



Integrated Morphological, Histochemical and Essential Oil Profiling of *Hedychium spicatum* Buch-Ham. ex D. Don from Different Eco-geographical Regions of Uttarakhand, India

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ABSTRACT

Background: The diversification of plant-based resources, particularly under-utilized species, can significantly strengthen human nutrition, livelihood security and ecological sustainability.

Methods: In this respect, the present studies was carried out on morphological traits, histochemical analysis and essential oil profiling of *Hedychium spicatum* Buch-Ham. ex D. Don, an aromatic and medicinal plant of *Zingiberaceae* family at Shri Guru Ram Rai University, Dehradun, Uttarakhand. Detailed assessments of vegetative and floral characteristics were conducted to document morphological variability across collected germplasm from different regions of Uttarakhand, India. Histochemical tests were performed on rhizome sections to localize lignin, tannins, starch grains, calcium oxalate crystals and vascular tissues, providing valuable markers for quality evaluation and identification of crude drugs. Essential oil content and composition were analysed from rhizomes.

Result: The present study revealed significant variability in the morphological traits. Histochemical assessments confirmed the presence of lignin, tannins, starch and calcium oxalate crystals. Oil yield showed significant variation, ranging from 0.22% to 1.05%. Maximum % oil content (1.05%) was reported from Giriya (1656 m altitude) followed by 1.0% from Deoria taal and minimum (0.22%) was observed from Ukhimath (1430 m altitude). Gas chromatographic analysis indicated 7-epi- α -eudesmol (28.33%) and 1, 8-cineole (12.12%) as major constituents. Altitude dependent differences in chemical composition highlight the influence of environmental factors on secondary metabolite accumulation. Therefore, the present findings elucidate that the morphological, histochemical and oil profiling traits offering a comprehensive understanding of plant's phenotype and serve a useful method for identifying and ensuring the quality of plant-based medicines.

Key words: Chemotaxonomy, *Hedychium spicatum*, Histochemistry, Morphological characters, Oil profile, Rhizome.

INTRODUCTION

Since the beginning of human civilization, plants have always been linked to health, serving as both food and medicine (Schaal *et al.*, 2019). Traditional herbal remedies laid the groundwork for modern medicine and are still widely used (Lemonnier *et al.*, 2017). This traditional knowledge, also called folk medicine, helps guide researchers in finding new compounds with therapeutic potential. As a result, medicinal plants and the natural compounds they produce have become a growing area of research and interest (Belokurov *et al.*, 2019; Kharbach *et al.*, 2020). Nearly one-third of pharmaceutical products have botanical origins and an estimated 17% of India's flowering plants are considered to have medicinal value (Dobriyal *et al.*, 2021; Humaira *et al.*, 2021).

The Himalayan Mountain range is rich in plants, herbs and shrubs with medicinal properties. Numerous plant species grow naturally in this region (Kandpal *et al.*, 2023). Among these, *Hedychium* genus is commonly found, known as spiked ginger lily, belongs to ginger family, *Zingiberaceae*. It includes 80 species found globally (He, 2000). Of these, 29 species are native to the tropical and subtropical regions of China, while 40 species are found in Indonesia (Riswan and Setyowati, 1996). These plants are both annual and perennial, have underground rhizomes

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and grow upright with flowers (Prakash *et al.*, 2016). They are known for their strong fragrance and contain compounds beneficial for medicinal purposes. *H. spicatum* Buch-Ham. ex D. Don, locally called Kapoor Kachri, grows naturally in mid-altitude zones of the Himalayas (Pushpangadan *et al.*, 2001; Samant *et al.*, 2007; Reddy *et al.*, 2009). Its rhizome is valued for aromatic oils possessing antimicrobial, antioxidant, anthelmintic, analgesic and anti-inflammatory properties (He, 2000; Lakhera and Srivastava,

2025). Their essential oils contain aromatic constituents such as α -pinene, ocimene, cineole and linalool (Gangwar *et al.*, 2024). The existing literature does not offer comprehensive morphological and histochemical studies on *Hedychium spicatum* from the Himalayan region. The present investigations offer the first detailed studies on the tissue-specific distribution of bioactive compounds. This study highlights the morphological, histochemical characterization along with oil profiling of collected germplasm of *Hedychium spicatum* from different altitude of Uttarakhand Himalayas and contributes to understanding the intricacies of its structural composition, aids in the recognition and differentiation of the species to constitute the efficacy of herbal drugs.

