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Diversified Pattern of Phytochemicals in Rhizomes of *Hedychium Spicatum* Sm. In Response to the Altitudinal Gradient in the Chopta-Tungnath Region of Uttarakhand

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Abstract: *Hedychium spicatum* Sm. Belonging to the family, Zingiberaceae is a rhizomatous perennial herb distributed in the temperate areas of the Himalayan region of India. It is traditionally used as a medicine by various indigenous communities of the region. The present study aimed to assess variation in the secondary metabolite content and antioxidant potential of the rhizomes of the *H. spicatum* collected from various locations along an altitudinal gradient in the Chopta-Tungnath region of Uttarakhand, India. The rhizome samples were collected from ten different locations of the Chopta-Tungnath region between 1253 and 2600 m asl. Variations in morphological characters of the studied 10 accessions were observed. Aqueous extracts of the dried and powdered rhizomes were prepared for phytochemical analysis. Qualitative phytochemical screening of the extract revealed the presence of flavonoids, phenolic compounds, alkaloids, reducing sugars (carbohydrates), proteins, steroids, saponins, terpenoids, and glycosides. Morphological characters varied along the altitudinal gradient. Plant height and leaf length decreased with increasing altitude. The total phenolic content ranged from 2.14 mg gallic acid equivalent (GAE)/g dry weight to 18.70 mg GAE/g dry weight, and the total flavonoid content varied between 2.00 mg quercetin equivalent (QE)/g dry weight and 10.61 mg QE/g dry weight. The *in-vitro* antioxidant assay using diphenyl-2-picrylhydrazyl (DPPH) demonstrated IC_{50} values between 70.79 and 170.94 µg/mL. A significant correlation ($p < 0.0262$) was observed between total phenolic content and altitude in the present study.

Keywords: Phytochemicals • *Hedychium spicatum* • Altitude

Introduction

Hedychium spicatum Sm., a perennial rhizomatous plant belonging to the *Zingiberaceae* family, is well known for its medicinal and aromatic properties (Fig 1). Native to temperate and subtropical regions of Southeast Asia, this species is commonly referred to as "spiked ginger lily," "Van haldi," or "Kapoorkachari." It thrives in the Himalayan region at altitudes ranging from 1000 to 2800 meters above sea level (m asl), where it is found growing in oak (*Quercus* spp.) and *Cedrus deodara* (Roxb. ex D.Don) G.Don forests (Kirtikar & Basu, 1975; Rawat et al., 2011). The plant is characterized by its fragrant rhizomes, which are used in traditional medicine to treat a variety of ailments, including respiratory problems, fever, cough, and wounds (Chopra et al., 1986; Srimal et al., 1984). The therapeutic and economic potential of *H. spicatum* is further exemplified by its wide usage in modern medicine and cosmetics, particularly in the perfumery industry (Kamble & Dale 2018).

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Fig. 1: General morphology of *H. spicatum*

Fig. 2: Collection of *H. spicatum* from the wildlings of Chopta-Thungnath region

The rhizomes of *H. spicatum* contain several bioactive compounds, including flavonoids, alkaloids, phenolic compounds, saponins, terpenoids, and glycosides, which contribute to its medicinal efficacy (Sravani & Parakh 2011). These phytochemicals have been shown to exhibit a wide range of biological activities, including anti-inflammatory, antimicrobial, and antioxidant properties. Recent studies have highlighted the variability of phytochemical composition in *H. spicatum* collected from different altitudes. This variability is significant for understanding how environmental factors, such as altitude, influence the concentration of bioactive compounds. For instance, the total phenolic and flavonoid content has been shown to vary significantly among populations from different elevations. This is crucial as phenolic compounds are known to play a key role in the

plant's antioxidant activity, which can be assessed through in vitro assays such as DPPH (Diphenyl-2-picrylhydrazyl) (Sravani & Parakh 2011).

