

Altitudinal Variation in Major Bioactive Phytoconstituents of *Dolomiaea macrocephala* DC. ex Royle in Himachal Pradesh, India

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ABSTRACT

Plant pharmacological activity is commonly associated with major classes of phytoconstituents, including phenolics, alkaloids, flavonoids, and saponins. *Dolomiaea macrocephala* DC. ex Royle is a critically endangered alpine herb of Himachal Pradesh with significant ethnobotanical value as an incense and traditional remedy. The present study investigated altitudinal variation in these key phytoconstituents in the leaves and roots across different locations in Himachal Pradesh. Total phenolic and flavonoid contents were quantified using colorimetric assays via spectrophotometry, while alkaloid and saponin contents were estimated using gravimetric methods. The results indicated that total phenolic and saponin contents exhibited a positive trend with increasing elevation in both leaves and roots, whereas alkaloid and flavonoid contents showed a decreasing trend along the altitudinal gradient. Elevation showed a significant positive correlation with phenolic content in leaves ($r = 0.634$, $p < 0.05$) and roots ($r = 0.578$, $p < 0.05$), as well as with saponin content ($r = 0.914$, $p < 0.01$). Other constituents exhibited weak or non-significant associations with the elevation.

Key words: *Jurinea*, Aromatic, Alpine herb, Gravimetric method, Quantitative analysis, Secondary metabolites, Dhoop

INTRODUCTION

Dolomiaea, a taxonomic genus within the family Asteraceae (tribe: Cardueae; subtribe: Saussureinae), comprises 25 documented species that predominantly inhabit the alpine terrain of China, India, Nepal, Myanmar, and Pakistan (Kasana et al. 2020, Wang et al. 2007). In the Indian subcontinent, the genus is represented by three distinct species: *D. macrocephala*, *D. baltalensis*, and *D. costus* (Kasana et al. 2020). *Dolomiaea macrocephala* DC. ex Royle, alternatively recognized as *Jurinea macrocephala* (DC. ex Royle) Benth. ex C.B. Clarke or *J. dolomiaea* Boiss., is a perennial species that predominantly inhabits the Western Himalayan expanse, which spans from Pakistan to Nepal (Singh et al. 2015). It thrives along elevational gradients ranging from 3200 to 3800 m above sea level (Kumar and Agnihotri 2020). Colloquially referred to as Himalayan *Dolomiaea* or Guggal Dhoop, the harvesting cycle of this species commences in July and extends through September (Awasthi et al. 2003). Morphologically, the species is characterized by

rosette formation and exhibits a stemless morphology, epitomized by its prostrate growth (Verma and Tewari 2016). This species proliferates into numerous sessile capitula, varying in number from 3 to 15 (Kasana et al. 2020). Phytochemical profiling has revealed the presence of phenolic compounds, such as caffeic acid, apigenin, rutin, myricetin, gallic acid, and catechin, in this species. Six compounds, β -sitosterol, Lupenone, Physcion, 20,21 α -epoxytaraxastan-3 β -ol, chlorogenic acid, and ptiloepoxide were isolated from the roots of *D. macrocephala* (Kumar and Agnihotri 2020). Its pharmacological repertoire encompasses antioxidant, antimicrobial, antidiabetic, analgesic, antiulcer, antileishmanial, and hepatoprotective properties (Bhat et al. 2023, Darshan et al. 2022, Dwivedi and Wagay 2014, Singh et al. 2015). The species has been categorized as endangered within the Indian Himalayan Region and Jammu & Kashmir, critically endangered in Himachal Pradesh, and placed in the negative export list by the Government of India (Ahmed et al. 2020, Awasthi et al. 2003, Rawat and Kumar 2024).

The phytochemical studies on *D. macrocephala* are limited, and there are no studies on altitudinal variations in the accumulation of secondary metabolites. Therefore, the current study aims to evaluate the altitudinal variations in four major secondary metabolites in this medicinally important herb. This study will help to identify the altitude-driven pattern in these bioactive phytoconstituents and potential populations for conservation and sustainable utilisation.