MATERIALS AND METHODS

Plant collection

Germplasm of *Hedychium spicatum* were collected from different regions of Uttarakhand, India during 2023-2025 basically found on hillsides or forest edges (Fig 1). The plant material was officially identified and corresponding herbarium voucher specimens were submitted to the internationally recognized herbarium at the Botanical Survey of India, Dehradun (Accession No. 1428).

Study site and experimental design

The experiment was laid out in randomized block design with three replications in the agriculture field of School of Agricultural Sciences, Pathribagh and Botany Laboratory of School of Basic and Applied Sciences, Dehradun, Uttarakhand during 2023-2025 (Fig 1). Suitable agricultural practices such as irrigation, fertilizer, weeding as per requirement followed for proper growth of the germplasm. Morphological observations were documented 4-5 months post-planting, once they had fully grown and the characteristics of the rhizome were recorded after harvesting (Sufaid *et al.*, 2024).

Histochemical analysis

For histochemical analysis, free-hand sections of the plant rhizome were prepared and treated with specific reagents to identify cellular components such as, lignin, tannins, starch grains, calcium oxalate crystals and vascular bundles. Then comparison of intensity of staining and

abundance of specific compounds in different samples was performed.

Test for vascular bundles

To visualize vascular tissues, rhizome sections were stained with a diluted safranin solution and gently rinsed before microscopic observation (usually a 1% aqueous solution) for about 1-5 minute. The stained sections were placed on a microscopic slide and a coverslip was placed carefully avoiding air bubbles. Now, the slide was examined under a microscope.

Test for starch

The sections were placed on a slide and a drop of KI solution was applied directly to the specimen. After allowing the solution to sit for a few minutes, excess stain was removed and a cover slip was placed on top and the specimen was then observed under a microscope (Linga and Savithramma, 2013).

Test for tannin

Sections were stained with 1% ferric chloride (FeCl_3) solution for 5 minutes. Then the sections were rinsed with water and a coverslip was added. The slides were examined under a microscope (Trimanto and Hapsari, 2021).

Test for calcium oxalate

Sections were placed on a glass slide and stained with 20% sulphuric acid solution. The acid was allowed to react with the section for about 5-10 minutes. This step helped to dissolve non-crystalline materials and highlights the calcium oxalate crystals. After staining, the slide was gently rinsed with distilled water and coverslip was placed over the stained sections and observed under microscope (Tütüncü *et al.*, 2014).

Test for lignin

Sections were stained with Phloroglucinol solution (1% in ethanol) and a drop of concentrated HCl were added to the slide. The slides were allowed to stand for few minutes to let the reaction occur. Now the sections were examined under microscope (Erst *et al.*, 2021).

Extraction of essential oil

The essential oil from moisture-free root powder of *Hedychium spicatum* was extracted using a hydro- distillation



Fig 1: Location of different regions of Uttarakhand, India from where germplasm has been collected for study.

apparatus and preserved in sealed amber glass vials at 4°C until further analysis. For chromatographic examination, 50 µL of pure essential oil and 0.5 µL of n-tetradecane were mixed in dichloromethane to obtain a final volume of 1.0 mL (Fig 4).