In the present study, we aimed to assess variation in the secondary metabolite content and antioxidant potential of the rhizomes of the *H. spicatum* collected from various locations along an altitudinal gradient in the Chopta-Tungnath region of Uttarakhand, India. We assessed the plant's morphological characteristics and chemical composition, with a particular focus on the relationship between altitude and the concentration of key bioactive compounds. This study seeks to enhance understanding of the secondary metabolite content and antioxidant potential of *H. spicatum* and provide insights for its future use in therapeutic and commercial applications.

Materials and Methods

Plant Material: Rhizomes of ten accessions of *H. spicatum* were collected from different altitudes in the Chopta-Tungnath region of Uttarakhand between September and November 2024 (Table 1 and Figure 2). The plant material was authenticated, and duly identified herbarium voucher specimens were deposited in the internationally recognized herbarium of the Botanical Survey of India, Dehradun (Accession No. 1428). The collected rhizomes were washed, dried in the shade, and powdered for further analysis. The powder was light to dark brown, fibrous, and had a bitter taste and camphoraceous odor.

Table 1: Details of the studied accessions of *H. spicatum* from the Chopta-Tungnath region of Uttarakhand, India.

S.No.	Accession code	Location name	Altitude (m asl)	Latitude (N)	Longitude (E)
1	HS01	Pinglapani	1253	30.505864	79.0943998
2	HS02	Ukhimath	1430	30.5057743	79.0948132
3	HS03	Giriya	1656	30.4986999	79.1070431
4	HS04	Mussoorie	1825	30.487404	79.199468
5	HS05	Sonprayag	1900	30.488212	79.15263
6	HS06	Sari	2034	30.55323	79.125747
7	HS07	Pothiwasa	2337	30.545034	79.130844
8	HS08	Gundagwar	2400	30.545054	79.130843
9	HS09	Deoriyatrek	2412	30.5197054	79.1296235
10	HS10	Duggalbitta	2600	30.5229454	79.1283676

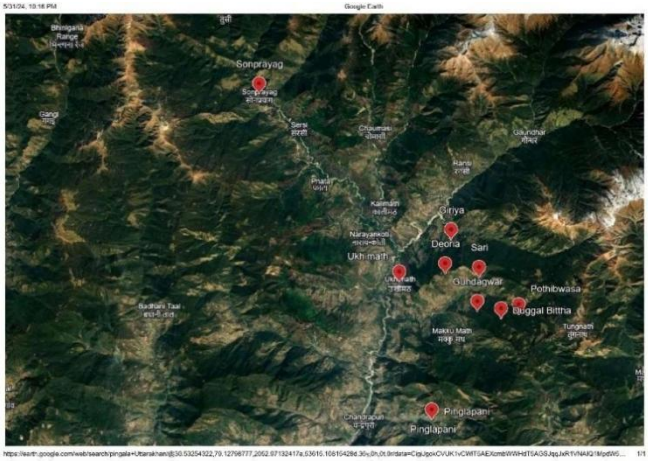

Fig 3. Map location of Plant rhizome collected from different sites of Chopta -Tungnath region, Uttarakhand

