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CONTENTS

Impact of altitude on photosynthetic and biochemical profile of *Didymocarpus pedicellatus* R.Br.: an antiurolithiatic Himalayan herb

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ABSTRACT: Altitude plays an important role in shaping plant's physiological and biochemical behavior. This ultimately helps the plant in acclimatization and adaptation in varying habitats spread across different altitudinal gradients. The current work focuses to study the biochemical and physiological profile of *D. pedicellatus* across varying elevations (700, 1186, 1370 and 1640 m). Total polyphenolic content including phenols, flavonoids and tannins were quantitatively estimated using standard protocols. Proteins, photosynthetic pigments and macronutrients were evaluated using standard available protocols with mild modifications. Data was statistically analyzed and means were compared for significance at $p< 0.05$. Results revealed statistically significant variation in all the studied parameters except for phosphorous content. Total phenol (23.26 %), tannin (63.75 %) and protein (175.7 %) content increased effectively whereas total flavonoids decreased (84.84 %) with rise in elevation. Photosynthetic pigments like chlorophyll a, b and carotenoids also followed the decreasing trend by 36.46 %, 39.98 % and 36.24 % respectively, with increasing altitude. Macronutrients like nitrogen, potassium and phosphorus also declined by 55.43 %, 57.67 % and 20.71 % respectively. The decline in photosynthetic pigments and macronutrient content highlights the decrease in photosynthetic and mineral uptake capacity of *D. pedicellatus* at higher altitudes. The study suggests that A4 populations (growing at 1640 m) are richer in polyphenolic compounds and hence more suitable for medicinal purposes. Hence, for bringing the plant in cultivation, A4 populations may be selected as elite planting material in order to get optimum yield of medicinally important compounds.

Keywords: Altitudinal variation, environmental stress, medicinal, nutrients, polyphenols, sustainability

Large number of environmental factors influence plant growth, development and its physiological and biochemical responses. Physical factors like altitude affect solar radiations, temperature, precipitation, atmospheric pressure and nutrient availability (Körner, 2007; Du *et al.*, 2017). Elevation shift leads to notable changes in plant morphology and metabolism, driving the development of altitude-specific mechanism to ensure plant survival (Zhao *et al.,* 2020; Dongdong *et al*., 2020). *Didymocarpus pedicellatus* (Gesneriaceae) is an endemic Himalayan herb used for curing kidney stones accompanied by nephroprotective, antibacterial, antioxidant and anti-inflammatory properties (Goyal *et al.*, 2015). The plant is distributed in the Indian Himalayan region at an altitude ranging from 700 m to 2000 m inhabiting moist shady places on rocks mostly near a river stream. Leaves and roots of this plant contain various phytochemical compounds like phenols, quinochalcones, chalcones, flavanines, terpenes, fatty acids and steroids (Nanjala *et al*., 2022). Didymocarpin, 5,8-dihydroxy-7-methoxyflavone, isopedicin, pediflavone, didymocarpin A, pedicellin, pedicin, pedicinin and methyl pedicin are the major polyphenolic constituents present in leaves of *D. pedicellatus*. (Saklani *et al.*, 2021) These chemical constituents vary among different populations depending not only upon the genotype but also influenced by the habitat conditions, altitudes and prevailing climatic conditions such as temperature, atmospheric pressure and humidity etc. It is necessary to see the impact of elevation on the polyphenolic content so as to identify the optimum yielding population that

can supply quality material for herbal industry. Further, the higher yielding population can also be used as elite planting material for cultivation purpose. Elevation also impacts the photosynthetic machinery, particularly chloroplast and chlorophyll pigment, which in turn affects the growth and other major metabolic processes besides affecting nutrient uptake and its assimilation (Mooney and Billings, 1961).

The current study aimed to investigate the effect of altitude on polyphenolic content, chlorophyll levels, protein content and concentrations of key nutrients (NPK) in plant samples collected from different altitudes in Uttarakhand. The study examined altitude-responsive traits of the plant to establish theoretical framework for comprehending its growth and adaptive mechanism to different environmental conditions aiming for sustainable agriculture.

MATERIALS AND METHODS

Collection of plant material and extract preparation

Plant samples of *D. pedicellatus* were collected from four different sites in Uttarakhand. Details of the elevation and GPS coordinates of the sites (A1, A2, A3 and A4) are given in Table 1. Voucher specimen was deposited in the herbarium of Department of Biological Sciences (GBPUH) with accession number 1441. Uprooted plant samples (along with the soil), were brought to the Plant Bioprospecting Laboratory in an aerated cool chamber (to keep the plant fresh). Leaves of the plants were immediately used for estimation of chlorophyll and protein content. Shade dried leaves were pulverized and methanolic extract was prepared using soxhlet extractor and stored at 4 °C for further use. All the chemicals used are of Hi Media make.