MATERIAL AND METHODS

Study area

The present study was carried out in the Himalayan state of Himachal Pradesh (India), spanning from 30°22'40" - 33°12'40" N to 75°45'55" - 79°04'20" E. The survey was conducted in the alpine and subalpine regions of the Inner and Greater Himalayan ranges. The high alpine region experiences low temperatures, high solar radiation with intense ultraviolet radiation, and a short growing season. The species was reported to inhabit mostly the southern dry slopes and open meadows of this Himalayan zone.

Collection of plant material and processing

A sample collection of roots and leaves was carried out between July and October over three consecutive years (2023-2025). As the species occur in high alpine regions, the collection sites ranged from 3386 to 4120 m amsl. A total of 12 sites were selected for the present study (Table 1). The elevation of each site was recorded using a Garmin GPS (accuracy < 5 m). The collected species samples were identified in the Herbarium of ICFRE-HFRI, Conifer Campus, Panthaghati, Shimla (India). After collecting plant parts, the plant material was washed, dried (open shade), ground and used for analysis.

Sampling was conducted across multiple alpine locations in Himachal Pradesh. Site-wise data were primarily used to capture continuous variation in phytoconstituent levels across elevation. The relationship between elevation and phytoconstituent levels was further evaluated using Spearman's Rank Correlation. For broader comparison, sites were additionally categorised into three elevation classes: lower alpine (<3500 m), middle alpine (3501-3700 m), and upper alpine (>3701 m). These classes represent ecologically distinct ranges within the alpine region, characterized by differences in

Table 1. Collection of *D. macrocephala* from different altitudinal regions of Himachal Pradesh

Zone/Site name	Elevation (m)	Division	District	Aspect	Habitat
Lower Alpine					
Laka	3386	Dharamshala	Kangra	NW	Alpine, Rocky meadow, moderately moist slope
Sundrasi	3446	Bharmour	Chamba	SW	Alpine, Rocky Meadows, Dry slope
Magen Jot	3459	Ani	Kullu	SE	Alpine, Rocky Meadows, Dry slope
Dhel Thach	3459	Great Himalayan National Park	Kullu	NE	Open alpine meadow (Moderately moist)
Middle Alpine					
Sural Bhatari	3506	Pangi	Chamba	SW	Alpine, Rocky Meadows, Dry slope
Bashleu Pass	3538	Ani	Kullu	SW	Alpine, Rocky Meadows, Dry slope
Bashal Kanda	3606	Sarahan WL	Shimla	SE	Alpine, Rocky Meadows, Dry slope
Chander Nahan	3662	Rohru	Shimla	SE	Alpine, Rocky Meadows, Dry slope
Upper Alpine					
Dehnasari	3727	Joginder Nagar	Kangra	SW	Alpine, Rocky Meadows, Dry slope
Kara	3809	Sarahan WL	Kinnaur	SE	Alpine, Rocky Meadows, Moist slope
Sach Pass	4090	Pangi	Chamba	SE	Alpine, Rocky Meadows, Dry slope
Chhitkul	4120	Sarahan WL	Kinnaur	SE	Alpine, Rocky Meadows, Dry slope

temperature, ultraviolet radiation, and growing season, which are known to influence plant secondary metabolism. Given the geographic dispersion of sampling sites, this stratification was adopted for comparative analysis across elevation ranges rather than to represent a single continuous environmental gradient.

Quantitative analysis

Four major secondary metabolites namely total phenolics, alkaloids, flavonoids, and saponins, were estimated in the roots and leaves of *D. macrocephala*. Total phenolic and flavonoid contents were determined using colorimetric assays as described by Chandra et al. (2014) and Chang et al. (2002), respectively, while alkaloid and saponin contents were estimated following the methods of Harborne (1984) and Mir et al. (2016), respectively.

RESULTS AND DISCUSSION

Total phenol content (TPC)

TPC in leaves of *D. macrocephala* ranges b/w 24.80 (Dhel Thach, Kullu) - 35.77 (Sural Bhatori, Chamba) mg GAE/g, and in the case of roots, it ranges b/w 18.32 (Sundrasi, Chamba) - 29.82 (Sach Pass, Chamba) mg GAE/g. The highest phenolic content in leaves and roots was reported in the samples collected from Sural Bhatori (leaves) and Sach Pass (roots) in Chamba district, respectively (Table 2).