Gas chromatography analysis

The gas chromatography-mass spectrometry (GC-MS) analysis of the oils was performed using an Agilent technology 8890 gas chromatograph with PAL RSI 85 auto sampler equipped with a split/splitless injector (split ratio 1:50) along with a data handling system at Centre for Aromatic Plants (CAP), Selaqui, Dehradun. The column was HP-5MS UI capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was the carrier gas at a flow rate of 0.80 mL/min. The GC was interfaced with (Agilent GC-MS/MS_7010 B system) mass detector operating in the EI⁺ mode. Temperature program used was the same as described above for GC analysis. The temperatures of the injector, transfer line and ion source were maintained at 280°C. Mass spectra were taken over *m/z* 40-450 amu that revealed the total ion current, using an ionizing voltage of 70 eV. The identification of constituents was performed by matching their recorded mass spectra with installed MS library (NIST 2.3 and Wiley FFNSC) and available literature (Adams, 2007).

Statistical analysis

Statistical analysis of morphological characters was carried out to understand the variation and relationship among different traits of the plant. The level of significance was tested at standard probability levels to ensure the reliability and accuracy of the results. Mean (\bar{X}), Standard error (SE) and critical difference at 5% probability were calculated using R 3.1.0 to assess the variations in different quantitative traits (R Core Team, 2024).

RESULTS AND DISCUSSION

Hedychium spicatum is a valuable plant known for its ornamental and medicinal importance. It possesses a tall, robust habit, aromatic rhizomes and broad lanceolate leaves that enhance its distinctive morphology. The rhizomes are rich in essential oils and have been widely used in traditional medicine for their therapeutic properties, including antimicrobial and anti-inflammatory effects (Tavares *et al.*, 2020; Choudhary and Singh, 2017 a, b).

Previous studies have highlighted its phytochemical richness and pharmacological potential as a source of bioactive compounds (Hartati *et al.*, 2014). The findings of the present study were presented under the following headings to provide a systematic understanding of its characteristics and uses.

Morphological characterization

In the present study, the morphological features of *Hedychium spicatum* exhibited significant variation across

different environmental conditions. Data of all the studied traits were presented in Table 1 highlighting the mean (\bar{X}), range, standard error (SE) and critical deviation (CD at 5%) by using standard statistical formulas. The height of the collected germplasm collected from different regions showed variation ranging from 140.66-46.33 cm. Maximum plant height was reported in HS-8 collected from Ukhimath and minimum in HS-9 from Duggalbitta (Table 1).

Stem was large, upright, erect, generally green when young, turns slightly brownish or reddish hue when matures. The rhizome diameter of the collected plant samples varied between 26.66 (HS-2) to 11.66 (HS-9) across different areas (Table 1 and Fig 2). The rhizome was thick, knobby, fleshy, tuberous and aromatic, covered with scales and brownish on the outside, with a pale yellow or whitish interior. It grows horizontally beneath the soil surface, with the roots emerging from the nodes along the rhizome.

The internodal distance of the collected plant varied across different areas, ranging from 16.66 cm (HS-5) to 8.33 cm (HS-4). The petiole length of the collected germplasm varied from 0.66 cm (HS-6) to 0.36 cm (HS-4 and HS-7) across different areas.

Leaves were alternate, distichous and lanceolate to oblong lanceolate in shape in all studied germplasm. Apex was acute or acuminate with base sheathing around the stem. Margin was entire with smooth and glabrous texture on the upper surface, with pubescence on the lower surface. Venations was parallel typical of monocot. The leaf length ranged from a minimum of 19.33 (HS-7 and -9) to a maximum of 41.33 (HS-1). The leaf width ranges from 12.66 (HS-8) to 7.16 cm (HS-2).

Inflorescence was terminal spike, dense, elongated and cylindrical, up to 10-25 cm long. It consists of numerous small, tubular flowers arranged in a spiral pattern. Bracts were green, ovate and enclosing flowers at the base. Flowers were arranged compactly on the spike. The inflorescence emerges from the rhizomes during the flowering season, which is typically in the summer to early autumn. Flowers were zygomorphic, bisexual, white or pale yellow with orange base on the labellum. Flower size was about 5-9 cm across and strongly aromatic. Calyx was tubular, greenish and about 2-3 cm long. Corolla was composed of three narrow petals, often hidden behind the labellum. Labellum was broad, petaloid and often orange in the centre. Single fertile stamen; the other two were staminodes. Ovary was inferior, trilocular, with axile placentation. Style was slender, passing through the stamen's filament. Stigma was slightly enlarged, sticky for pollen adherence. Fruit was globose to ellipsoid capsule that dehisces to release seeds. Initially green in colour, turns reddish-brown when mature. Seeds were numerous, small, black or brown and covered with a bright red aril.