Qualitative Phytochemical Screening

To prepare the aqueous rhizome extract, 15 grams of air-dried rhizome powder was macerated with 100 ml of distilled water in a conical flask for 24 hours, including 6 hours of continuous shaking followed by 18 hours of standing. After 24 hours, the mixture was filtered using Whatman No. 1 filter paper. A 20 ml portion of the filtrate was dried at 105 °C in a tared shallow dish and weighed to determine yield. The dried extract was stored at 4 °C for future use. For phytochemical screening, 200 mg of this dried extract was dissolved in 20 ml of distilled water to make a 10 mg/ml aqueous solution. Standard procedures, as described by Shaikh et al. (2020), were followed to test for various phytochemicals. The Biuret test for proteins involved mixing 100 µl of extract with 100 ml of ethanol, adding 1 ml of Biuret reagent, and observing a color change. The Benedict test for carbohydrates required mixing the extract with 2 ml of Benedict reagent, boiling it, and checking for reddish-brown precipitate. For phenols and tannins, 1.5 gm of $FeCl_3$ was dissolved in 30 ml water; 50 ml extract was mixed with ethanol and $FeCl_3$ solution, and black coloration indicated their presence. Flavonoids were detected by mixing the extract with 1 ml of 1% NaOH, forming an intense yellow color that turned colorless on adding diluted HCl. Saponins were tested by shaking the extract with 2 ml of water; stable foam confirmed their presence. For glycosides, the Salkowski test was performed by mixing the extract with 2 ml of chloroform and 2 ml of concentrated H_2SO_4, with reddish-brown color indicating steroidal glycone presence. Steroids were tested by adding 1 ml each of concentrated H_2SO_4 and acetic acid to 2 ml of extract-chloroform mix; green coloration indicated a positive result. Terpenoids were confirmed by diluting 20 µl extract with 200 µl ethanol, mixing with 2 ml chloroform, evaporating to dryness, adding 2 ml concentrated H_2SO_4, and heating for 2 minutes; a greyish color was observed. Lastly, alkaloids were detected using the picric acid test, where adding 1% picric acid to 2 ml extract resulted in a yellow precipitate.

Quantitative Phytochemical Analysis

Total Phenolic Content (TPC): The amount of phenolic content in the aqueous extract of *H. spicatum* was determined with the Folin-Ciocalteu reagent (Spanos & Wrolstad 1990; Lincoln 2001). 50 µl of Distilled water, 50 µl Folin- Ciocalteu reagent, and 300 µl of Na_2CO_3 were added to 50 µl of the sample (3 replicates) of each plant extract solution. The resulting mixture was incubated in the dark for 30 min. After incubation, 5 ml of distilled water was added to each test tube. The absorbance of each sample was measured at

725 nm using a UV Visible Spectrophotometer. Gallic acid (20-200 µg/ml) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration (µg/ml) versus absorbance (nm) (y= 0.0028x + 0.0086; R2= 0.9914), where y is the absorbance at 725 nm, and x is the concentration. The total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent/ g of sample) and was calculated by the following formula as per Chakraborthy & Ghorpade (2010):

$T = (C \times V)/M$

Where T= total content of phenolic compounds, mg/g plant extract, in GAE; C= concentration of gallic acid established from the calibration curve, µg/ml; V= volume of extract, ml; M = weight of water extract of the plant, g.

Total Flavonoid Content (TFC)

The amount of flavonoid content in the aqueous extract of *H. spicatum* was determined with an Aluminium chloride colorimetric assay. 750 µl of Ethanol, 75 µl $NaNO_2$ (5% $NaNO_2$ solution), and 150 µl $AlCl_3$ (10 % $AlCl_3$) were added to 100 µl of the sample (3 replicates) of each plant extract solution. The solution was then incubated for 5 min at room temperature. Then 500 µl of NaOH (1 mm NaOH solution) and 5 ml of distilled water were added to each test tube. The resulting solution was then incubated for 20 min in the dark. The absorbance of each sample was measured at 510 nm using a UV Visible Spectrophotometer. Quercetin (0-1350 µg/ml) was used as a standard compound. The Quercetin standard calibration curve was established by plotting concentration (µg/ml) versus absorbance (nm) (y= 0.0024x + 0.02; R2= 0.9901), where y is the absorbance at 510 nm, and x is the concentration.

DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a common method used to evaluate the free radical scavenging ability of antioxidants. We dissolved 4 mg of DPPH in 40 ml of Ethanol. Stored this solution in a dark bottle for 2 h until absorbance stabilized. We prepared a 0.1 M Tris-HCl buffer, pH 7.4. Dissolve 0.01gm gallic acid in 50 ml of distilled water. Prepared a series of dilutions of the standard stock solution in Tris-HCl buffer to obtain different concentrations for testing. Prepared a 5 ml blank solution by mixing 3.4 ml of Ethanol with 1.6 ml of tris HCl buffer. In the analytical procedure distributed, the measurements at six points of concentration, including control, were required. After 2 h, 1 ml of the DPPH solution was added into a test tube, and then 700 µl of Ethanol and 800 µl of 0.1 M tris HCl buffer were added to make a 2.5 ml solution. For each sample, five different concentrations were made and were prepared in duplication (10 for each sample). Vortex the mixture to ensure thorough mixing. Incubated the reaction mixture in the dark at room temperature for 30 min. After mixing, the absorbance at 517 nm was measured.

