuticle (f).

root constituted a special type of multilayer before the epidermis called velamen. The vascular bundles are radial in nature with well -developed pith in the center (Fig. 3).

Phytochemical analysis

The qualitative phytochemical analysis (Table 4) revealed that roots contained a higher accumulation of alkaloids compared to the leaves. In contrast, leaves exhibited a greater presence of tannins, steroids and phytosterols. Terpenoids, anthraquinone and coumarin were absent in both leaves and roots. Carbohydrates, proteins, flavonoids, phenolics, saponins, phylobatannin, quinone, fats and oils and lignin were present in both leaves and roots.

GC-MS analysis

The methanolic leaves extract of *G. calceolaris* had revealed the presence of 43 different phytoconstituents through GC-MS chromatogram identified by the National Institute of Standards and Technology Library (Fig. 4). A major percentage of phytoconstituents (Table 4) is 3-heptyn-1-ol (31.07 %), 4-oxepincarboxylic acid, 2,3,6,7-tetra (7.63 %), n-hexadecanoic acid (7.11 %) and the least percentage of phytoconstituents is eicosanoic acid, methyl ester (0.06 %). Among these, 4-oxepincarboxylic acid, 2,3,6,7-tetra is reported to possess antihistamine activity (15). n-hexadecanoic acid exhibits several pharmacologically relevant activities such as anti-androgenic, antioxidant, hypocholesterolemic, haemolytic, nematicidal, pesticide and 5-α-reductase inhibitory properties (16). Additionally, minor constituents detected in the extract also contribute to significant activities such as anti-inflammatory properties of 11,14,17-eicosatrienoic acid methyl ester (17), hepatoprotective, anticancer, antiacne, antihistaminic and anticoronary activities of 9,12-octadecadienoic acid (Z,Z) methyl ester (18) and analgesic and antioxidant potential of 10E,12Z-octadecadienoic acid (19). The presence of cis-cis-cis-7,10,13-hexadecatrienal associated with neurological benefits such as in Alzheimer's disease, epilepsy, depression and stroke (20), further highlights the therapeutic potential of the species.

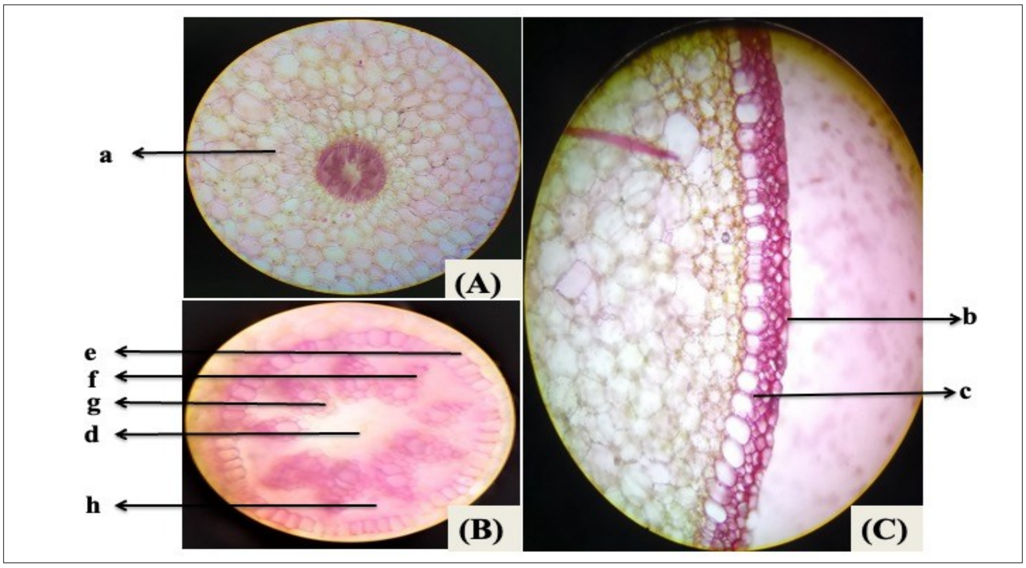


Fig. 3. Transverse section of *G. calceolaris* root - (A): shows cortex region (a); (B): shows endodermis (e), well developed pith (d), radial vascular bundles- phloem(h)and xylem is differentiated into metaxylem (g) and protoxylem (f); (C): shows a special layer called velamen (b) and epidermis (c).