In the present study, the morphological features of *Hedychium spicatum* exhibited significant variation across different environmental conditions especially altitude.

The high variation in plant height suggests that environmental factors, such as soil type, climate and available resources, might influence the growth of the plant (Table 1 and Fig 2). Smaller rhizome diameters might indicate less favourable growing conditions, whereas larger diameters could suggest optimal environmental support for growth. The rhizome serves as the primary means of vegetative reproduction for the plant, so it is a key part of the plant's survival. The plant's rhizomes are strongly fragrant and are a key feature used for medicinal and aromatic purposes (Singh *et al.*, 2023). A greater internodal distance may indicate favourable growth conditions, while a shorter distance could result from environmental stress or genetic traits.

Histochemical characterization

Histochemical analysis is an important method for checking the quality of crude drugs by identifying the cell contents found in the histological areas of plant organs. Stains help improve the visibility of the specimen by enhancing contrast. Furthermore, different stains show varying affinities for specific organelles and macromolecules, so choosing the right stains can also provide insights into the chemical composition of substances within the cell. Histochemical stains enhance visibility, enabling the clear differentiation of structural components and metabolite distribution. This approach not only supports pharmacognostic identification but also assists in understanding the functional roles of biochemical constituents within the plant (Kumar and Verma, 2018). Nissar *et al.* (2015) worked on the histochemical analysis of *Curcuma neilgherrensis* leaf and rhizome to identify and locate key phytochemicals such as alkaloids, saponins, tannins, oils and starch grains. These observations support chemotaxonomic studies and help detect drug adulteration. The study conducted by Chittaragi and Menon (2022) and Johri and Singh (2016) on the anatomical morphology of ginger seed rhizomes and examined the histochemical changes during the storage.

In the present study on detailed cross-section (T.S.) with 45X magnification (FOV-2 mm) of rhizome revealed the presence of starch after the sections were treated with iodine solution. The starch grains were found in the cortex, upper epidermis and endodermal areas of the rhizome (Table 2 and Fig 3). Throughout the rhizome, the parenchymatous cells were packed with starch grains and oleo resin cells. The starch grains were oblong to oval in shape, violet in colour and dispersed across the cortex and stellar region. These starch grains were important for the storage of carbohydrates in the rhizome of *Hedychium spicatum*, contributing to its medicinal and economic value (Sass, 1958).

In all the samples of *H. spicatum*, transparent calcium oxalate crystals were revealed when rhizome sections were stained with 20% sulphuric acid. These crystals remain intact and became more prominent because the surrounding tissue was degraded. These crystals occur in

Table 1: Morphological characterization of *H. spicatum*.

Germplasm	Location	Altitude (m)	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Internodal distance (cm)	Petiole length (cm)	Rhizome diameter (cm)	Stem girth (cm)	Inflorescence length (cm)
HS-1	Pinglapani	1253	100.33	41.33	11.7	15.66	0.46	20.66	2.4	17.66
HS-2	Ukhimath	1430	140.66	38.66	7.16	11.33	0.53	22.66	2.7	18.33
HS-3	Giriya	1656	68.16	35.33	12.33	10.33	0.56	16.33	2.06	14.33
HS-4	Sonprayag	1900	50.66	24.66	10.33	8.33	0.36	17.66	2.6	15.66
HS-5	Sari	2034	96.66	26.33	11.83	16.66	0.46	19.33	2.03	13.33
HS-6	Pothibasa	2337	60.5	24.33	10.66	10.66	0.66	26.66	2.23	12.33
HS-7	Gundagwar	2400	49.33	19.66	11.66	10.16	0.46	19.33	2.7	11.33
HS-8	Deoria Taal	2412	84.5	33.33	12.66	11.66	0.46	15.33	2.43	12.66
HS-9	Duggalbitta	2600	46.33	19.33	7.66	10.83	0.36	11.66	2.03	15.66
Range			140.66-46.33	41.33-19.33	12.66-7.16	16.66-8.33	0.66-0.36	26.66-11.66	2.7-2.03	18.33-11.33
Mean			77.46	29.22	10.67	11.74	0.49	18.85	2.36	14.59
SEM			0.29	0.35	0.319	0.32	0.03	0.35	0.06	0.34
CD at 5%			0.89	1.04	0.96	0.96	0.08	1.05	0.17	1.01