Estimation of total phenolics, flavonoids and tannins

Spectrophotometric estimation of total flavonoid content (TFC) was done using the method of Djeridane *et al* (2006) with Quercetin as the standard. Total phenolic content (TPC) was evaluated using Folin-Ciocalteau reagent (Wolfe *et al.,* 2003) with Gallic acid as the standard; absorbance was taken at 665 nm and 765 nm respectively. The results were expressed in mg quercetin equivalent (QE) /g of extract for flavonoids and mg gallic acid equivalent (GAE) /g of extract for phenolic content. Total tannin content was estimated using protocol of Price and Butler (1977) with minor modifications. For this, diluted extracts were mixed with 0.1 M $FeCl_3$ and 8 mM potassium ferricyanide followed by incubation for 10 minutes at 27± 2°C. The optical density was measured at 720 nm using GENESYS 10S UV-VIS spectrophotometer (Thermo Scientific). Tannic acid standard curve was prepared for quantification of tannin content in the extract and results were expressed in mg tannic acid equivalent (TAE)/ g of extract.

Estimation of protein content

Spectrophotometric estimation of protein was done from the fresh leaves of *D. pedicellatus* following Bradford (1976) method using BSA (bovine serum albumin) for preparation of standard curve. The absorbance was recorded at 595 nm and protein content was expressed in mg BSA/g of fresh leaves.

Estimation of macronutrients

Leaf samples were used for evaluation of macronutrients (Nitrogen, Phosphorus and Potassium) using standard protocols (Okaebo *et al*., 1993). Leaves were washed, shade dried and powdered for estimation of macronutrients. Nitrogen was quantified using Kjeldahl method (KJEL PLUS system, Pelican, India), by digestion, distillation and titration of leaf sample and content was expressed in mg /100 g dry weight. For phosphorus evaluation, a reaction mixture of ammonium molybdate, conc. H, SO,, and stannous chloride was added to the digested leaf samples. Absorbance was read at 420 nm using RAY LEIGH UV 2601 spectrophotometer. Absorbance vs. concentration standard curve of KH_2PO_4 was plotted and phosphorus content was estimated using the curve and expressed in mg/100 g dry weight. Potassium content was determined with digested sample using flame-photometer (128 Systronics). Potassium chloride (KCl) standard curve was prepared and concentration was read directly from the photometer.

Estimation of photosynthetic pigments

Spectrophotometric evaluation of chlorophyll a, b and carotenoids was done from the leaf samples of the plants collected from different altitudes using Hiscox and Israelstam (1979) adopted by Richardson *et al* (2002). Pre heated 1 ml dimethylsulfoxide (DMSO) per 10 mg of fresh leaf tissue was incubated for 30 min. at 65 °C. Absorbance was measured at 470, 645 and 663 nm, calibrating to zero with pure DMSO.

The following formula was used which was based on Arnon's (1949) equations, where:

$$Chlorophyll\ a = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times \frac{V}{1000 \times W}$$

$$Chlorophyll\ a = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \frac{V}{1000 \times W}$$

$$Carotenoids = \left(\frac{1000 \times A_{470}\ 1.82\ Chl\ a\ 85.02\ Chl\ b}{214}\right) \times \frac{V}{1000 \times W}$$

Where; V= volume of extract (ml), W= fresh weight of sample (g)

Statistical analysis

All the experiments were done in triplicates. Data was subjected to one way ANOVA and values were expressed in mean ± standard deviation using MS-Excel and Origin pro software. Tukey's mean comparison test was used to evaluate significance of data at $P < 0.05$.

RESULTS AND DISCUSSION

Total phenolics, flavonoids and tannin content

Total phenolics, flavonoid and tannin content analysed quantitatively across plant samples collected from different altitudes (A1 to A4 - where A1 is the lowest altitude, 700 m and A4 is the highest altitude, 1640 m), showed significant variations ($p < 0.05$) as depicted in Table 2. Total phenolic content ranged from 27.94 ± 0.35 mg GAE/g extract in A1 to 34.44 ± 0.43 mg GAE/g extract in A4 showing 23.26% increase with increasing altitude. The results revealed an increase in phenolic content with increasing altitude. Similar trend was observed in leaves of *Crataegus x sinaica, Thalictrum foliolosum, Tanacetum sinaicum* and *Cotoneaster orbicularis* that also showed elevated phenols with increasing elevation (Ibrahim *et al.*, 2022). *Asarum sieboldii* showed significant increase in total phenols with increasing altitude (Pan *et al*., 2023).