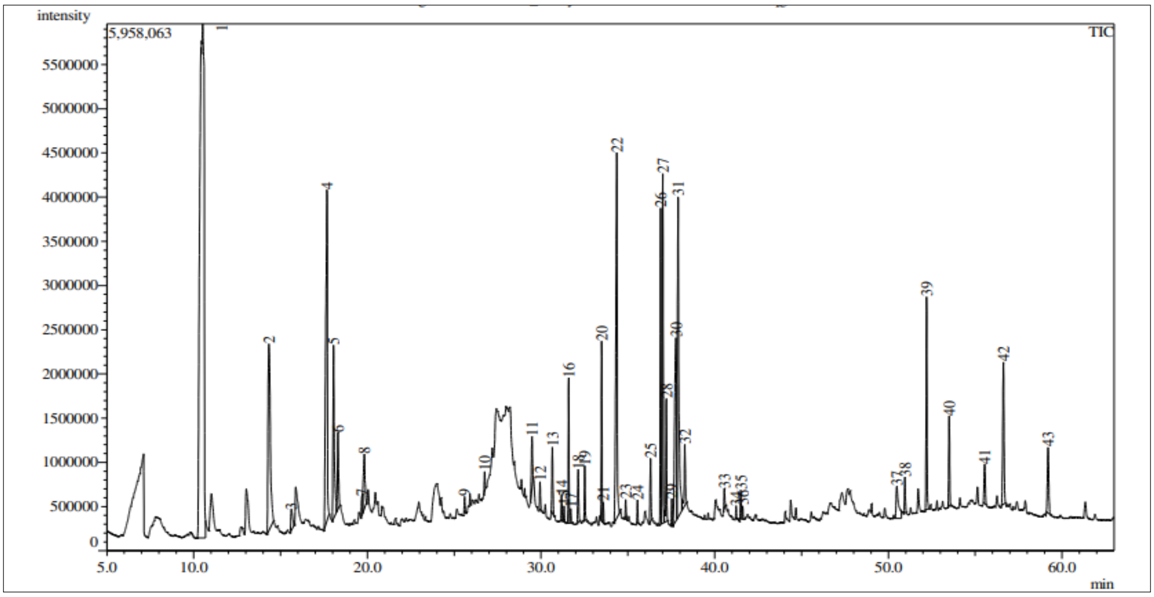


Fig. 4. GC MS chromatogram of *G. calceolaris.*

Table 4. Qualitative test for phytochemicals of *G. calceolaris*

Phytochemicals	Tests	Roots	Leaves
	Dragendroff`s test	+++	++
	Wagner`s test	+++	++
Alkaloids	Picric acid test	+++	++
	Iodine test	++	+
	Benedict`s test	+++	+++
	Fehling`s test	+++	+++
Carbohydrates	Resorcinol test	+++	+++
	Starch test	+++	+++
	Million test	+++	+++
Protein	Ninhydrin test	+++	+++
	Xanthoproetic test	+++	+++
	Lead acetate test	+++	+++
	Ferric chloride test	+++	+++
Flavonoids	Conc. Sulfuric acid (H_2SO_4) test	+++	++
	Shinoda`s test	+++	+++
	Lead acetate test	+++	+++
Phenolics	Ferric chloride test	+++	+++
	Iodine test	+++	+++
Saponins	Foam test	+	+
Tannin	10 % sodium hydroxide (NaOH) test	+	++
Steroids	Liebermann-Burchard test	++	+++
Phytosterol	Salkowski`s test	+	++
Phylobatannin	Hydrochloric acid (HCl) test	+	+
	Sulphuric acid test	++	++
Quinone	Alcoholic potassium hydroxide (KOH) test	++	++
Terpenoid	Sulphuric acid test	-	-
Anthraquinone	Ammonium hydroxide test	-	-
Coumarin	NaOH test	-	-
Fats & oils	Spot/stain test	+++	+++
Lignin	Labat`s test	+++	++

Note: The '+++' sign indicates high presence, '++' sign indicates moderate presence, '+' sign indicates low presence and '-' sign indicates the absence of the phytochemical.