TPC in leaves and roots showed a significant positive correlation with elevation, with correlation coefficients of $r = 0.63$ and $r = 0.578$, respectively ($p < 0.05$) (Table 3). This indicated a higher accumulation of TPC at higher elevations in both leaves and roots. The present findings also demonstrate a progressive increase in total phenolic content (TPC) across the three elevation zones in both leaves and roots of *D. macrocephala*. However, this altitudinal effect was statistically significant only in leaves, suggesting a stronger sensitivity of aerial tissues to environmental variation (Table 4). The significantly higher TPC observed at 3501-3700 m and >3700 m compared to ≤ 3500 m indicates that phenolic accumulation intensifies beyond a certain elevation threshold. However, the absence of a significant difference between 3501-3700 m and

>3700 m may suggest a plateau in phenolic synthesis at extreme elevations, possibly reflecting metabolic stabilisation once plants acclimatise to high-altitude stress conditions. In contrast, no statistically significant differences were detected among the three defined altitudinal zones for root TPC. Research studies on the phytochemical investigation of *D. macrocephala* are very limited. In the present investigation, leaf tissues exhibited higher TPC compared to roots. This observation is consistent with the findings of Bhat et al. (2022), who also reported greater phenolic accumulation in leaves than in underground parts. Such tissue-specific distribution can be explained ecologically, as leaves are directly exposed to environmental stress, including UV radiation, temperature fluctuations, and herbivory. Therefore, their higher concentration in aerial parts likely reflects an adaptive strategy to cope with external stress conditions. A clear increase in TPC with rising altitude was observed in the present study. Similar altitudinal trends have been reported by Mangral et al. (2023) in *Rhododendron anthopogon*, another Himalayan alpine species. From an ecological perspective, this pattern suggests that phenolic accumulation may represent a form of biochemical adaptation that enhances stress tolerance under harsh alpine conditions.

Alkaloid content

The alkaloid content was expressed as a percentage of the dry weight of the sample. In the current study in *D. macrocephala*, the alkaloid content ranges b/w 0.71-3.33% in leaves. The highest content was reported in Sundrasi (Chamba), while the least was in Kara (Kinnaur). In roots, the alkaloid content ranges b/w 3.35-6.62% with the highest content at Bashal Kanda (Shimla) and the least in the samples collected from Dehnasar (Kangra) (Table 2). Overall, roots exhibited greater alkaloid content than leaves, suggesting tissue-specific allocation of these nitrogen-containing secondary metabolites.

Correlation analysis indicated a negative relationship between alkaloid content and elevation in both leaves ($r = -0.518$) and roots ($r = -0.378$) (Table 3). Alkaloid levels tended to decrease with increasing elevation; however, this trend was not statistically significant ($p > 0.05$). This suggests that

Table 2. Phytochemical screening of leaves and roots of *D. macrocephala* in Himachal Pradesh