bundles within the parenchyma cells of the rhizome and exhibit variation in shape, size and form, such as druses, dumbbell shape, round and rosette shape. Tütüncü Konyar

et al. (2014) reported calcium oxalate crystals with different morphological forms: As druses, prism, styloids, raphides and crystal in many organs of the leaves and stems of the



Fig 2: (A) *H. spicatum* plant growing in natural habit; (B, C) Plant with Inflorescence; (D) Leaves; (E) Rhizome and (F) Fruit with seeds.

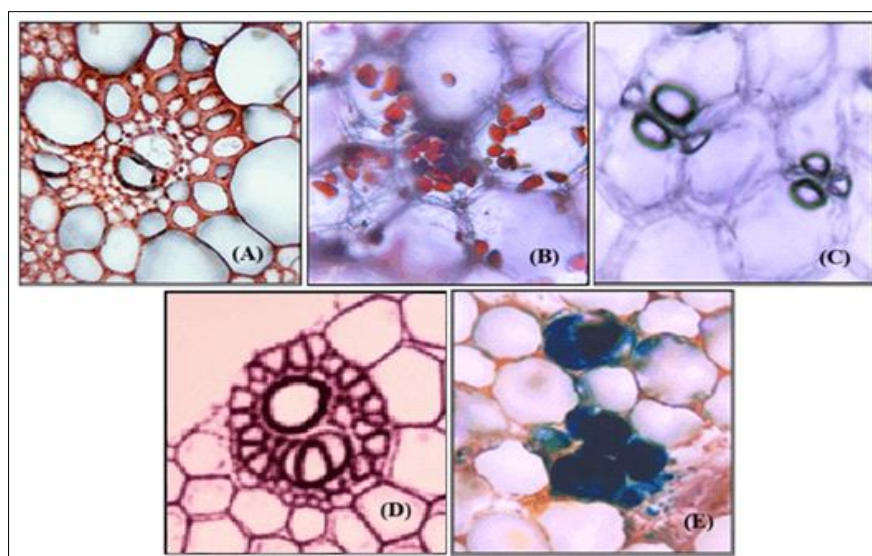


Fig 3: Histochemical studies in cross-sectioned rhizome of *H. spicatum* (with magnification 45X): (A) Vascular bundle, (B) Starch grains, (C) Calcium oxalate crystals, (D) Lignin, (E) Tannin.

Table 2: Histochemical estimation on rhizome sections of *H. spicatum*.

Test for compounds and tissues	Reagents	Colour	Histological zone
Starch	Iodine solution	Blue/orange	Cortical and medullary ray cells
Calcium oxalate	Sulphuric acid (20%)	Crystals of calcium oxalate	Stele
Vascular bundles	Safranin	Pink	Stellar tissue
Tannin	Aqueous FeCl_3	Greenish blue	Cork and cortical cells
Lignin	Phloroglucinol + conc. HCl	Pink	Stellar tissue

eight species of poisonous plants and one species of non-poisonous plant. Their studies reported that there was no absolute correlation between the presence and type of calcium oxalate crystals and toxic plant organs. However druse crystals may function as main irritant in toxic organs of the plants.