Results and Discussion

Morphological Variations along the altitudinal gradient : The morphological characteristics of *H. spicatum* exhibited considerable variation in response to the altitudinal gradient (Table 2). Specifically, as altitude increased, both plant height and leaf length showed a consistent decline. This trend aligns with similar patterns observed in other alpine species, where environmental factors inherent to higher altitudes induce morphological adaptations (Chaves et al., 1997; Körner 2003).

The reduction in plant height and leaf length with increasing altitude may be a direct consequence of several interrelated environmental factors, primarily temperature, sunlight, and atmospheric pressure. At higher altitudes, lower temperatures tend to reduce metabolic rates, resulting in stunted growth compared to those plants situated at lower elevations where the climate is more

temperate. The reduction in leaf length could also be attributed to the need for plants to conserve energy and water in harsher environmental conditions, as larger leaves may increase transpiration rates and water loss. Smaller leaf size at higher altitudes is often an adaptive response to limit water loss, a critical factor in alpine ecosystems where water may be less readily available due to lower temperatures and restricted precipitation (Körner 2003; Desender et al 2013).

Additionally, the decrease in plant height could be associated with increased atmospheric pressure and reduced oxygen levels at higher altitudes. These changes could limit the plant's ability to perform photosynthesis and grow vertically, as oxygen is crucial for respiration and energy production. Furthermore, the intensity of sunlight increases with altitude, and in response, some species may exhibit shorter, more compact growth forms as a way to minimize exposure to intense UV radiation and prevent damage to their cellular structures (Grabherr et al 1994).

In alpine environments, the combination of shorter growing seasons and the increased likelihood of extreme weather events further influences the growth patterns of *H. spicatum*. As a result, the plant appears to adopt a more conservative growth strategy, allocating resources more efficiently for survival rather than rapid vertical expansion. Such morphological responses are critical for surviving in environments where resources are limited, and environmental pressures are amplified (Chaves et al 1997; Körner 2003).

In conclusion, the morphological variations observed in *H. spicatum* along the altitudinal gradient underscore the adaptability of this species to the complex and fluctuating environmental conditions encountered at different elevations. The observed trends highlight the broader ecological principle that plants must continuously adapt their growth strategies to cope with altitude-related stresses, ensuring their survival in challenging alpine environments.

The phytochemical composition of *H. spicatum* rhizomes demonstrated notable variation across the altitudinal gradient (Table 3). Phytochemical analysis revealed the presence of a range of bioactive compounds, including carbohydrates, phenols, flavonoids, saponins, and steroids. Of particular interest was the higher concentration of phenolic compounds and flavonoids in populations located at higher altitudes. These compounds have long been recognized for their protective roles in plants, especially in response to environmental stressors such as UV radiation, temperature extremes, and oxidative stress, which are more prevalent at higher elevations.

Table 2: Morphological variation in ten accessions of *H. spicatum* collected from different regions of the Chopta-Tungnath region.