In contrast, total flavonoid content declined from 19.00 ± 1.33 mg QE/g extract (A1) to 2.88 ± 0.52 mg QE/g extract (A4) resulting in 84 % decrease with ascending elevation (Table 2). Similar trend was observed in *Polygonatum verticellatum* which also showed negative correlation with altitude ($r = -0.708$, $p < 0.01$) (Suyal *et al.*, 2019). Generally, flavonoid content increase with increasing altitude but this unusual trend suggests potential redistribution of resources towards other secondary metabolites during stress. Tannins also showed a marked increase of 63.75 % with increasing altitude, showing maximum tannin content at A4 (31.03 ± 0.85 mg TAE/g extract), suggesting an adaptive response to oxidative stress prevalent at higher elevations.

In the present study, phenols and tannin content showed an increasing trend with increasing altitude whereas flavonoid showed a reverse trend with increasing altitude. Rana *et al* (2020) showed increase in phenols, flavonoids and terpenoid content with increasing altitude in *Coleus forskohlii* collected from different altitudes of Garhwal region of Uttarakhand. Various abiotic stress like heavy metal, drought, high/low temperature and ultraviolet radiations elevate biosynthetic pathway of phenylpropanoid triggering increase of polyphenolic content in plants (Sharma *et al.*, 2016; Wang *et al.*, 2019; Nataraj *et al*., 2022). Flavonoid pathway might be more dependent on other abiotic factors *viz*., UV light, topography of the habitat.

Total protein content

Protein also showed a significant upward trend from 27.06 ± 1.21 mg BSA /g (A1) to 74.61 ± 0.47 mg BSA /g FW (A4) giving an impressive 176 % increase in protein profile when moving up the hill, Table 2. Similar reported in *Kobresia pygmaea*, *Nepeta septtemcrenata* and *Rosa arabica* yielding significantly high protein content at higher elevations (Li *et al*., 2014; Hashim *et al.,* 2020). Dhatwalia *et al* (2024), also observed significant increase in protein content in *Rubus ellipticus* being minimum at 500 m and maximum at 2000 m. Protein accumulation actually fulfil the energy requirement of a plant in response to stress as well as scavenge

Table 1: Collection sites of *D. pedicellatus* in Uttarakhand, India

S.No.	Collection sites	Altitude (m)	Latitude	Longitude
1	Nainital (A1)	700	29° 17' 43.55" N	79° 32' 25.57" E
2	Nainital (A2)	1186	29º 20' 17" N	79º 29' 14" E
3	Chamoli (A3)	1370	30º15' 18 N	79º26' 48 E
4	Bageshwar (A4)	1640	29 º 54' 49″ N	79 º 46' 34 ‟ E

Where A1 is the lowest altitude and A4 is the highest altitude.

Table 2: Total phenolics, flavonoids, tannin and protein content in leaves of *D. pedicellatus* collected from different altitudes

Collection sites	TPC ± SD mg GAE/ g of extract	TFC ± SD mg QE/g of extract	TTC ± SD mg TAE/g of extract	Protein (mg BSA/g FW)
A1	27.94 ± 0.35 c	19.00 ± 1.33 a	18.95 ± 0.71 d	27.06 ± 1.21 d
A2	28.83 ± 0.79 c	10.26 ± 0.63 b	20.40 ± 1.80 c	50.44 ± 1.57 c
A3	31.06 ± 0.43 b	6.77 ± 0.56 c	25.55 ± 0.76 b	58.26 ± 0.86 b
A4	34.44 ± 0.43 a	2.88 ± 0.52 d	31.03 ± 0.85 a	74.61 ± 0.47 a
CV	0.017	0.085	0.047	0.021

Data are represented as mean ± standard deviation. Tukey's mean comparison at significance level P < 0.05. CV – Coefficient of variation. A1- 700 m, A2- 1186 m, A3- 1370 m and A4- 1640 m.

Table 3. Photosynthetic pigments in leaves of *D. pedicellatus* collected from different altitudes

Collection sites	Chlorophyll a (mg/ 100 g fresh weight)	Chlorophyll b (mg/ 100 g fresh weight)	Carotenoids (mg/100 g fresh weight)
A1	78.20 ± 0.95 a	35.12 ± 0.34 a	32.89 ± 0.70 a
A2	75.84 ± 0.65 b	31.95 ± 0.75 b	29.55 ± 0.56 b
A3	69.48 ± 0.55 c	24.97 ± 1.61 c	24.83 ± 0.89 c
A4	49.69 ± 0.44 d	21.08 ± 0.67 d	20.97 ± 0.17 d
CV	0.01	0.034	0.02

Data are represented as mean ± standard deviation. Tukey's mean comparison at significance level P < 0.05. CV – Coefficient of variation. A1- 700 m, A2- 1186 m, A3- 1370 m and A4- 1640 m.