Anatomical characterization of rhizome section of *Hedychium spicatum* showed the vascular bundles appear distinctly stained, usually red or pink, due to the safranine Chittaragi and Menon (2022) making it easy to distinguish the lignified xylem and fibres from the less lignified phloem and surrounding tissue. In all samples, vascular bundles were closed, collateral and arranged in a scattered manner. The prominent staining of xylem compared to phloem facilitated clear assessment of tissue organization and developmental maturity important criteria in determining appropriate harvesting stages for medicinal purposes (Esau, 1977).

Tannins in the rhizome were confirmed when bluish-green coloured cells appeared after the sections were treated with aqueous FeCl_3 solution. These tannins were found in the epidermal and parenchymatous areas of the rhizome. The study highlights significant geographical variations in tannin content in *H. spicatum* through histochemical analysis. These secondary metabolites are known to contribute to antimicrobial defence, antioxidant activity and herbivore deterrence (Singh *et al.*, 2010).

The presence of lignin revealed, when stained with phloroglucinol - HCl solution. It typically showed a reddish-pink colour. The xylem vessels stain deep red, appearing prominently due to the high lignin content in their cell walls whereas the phloem tissues, were less prominently stained due to the absence of lignin. The intensity of the staining varied depending on the concentration of lignin in different tissues. Lignin is commonly found in the epidermis and parenchyma areas of the rhizome of *H. spicatum*. The results indicate significant variations in lignin deposition, which might be influenced by environmental

factors such as altitude, temperature and soil composition. Lignin is an important component of the plant's cell wall, providing structural support (Erst *et al.*, 2021). Although its exact function is still unknown, lignin effectively strengthens structural bonds to enhance the stability of water transport vessels. It also helps keep these vessels open, allowing water, dissolved nutrients and waste products to flow through the plant (Erst *et al.*, 2021). Because lignin provides mechanical support and enhances water transport efficiency, differences in staining intensity may reflect ecological influences on plant development, including moisture availability and climate (Boerjan *et al.*, 2003).

Collectively, the histochemical characteristics of *Hedychium spicatum* rhizomes offer comprehensive insights into their structural integrity, chemical profile and adaptive strategies. These findings reinforce their pharmacognostic identity and highlight environmental impacts on bioactive constituent formation. Similarly, Studies conducted by Rasool and Maqbool (2019) on *H. spicatum* and stated that the rhizome contain diverse terpenoids and essential oils, contributing to their traditional use in treating respiratory, inflammatory, microbial and neurological disorders. Despite limited widespread ethnobiological popularity in the Indian Himalayas, the Zingiberaceae family including *H. spicatum* remains a valuable resource for food, medicine, spices and other cultural uses.

Essential oil content and its profiling

Essential oil content (%)

The % oil content ranged from 0.22 to 1.05. The maximum essential oil content (1.05%) was reported in germplasm HS-3 collected from Duggalbitta (2600 m), whereas the lowest (0.22%) was recorded in germplasm HS-2 at Deoriyatal (2412 m) (Table 3 and Fig 4). This notable variation in yield across locations aligns with changes in altitude, supporting earlier research results (Ozguven and Tansi, 1998).

Table 3: Essential oil (%) and its main constituents.

Oil yield (%) and its components	HS-1	HS-2	HS-3	HS-4	HS-5	HS-6	HS-7	HS-8	HS-9
Oil yield (%)	0.33	0.22	1.05	0.44	0.85	0.40	0.65	1.00	0.89
1,8-Cineole	5.54	22.95	20.21	30.23	3.23	20.84	12.03	2.65	3.55
Linalool	21.26	0.34	0.74	4.38	0.45	2.81	13.44	0.41	0.72
δ -Cadinene	1.85	0.42	1.52	1.95	1.46	2.74	2.26	3.03	3.35
Cis-Nerolidol	3.67	5.16	5.45	2.83	9.91	7.12	5.86	8.28	3.50
γ -Cuprenene	1.08	1.15	0.84	0.85	1.90	0.75	0.84	1.15	1.07
β -Vetivenene	1.36	0.75	1.57	1.56	1.32	2.11	1.77	2.01	1.25
10-epi- γ -eudesmol	1.59	12.22	1.95	0.64	6.25	0.47	1.16	2.23	1.64
γ -eudesmol	4.45	5.43	7.87	2.86	10.19	1.16	5.35	7.55	4.43
α -muurolol	5.13	1.25	6.36	8.25	1.97	7.10	6.16	7.32	9.01
7-epi- α -eudesmol	29.91	32.52	32.85	24.42	41.95	27.18	26.75	33.47	34.25