Accession code	Plant height (cm)	Leaf Length (cm)	Leaf Width (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Internodal distance (cm)	Petiole length (cm)	Stem girth (cm)	Rhizome Diameter (cm)
HS01	100	42	11	4.0	3.0	20	0.5	2.0	20
HS02	150	36	8	3.5	2.0	13	0.5	2.5	24
HS03	60	34	15	9.0	3.0	11	1.2	2.8	18
HS04	50	25	7	5.0	2.0	10	0.4	3.0	16
HS05	47	24	13	8.0	2.1	18	0.3	2.0	17
HS06	40	20	11	6.0	2.3	9	1.0	2.0	26
HS07	38	19	10	8.0	3.0	13	0.5	3.0	18
HS08	35	18	9	7.0	3.0	9	0.5	2.5	17
HS09	24	17	6	5.0	2.5	12	1.0	2.3	15
HS10	27.94	17.78	4.5	8.0	4.0	6	0.4	2.7	8

Phenolic compounds, which include flavonoids, are well known for their antioxidant properties, which help mitigate damage caused by reactive oxygen species (ROS) generated under stress conditions, particularly UV radiation (Chaves et al 2009). At elevated altitudes, where UV radiation is more intense due to thinner atmospheric layers, plants are often exposed to heightened oxidative stress. The increased presence of phenolics and flavonoids in higher altitude populations of *H. spicatum* likely represents an adaptive mechanism to protect cellular structures from UV-induced damage (Hernández et al., 2008). These compounds act as natural sunscreens, absorbing harmful UV radiation and neutralizing free radicals, which would otherwise lead to DNA mutations and oxidative damage to cellular components (Rao et al 2014).

Table 3: Phytochemical constituents of aqueous extracts of *H. spicatum*.

Accession code	Carbohydrate	Phenols/ Tannins	Saponins	Protein	Flavonoid	Terpenoid	Glycosides	Steroids	Alkaloids
HS01	+	+	-	+	+	+	-	+	_
HS02	+	+	+	+	+	-	-	+	_
HS03	+	-	+	+	+	-	+	+	+
HS04	+	+	+	+	+	-	+	+	-
HS05	+	+	-	+	+	-	+	+	+
HS06	+	+	-	+	+	-	+	-	-
HS07	+	-	+	+	+	+	+	-	+
HS08	+	+	-	+	+	-	+	+	+
HS09	+	+	+	+	+	+	+	-	+
HS10	+	+	+	+	+	+	+	+	+

Phytochemical Variation along the Altitudinal Gradient

Moreover, the elevation-related increase in phenolics and flavonoids may also be linked to other abiotic stress factors commonly encountered at higher altitudes, such as reduced temperature and atmospheric pressure. It is well-established that plants growing in these conditions often increase the production of secondary metabolites as a strategy to enhance their resistance to cold stress, as well as to control water loss and to limit the effects of reduced oxygen availability (Körner, 2003). These biochemical responses allow the plant to maintain metabolic stability and overall fitness under challenging climatic conditions.

Interestingly, while flavonoids and phenolics dominated the phytochemical profile of higher-altitude populations, other bioactive compounds such as saponins and steroids did not show significant variation with altitude. Saponins, known for their antimicrobial and antifungal properties (Cheng et al., 2015), were present at relatively consistent levels across all altitudinal ranges, suggesting that their primary role might be related to defense against soil-borne pathogens rather than environmental stressors linked to altitude. Similarly, steroids, which have various biological functions including hormone regulation and membrane stabilization, did not exhibit significant variation with altitude, indicating that their biosynthesis might be more stable across different environmental conditions.

The presence of carbohydrates across all altitudes suggests their fundamental role as primary metabolites in energy storage and as structural components. These compounds did not show significant variation across the altitudinal gradient, indicating that they are

essential for basic metabolic functions, regardless of altitude.

In conclusion, the phytochemical variation observed in *H. spicatum* along the altitudinal gradient highlights the plant's capacity to adapt to the stressors associated with higher elevations. The increased synthesis of phenolic compounds and flavonoids in high-altitude populations underscores their critical role in protecting plants from intensified UV radiation and oxidative stress. These findings provide further insights into how plants, particularly in alpine ecosystems, alter their biochemical profiles to enhance survival in harsh and variable environmental conditions.

Correlation between Total Phenolic Content and Altitude

A significant positive correlation ($p < 0.0262$) was observed between the total phenolic content and altitude, with higher concentrations of phenolic compounds found in *H. spicatum* populations at altitudes exceeding 2300 m asl (Table 4). This finding suggests a clear relationship between increased elevation and the accumulation of phenolic compounds, potentially reflecting the plant's adaptive response to the harsher environmental conditions characteristic of higher altitudes.