Site name	Phenol (%)		Alkaloid (%)		Flavonoid (%)		Saponin (%)	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Laka	28.35±2.88	23.80±2.48	2.91±0.32	6.59±0.42	0.24±0.02	2.13±0.10	2.16±0.06	0.32±0.11
Sundrasi	26.33±3.64	18.32±1.97	3.33±0.20	6.43±0.39	0.27±0.01	3.79±0.04	2.19±0.07	0.35±0.09
Magen Jot	29.73±2.63	22.85±0.64	1.71±0.31	4.11±0.66	0.29±0.01	3.57±0.12	2.14±0.11	0.29±0.14
Dhel Thach	24.80±1.28	25.44±2.67	2.45±0.38	5.71±0.64	0.22±0.03	2.28±0.08	2.12±0.10	0.41±0.08
Sural	35.77±4.23	24.09±0.23	3.25±0.25	7.65±0.55	0.26±0.01	2.51±0.06	2.66±0.13	0.63±0.09
Bashleu Pass	32.44±0.55	23.58±3.59	1.72±0.15	6.15±0.32	0.21±0.01	2.49±0.04	2.61±0.00	0.43±0.09
Bashal Kanda	30.73±1.67	24.16±2.20	2.56±0.35	6.62±0.11	0.26±0.01	3.62±0.30	2.54±0.11	0.66±0.04
Chander Nahan	34.42±1.94	26.89±1.26	2.58±0.13	3.99±0.82	0.17±0.01	2.04±0.13	2.56±0.05	0.62±0.09
Dehnasar	32.97±3.53	25.58±2.67	2.62±0.13	3.35±0.65	0.22±0.01	2.00±0.17	2.45±0.12	0.70±0.04
Kara	35.64±2.24	22.76±3.66	0.71±0.11	4.79±0.15	0.28±0.00	3.57±0.33	2.49±0.07	0.72±0.02
Sach Pass	34.53±4.54	29.82±3.72	1.48±0.33	6.49±0.22	0.27±0.01	2.63±0.14	2.72±0.16	0.78±0.07
Chhitkul	31.24±0.31	25.75±3.29	2.03±0.20	3.64±0.35	0.20±0.03	2.74±0.14	2.35±0.07	0.71±0.06

Table 3. Spearman’s rank correlation coefficients (ρ) among elevation and secondary metabolites of *D. macrocephala*

	Elevation	Alkaloid		Phenol		Flavonoid		Saponin	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Elevation	1.000								
Alkaloid									
Leaf	-0.518	1.000							
Root	-0.378	-0.049	1.000						
Phenol									
Leaf	0.634*	-0.245	-0.385	1.000					
Root	0.578*	-0.035	-0.231	0.266	1.000				
Flavonoid									
Leaf	-0.147	-0.168	0.336	0.056	-0.503	1.000			
Root	-0.042	-0.147	0.357	-0.175	-0.503	0.643*	1.000		
Saponin									
Leaf	0.368	-0.028	-0.168	0.762**	0.112	-0.231	-0.133	1.000	
Root	0.914**	-0.378	-0.203	0.692*	0.503	0.000	0.000	0.427	1.000

** . Correlation is significant at the 0.01 level (2-tailed); * . Correlation is significant at the 0.05 level (2-tailed); Abbreviation: L- Leaf; R- Root

altitude may exert a limited or inconsistent influence on alkaloid accumulation. Also, across three elevation ranges, leaf and root alkaloids have shown a decreasing trend, although this decrease was not statistically significant (Table 4). A slightly greater decline in leaf and root alkaloid content was observed between 3501-3700 m and >3700 m compared to the decline from ≤3500 m to 3501-3700 m. This pattern indicates a gradual altitudinal decrease rather than abrupt shifts between elevation categories. The higher alkaloid concentration in roots may be due to

its role in below-ground defence against pathogens. Alkaloid synthesis is closely linked to nitrogen metabolism; therefore, reduced nutrient availability and lower temperatures at higher elevations may limit nitrogen uptake, leading to decreased accumulation of nitrogen-based secondary metabolites. The root alkaloid levels recorded in the present study are comparable to the values reported by Bhat et al. (2023) from the Kashmir Himalaya, indicating relative consistency in alkaloid accumulation within this species across regions. A decreasing trend in

Table 4. Phytochemical content in leaves and roots of *D. macrocephala* in three elevation ranges

Elevation Range (m)	Total Phenol (mg GAE/g)		Alkaloid (%)		Flavonoid (mg QE/g)		Saponin (%)	
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Lower Alpine	27.30±1.09 ^a	22.60±1.52	2.60±0.35	5.71±0.57	0.26±0.01	2.94±0.43	2.15±0.01 ^a	0.34±0.02 ^a
Middle Alpine	33.34±1.11 ^b	24.68±0.75	2.31±0.51	5.15±0.72	0.23±0.02	2.67±0.34	2.59±0.03 ^b	0.59±0.05 ^b
Upper Alpine	33.60±0.96 ^b	25.98±1.45	1.71±0.41	4.57±0.71	0.24±0.02	2.74±0.32	2.43±0.03 ^c	0.73±0.02 ^c
F _{Calculated} (2,9)	11.45	1.74	1.13	0.725	0.654	0.152	79.63	30.93
p-value	0.003	0.23	0.36	0.51	0.54	0.86	0.00	0.00
CD (p≤0.05)	3.89	NS*	NS	NS	NS	NS	0.09	0.13