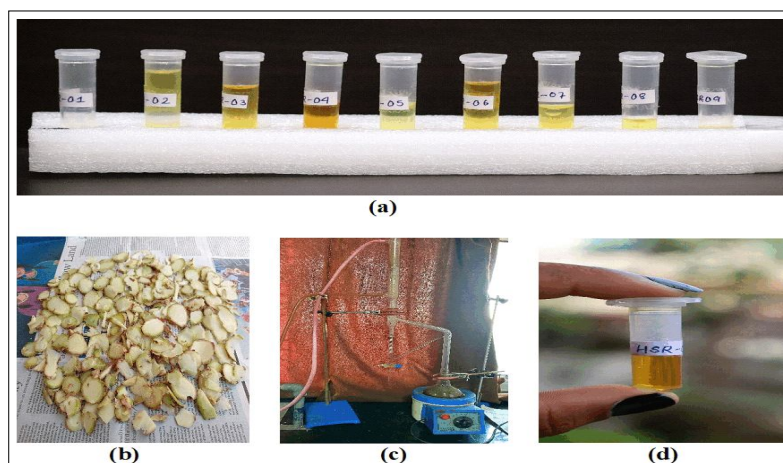


Fig 4: (a) Essential oil extracted from plant samples; (b) Rhizome slices, (c) Clevenger apparatus, (d) Essential oil.

Composition of essential oil

The chemical composition of *H. spicatum* essential oil through gas chromatography analysis shows marked qualitative and quantitative variation among the nine samples (HS-1 to HS-9), indicating strong chemotypic diversity (Table 3). Across all samples, 7-epi- α -eudesmol was the dominant constituent (24.42–41.95%), confirming a primarily sesquiterpenoid-rich profile (Table 3). Oxygenated monoterpenes such as 1,8-cineole and linalool display the greatest variability, with 1,8-cineole ranging from 2.65% to 30.23% and linalool from 0.34% to 21.26%, revealing distinct cineole-rich (HS-2, HS-3, HS-4, HS-6) and linalool-rich (HS-1, HS-7) subgroups. Sesquiterpenes including δ -cadinene, cisanerolidol, γ -cuprenene, β -vetivenene and α -muurolol occur consistently but at moderate levels, contributing to the characteristic woody earthy profile of the oil. The presence of high levels of eudesmol isomers (γ -eudesmol and 10-epi- γ -eudesmol), especially in samples HS-2 and HS-5, further supports a eudesmol-dominant chemotype across most populations. Overall, the oil profiling indicates that while *H. spicatum* essential oil maintains a consistent sesquiterpene backbone, significant variation in monoterpene content creates multiple chemotypes that may influence the oil's aroma and diverse biological activities with wide commercial potential in pharmaceutical industries. Altitude affected both the oil yield and chemical composition, with elevated regions promoting a greater concentration of oxygenated monoterpenoids. These results were consistent with previous research on other medicinal plants (Mahdavi *et al.*, 2013).

CONCLUSION

The present study on morphological traits, histochemical characteristics and essential-oil composition of *Hedychium spicatum* germplasm demonstrate intra-

specific diversity due to environmental and altitudinal differences. This study supports reliable identification of the species and enhances the authentication of crude herbal materials. Collectively, the results highlighted the chemotaxonomic knowledge of the species and revealed its utility in pharmaceutical, aromatic and value-added industries, supporting both conservation measures and reliable quality evaluation.

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Disclaimers

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Conflict of interest

All authors declare that they have no conflict of interest.

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