The observed increase in phenolic content with altitude may be attributed to the protective role that these compounds play in defending plants against various environmental stressors, particularly ultraviolet (UV) radiation. As altitude increases, atmospheric pressure decreases, and the intensity of UV radiation increases due to the thinning of the protective atmospheric layers (Chaves et al 1997). This heightened UV exposure can lead to cellular damage caused by reactive oxygen species (ROS), which in turn can impair plant growth and metabolism (Hernández et al 2008). Phenolic compounds, especially flavonoids, are well known for their ability to absorb UV radiation and neutralize ROS, thus mitigating the harmful effects of oxidative stress (Dixon & Paiva 1995).

Flavonoids, a subclass of phenolic compounds, are particularly adept at absorbing UV radiation, thereby protecting plant tissues from UV-induced damage (Agati et al., 2013). Their synthesis is often upregulated in response to environmental stress, which could explain the higher phenolic content in populations from higher altitudes. Additionally, phenolics act as antioxidants, scavenging free radicals and reducing the potential for oxidative damage to DNA, lipids, and proteins (Czégény et al 2014). The increased production of these compounds in higher-altitude populations of *Hedychium H. spicatum* likely represents an adaptive mechanism to mitigate the damaging effects of elevated UV exposure, which is more prevalent at greater elevations.

Moreover, the correlation between altitude and phenolic content may also reflect other environmental stresses that are intensified at higher altitudes, such as lower temperatures, higher winds, and reduced oxygen availability (Körner,

2003). Plants in these environments often enhance the production of secondary metabolites like phenolics not only for UV protection but also to stabilize cellular structures and maintain metabolic function under cold and hypoxic conditions. By producing more phenolics, *H. spicatum* may be able to cope better with these stresses, thereby ensuring its survival in challenging alpine environments.

Table 4. Total phenolic compounds (TPC), Total Flavonoid Content, and antioxidant capacity (DPPH) in the rhizome of *H. spicatum* collected from different altitudes. Data is presented as the Mean ± Standard Deviation.

Accession code	TPC (mg GAE/g dry weight)	TFC (mg QE/g dry weight)	DPPH (mMAAE per 100 g dry weight)
HS01	2.14± 0.21	2.00± 0.34	153.81± 0.57
HS02	4.80± 0.62	2.83± 0.34	162.79± 0.97
HS03	3.23± 0.18	2.59± 0.19	170.94± 0.44
HS04	7.46± 0.20	2.74± 0.19	133.87± 0.83
HS05	11.84± 0.54	3.88± 0.19	147.28± 0.60
HS06	9.06± 0.35	5.70± 0.33	98.19± 0.41
HS07	10.93± 0.29	8.74± 0.19	125.96± 0.52
HS08	12.73± 0.39	8.11± 0.34	70.79± 0.58
HS09	18.77± 1.24	10.61± 0.34	157.07± 0.74
HS10	16.35± 0.55	9.63± 0.20	166.67± 0.47

In conclusion, the significant positive correlation between total phenolic content and altitude in *H. spicatum* highlights the plant's biochemical adaptation to the environmental pressures found at higher elevations. The increased synthesis of phenolic compounds at greater altitudes underscores their protective role against UV radiation and oxidative stress, essential for the plant's survival in alpine habitats. These findings contribute to a better understanding of how plant biochemical responses to environmental gradients, particularly UV radiation, help species thrive in high-altitude ecosystems.

Conclusion

The results of the present study demonstrated that *H. spicatum* contains a diverse array of phytochemicals, with significant variations observed across populations at different altitudes. The plant's high phenolic and flavonoid content, coupled with its antioxidant activity, highlights its potential for pharmaceutical and cosmetic applications. Further research could focus on isolating and characterizing specific bioactive compounds for the development of novel therapeutic agents.

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