ANOVA results: Values are Mean ± SE (n = 4) followed by different letters that differ significantly according to Tukey's HSD test; F_{Calculated} (2,9) shows F value calculated and degrees of freedom (degrees of freedom of replication, degrees of freedom of error); *NS- non-significant difference of phytoconstituent among the three elevation ranges

alkaloid content at higher elevations has also been documented in *Taxus contorta* by Chauhan et al. (2024), as well as in high-altitude lupine species studied by Carey and Wink (1994) and Jan et al. (2016). The altitudinal patterns seen for TPC (increasing) and alkaloids (decreasing) show a different regulation of carbon-based and nitrogen-based secondary metabolites with environmental gradients. While carbon-rich TPCs show an increase under high UV and oxidative stress, nitrogen-containing alkaloids declined due to less nutrient availability at higher elevations.

Total flavonoid content

The regression equation $y = 0.8576x - 0.0395$, with $R^2 = 0.9892$ was used to calculate the flavonoid content. In *D. macrocephala* samples, the flavonoid content ranges b/w 0.17-0.29 mg QE/g of dry weight in leaves. The highest content in leaves was in Magen Jot (Kullu), and the least was in Chander Nahan (Shimla). In the roots of this species, the variation in flavonoid content ranges b/w 2.0-3.79 mg QE/g of dry weight. The highest content was reported in Sundrasi (Chamba), and the least in the samples collected from Dehnasar (Kangra) (Table 2). Overall, roots exhibited significantly higher flavonoid content than leaves.

Flavonoid content in both leaves and roots exhibited a weak negative correlation with elevation, with correlation coefficients of $r = -0.147$ and $r = -0.042$, respectively, indicating a negligible influence of altitude on flavonoid accumulation (Table 3). The variation in flavonoid content along the altitudinal

gradient does not exhibit a directional trend in either leaves or roots; it shows higher content in both the lower and higher elevational zones, but this difference is not statistically significant (Table 4). This non-linear distribution indicates that flavonoid accumulation in *D. macrocephala* may not be directly regulated by elevation alone. Previous studies on Himalayan plants have reported an increasing trend in flavonoid content with altitude (Dhiman et al. 2025, Mangral et al. 2023), whereas the present study on *D. macrocephala* did not exhibit a similar linear pattern across the three elevation zones. This suggests that flavonoid synthesis in this species may be influenced by multiple interacting environmental and physiological factors rather than altitude alone. Nataraj et al. (2022) reported a decline in flavonoid content of *Artemisia brevifolia* with increasing altitude in the Ladakh region of Western Himalaya, indicating that elevation alone may not determine variations in secondary metabolite accumulation. These observations suggest that secondary metabolite production in *D. macrocephala* is governed by complex ecological regulation rather than a simple response to altitude.

Saponin content

The saponin content in the leaves and roots of *D. macrocephala* ranges between 2.12 and 2.66%. The highest saponin content was found in the Sural Bhattori region (2.66%) of Chamba, and the least in the Dhel Thach region (2.12%) of GHNP, Kullu. In roots, the saponin content ranges between 0.29 and 0.78%, with the highest value found in Sach Pass