Discussion

In the present study, *G. calceolaris* showed several morphological and anatomical characters that differentiate it from other plant species and exhibited similarities with the family. The epidermis contained an extra layer made up of dead cells forming velamen, which prevents water loss and facilitates water absorption. This is consistent with earlier descriptions of epiphytic orchids, which are characterized by velamen-covered roots, succulent leaves with thick cell walls, cuticles and specialized water storage structures that help them cope with drought and canopy microclimates (21).

The methanolic extract of leaves and roots showed the positive result for 13 secondary metabolites (proteins, carbohydrates, flavonoids, phenolics, tannin, lignin, etc.) and absence of 3 secondary metabolites (terpenoids, anthraquinone and coumarin). The methanolic extract of roots showed a more positive result than the leaves. The methanolic leaves extract of *G. calceolaris* has revealed 43 phytoconstituents through the GC-MS analysis, many of which are associated with diverse biological activities, including anti-inflammatory, antifungal and anti-diabetic properties. Several of these compounds also exhibited antioxidant, anticancer, anti-bacterial, antifungal, anti-microbial, nematicidal, pesticidal, hypocholesterolemic, anti-arthritic effects hepatoprotective activities and anti-microbial properties. Some of the phytoconstituents are adhesive in nature and contain bioactive chemicals effective against pests and nematodes.

Comparable GC-MS-based phytochemical studies in orchids further support the medical relevance of *G. calceolaris*. A previous study identified the major bioactive compounds such as 3-methoxy-hexane-1,6-diol, 1-methylene-2b-hydroxymethyl-3, 3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane and butyl 9,12,15-octadecatrienoate in *Rhynchostylis retusa* leaf extract by GC-MS (22). The phytochemical examination of *Dendrobium candidum* by GC-MS reveals 21 bioactive compounds in the chromatogram and the major compound was heneicosane, showing insecticidal activity against mosquitoes (23). Another study identified a wide range of bioactive compounds by GC-MS in the stem and leaf methanolic extract of *Dendrobium jenkinsii* (24). The main constituents of stem were hexadecanoic acid, methyl ester, dotriacontane, gamma-sitosterol, mandelic acid, 3,4-dimethoxy-, methyl ester, whereas in leaf, the most prevalent compounds were 9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)-; gamma-sitosterol, hexadecanoic acid, methyl ester and n-hexadecanoic acid. Eight phytochemical compounds, namely phytol, 2-butyne, 2-cyclopenten-1-one, n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z), 9,12,15-octadecatrienoic acid (Z,Z,Z), octadecanoic acid and 1,4-benzenedicarboxylic acid, were identified through GC-MS in *Cymbidium aloifolium* (25).

The presence of these compounds marked it as a highly medicinal plant and has potential for new drug manufacturing in the future. It also holds promise to produce novel drugs with the isolation of specific compounds. *G. calceolaris* contains numerous biologically active compounds. Therefore, it is advised to consider this plant significant in the field of phytopharmaceuticals. However, additional research is recommended to thoroughly study the plant to fully evaluate its biological activity, pharmacological properties, toxicity profile, ecological impact and effects on agricultural products.

Conclusion

The present study presents the first comprehensive morpho-anatomical and metabolic profiling of *G. calceolaris*, a critically endangered epiphytic orchid of the Western Himalaya. The xeromorphic anatomical traits identified, such as a multi-layered velamen, thick cuticle and compact mesophyll, highlight the species' ecological adaptation to intermittent water availability and arboreal habitats. GC-MS analysis revealed a rich profile of 43 phytoconstituents, including major bioactive compounds such as n-hexadecanoic acid, phytol, stigmasterol and γ-sitosterol, which are known for their antioxidant, anti-inflammatory and antimicrobial activities. These findings underscore the significant pharmacological potential of *G. calceolaris* and provide foundational data for its future bioprospecting. Given its threatened status and declining natural populations, the study also emphasizes the urgent need for conservation and further research, including *in vitro* propagation, targeted metabolomics and biological activity validation.

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Authors' contributions

A and NG performed the study. A, NG, KC, MV, S, HS and PB wrote the manuscript. JK edited the final version of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflicts of interest to declare.

Ethical issues: None

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