Table 4: Nitrogen (N), phosphorus (P) and potassium (K) level in leaves of *D. pedicellatus* collected from different altitudes

Collection sites	Nitrogen (mg / 100 g)	Phosphorus(mg / 100 g)	Potassium(mg / 100 g)
A1	789.00 ± 1.00 a	214.00 ± 5.29 a	1315.00 ± 13 a
A2	624.67 ± 4.73 b	204.67 ± 6.43 a	989.67 ± 10.02 b
A3	484.33 ± 7.37 c	186.33 ± 5.51 b	633.67 ± 11.02 c
A4	351.67 ± 3.79 d	169.67 ± 9.07 b	556.67 ± 7.02 d
CV	0.009	0.035	0.012

Data are represented as mean ± standard deviation. Tukey's mean comparison at significance level P < 0.05. CV – Coefficient of variation. A1- 700 m, A2- 1186 m, A3- 1370 m and A4- 1640 m.

ROS as a defence response (Khan *et al*., 2016).

Macronutrient content

Nutritional content of the leaves also significantly varied with the altitude (Table 4). Analysis of macronutrients in the leaves of *D. pedicellatus* collected from different altitudes showed significant decreasing trend of nitrogen (A1- 789.00 ± 1.00 to A4 - 351.67 ± 3.79) and potassium (A1- 1315.00 ± 13 mg /100 g, A4 – 556.67 ± 7.02 mg/100 g) with rising elevation. Decrease in phosphorus, however (A1- 214.00 ± 5.29 mg/ 100 g, A4 - 169.67 ± 9.07 mg/100 g) was not very significant in relation to varying elevations. More than half of the nitrogen (55.43 %) and potassium content (57.67 %) decreased with increase of about 940 m altitude whereas phosphorous had a decrease of only 20.71 %. The results are in conformity with other recent studies on altitudinal variation in leaf nutrient content (Tsombou *et al*., 2025). Chandra and Lata

(2022) also found decrease in potassium and phosphorous content in *Aconitum balfourii* and *Podophyllum hexandrum* with an exceptional increase in nitrogen content. In the present study, nitrogen decrease may be possibly due to over accumulation of protein content in the leaves of *D. pedicellatus* at higher altitudes. Phosphorus and potassium being involved in maintaining other metabolic processes for sustaining life at higher altitudes, also showed a decline. This decrease can be explained owing to comparatively lower soil temperature and hence decreased soil microbial activity causing poor nutrient absorption (Zhu *et al*., 2024).

Photosynthetic pigments

Altitude and environmental conditions greatly impact photosynthesis and its pigments. The results revealed a significant decrease in chlorophyll a, b and carotenoids content with increasing altitude (36.46%, 39.98 % and 36.24 % respectively). Maximum chlorophyll-a content was 78.20 ± 0.95 at A1 elevation whereas lowest was 49.69 ± 0.44 at A4 elevation as shown in Table 3. Similarly, Chlorophyll b and carotenoids were maximum at A1 (35.12 ± 0.34, 32.89 ± 0.70) and minimum at A4 (21.08 ± 0.67, 20.97 ± 0.17) showing significant difference at $p < 0.05$ (Table 3). This trend aligns with the studies of Ahmad *et al.* (2018), Cui *et al.* (2018), Zhao *et al.* (2020) and Chandra and Lata (2022) and indicating decrease in all the photosynthetic pigments with increasing altitude which ultimately affect the photosynthetic capacity of the plants growing at higher altitude leading to slower growth and poor development. Low-temperature stress at higher elevations affect synthesis of chlorophyll pigments and also its ability to harvest light due to increased photo-oxidation (Ibrahim *et al*., 2022).

CONCLUSION

This study reveals notable effect of altitude on various biochemical and physiological parameters (phenols, flavonoids, tannins, chlorophylls, macronutrients and proteins) of a plant. The results divulged that phenol, tannin and protein content of the plants are directly proportional to altitude whereas photosynthetic pigments (chl a, chl b and carotenoids), flavonoids and macronutrients (nitrogen, phosphorus and potassium) decrease in content with elevation. Our findings reveal distinct variation patterns across four different altitudes, suggesting adaptive response of *D. pedicellatus* to changing environmental conditions of temperature, precipitation, humidity, soil microbial activity and soil nutrient profile. This research fills a gap by providing comprehensive data on how altitudinal variation influence not just nutrient status but the overall physiological profile of plants. The study will also be helpful in selecting the elite population and its adaptability to plan its sustainable propagation in suitable mountainous ecosystems.

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