(0.78%) and the lowest in Magen Jot (0.29%) (Table 2). Overall, leaves exhibited significantly higher saponin content than roots. Saponin content exhibited a positive relationship with elevation in both plant parts. This correlation was strong and statistically significant in the roots ($r = 0.914$) of *D. macrocephala*, whereas it was comparatively weak in the leaves ($r = 0.368$) (Table 3). This strong positive relation in roots shows a greater role of roots in stress response and metabolite storage under increasing environmental stress at higher elevations. Across three elevation zones, the saponin content has also shown a significant increase ($p \leq 0.05$) in both the leaves and roots (Table 4). In leaves, the middle elevation range (3501-3700 m) has shown the highest saponin content, while the least was reported below 3500 m. In roots, the saponin content showed a linear increasing trend with the maximum saponin value at >3700 m and the least below 3500 m. It was observed that the saponin synthesis responds differently in aerial and underground tissues with the rise in elevation. Saponins help in defence against herbivores and pathogens, and may contribute to stress tolerance under changing environmental conditions. The high root saponin at higher elevations shows enhanced below-ground defence against soil pathogens and environmental stress. In leaves, the peak at mid-elevation followed by a slight decline at higher elevations may indicate an optimal altitudinal range for saponin synthesis, beyond which metabolic constraints such as low temperature or reduced nutrient availability may limit further accumulation. A comparable altitudinal pattern has been reported by Kimta et al. (2024) in *Adiantum venustum* fronds from different elevations of Himachal Pradesh, where maximum saponin content was recorded at intermediate elevations, followed by reduced levels at lower and higher altitudes. Such non-linear responses suggest that secondary metabolite production may be influenced by a balance between environmental stimulation and physiological limitation. So, the saponin accumulation in *D. macrocephala* appears to be affected by altitude-related ecological factors rather than elevation alone.

The findings of the present study indicate that phenolic compounds and root saponins tend to increase progressively along the continuous

altitudinal gradient, even when differences among broad elevation zones were not always statistically distinct. Additionally, leaf total phenolic content showed significant positive correlations with saponin content in both leaves ($r = 0.76$; $p < 0.01$) and roots ($r = 0.69$; $p < 0.05$), suggesting a coordinated pattern of secondary metabolite accumulation. This study aims to explain the influence of altitude on the accumulation of these phytochemicals; however, we do not claim that altitude is alone responsible for these variations. The results indicate that secondary metabolite accumulation in *D. macrocephala* responds differently along the altitudinal gradient. The increase in TPC and saponins at higher elevations shows an adaptive response to environmental stress, whereas the decrease in alkaloids may be due to metabolic or less nutrient availability in higher regions. Overall, our findings show that altitude and its related factors play an important role in determining phytochemical profiles in alpine flora, although additional ecological factors like soil, precipitation, temperature, and the plant's physiology (Nobel 1991), Genetic differentiation (Hashimoto and Yamada 1994) and developmental stages (Singh et al. 2025), etc., may also contribute to the observed patterns. Therefore, while elevation-associated environmental conditions appear to play an important role, the regulation of secondary metabolite accumulation in *D. macrocephala* is likely shaped by multiple interacting ecological and physiological factors.

D. macrocephala is a threatened species of the Indian Himalayan region due to its overexploitation for commercial purposes. The current study will help identify superior germplasm of this species in the study area, which can act as a potential source for future propagation, cultivation, and other conservation programs. The observed altitudinal variation in phenolics and saponins suggests that populations from higher elevations can be prioritized for the selection of superior chemotypes with high medicinal value. The selection of superior germplasm for conservation planning (in-situ or ex-situ) will improve the success of the reintroduction program while maintaining the desired phytochemical profiles. Such selection of superior germplasm may also reduce harvesting pressure on wild populations by encouraging the cultivation and domestication of

high-value genotypes.

CONCLUSIONS

Dolomiaea macrocephala, a valuable medicinal plant native to the alpine regions of the Himalayas, holds significant value due to its various therapeutic properties. The species is generally reported from the windward southern slopes of the high Himalayas, which receive heavy monsoon rainfall but remain effectively dry for most of the year. Primarily, the roots of this species are used in religious rituals and the incense industry to produce Dhoop, a sacred substance employed in spiritual practices. The medicinal benefits of *D. macrocephala* can be attributed to the presence of essential phytoconstituents like alkaloids, phenolics, and saponins. In the present study, it was reported that in *D. macrocephala*, the TPC and saponin showed an upward trend with altitude, whereas the alkaloid showed a downward trend with the same. Also, TPC and saponin content were higher in leaves than in roots, whereas alkaloid and flavonoid content were significantly higher in roots than in leaves. Due to its considerable significance in both pharmacology and incense production, there is a pressing need for more focused research aimed at elucidating the detailed phytochemical composition, aromatic resin characteristics, and ethnomedicinal formulations of this species.

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