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The effect of elevational gradient on Potentilla *fruticosa from Pir Panjal range of* Jammu &Kashmir, India.

Farzana Kouser[1], Gurdev Chand*[1], Bhav Kumar Sinha[1], Nazia Parveen[2], Sapalika Dogra[3], Muneeba Banoo[1], Marvi Sharma[1], Monika Kumari[1].

[1] Division of plant physiology, Faculty of Basic science, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu- Chatha

[2] Department of Environmental sciences, center for Biodiversity Studies, Baba Ghulam Shah Badshah university Rajouri

[3] Faulty of agriculture, division of Basic science and Humanities, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

*Corresponding author mail id: gurdev74@gmail.com

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ABSTRACT

There is significant interest in understanding how medicinal plants vary in morphological and physiological responses with changes in elevation. And to assess the soil properties of these altitudinal gradients. The mountainous areas of Pir Panjal range of Jammu region offer a unique opportunity to study how plants adapt to rapid changes in elevation. This study focused on *Potentilla fructicosa* examining their responses at three different elevations of Pir Panjal range of Jammu region: 930 m, 1183 m, and 1843 m above sea level (ASL). In the current study, several parameters have been evaluated along this vertical gradient, including soil physio-chemical parameter along with morpho-physiological parameters (Plant height, leaf area and photosynthetic pigments). The results revealed a negative correlation between soil pH, phosphorus and potassium with increasing altitude. Further, a positive correlation was found between soil total nitrogen, organic carbon, and soil organic matter. The results indicated that with increasing elevation, plant height and leaf area decrease, while photosynthetic pigments increase. Additionally, it was observed that the variations in photosynthetic pigments, leaf area, and plant height in *Potentilla fruticosa* are attributed to local adaptation. These findings offer valuable insights into how a narrow elevational gradient influences plant morphology and physiology.

INTRODUCTION

Medicinal plants have traditionally been used in many applications including treatment of various ailments/diseases such as cough, malaria, diarrhea, severe headaches, lung cancer, seizures etc (Pan *et al*., 2013, Sindhu *et al*., 2022; Matera *et al*., 2023). Various plant parts, including roots, leaves, stems, bark, fruits, and seeds, have been utilized to treat a wide range of diseases and enhance the immune system. (Butnariu, 2021, Matera *et al*., 2023). These herbal remedies are eco-friendly and are considered safer for the general population. There is a growing global trend toward herbal healthcare products as alternatives to synthetic drugs, due to issues like resistance, side effects, and limited efficacy in chronic conditions [Karimi *et al*., 2015, Sharma *et al*., 2019]. The majority of medicinal plants are located in mountainous regions, which influence their distribution and population structure. Under abiotic stress, cellular homeostasis becomes imbalanced, leading to the generation of free radicals that can cause oxidative damage. In response to environmental stress, nearly all plants produce specific secondary metabolites, such as flavonoids, phenolic compounds, alkaloids, carotenoids, steroids, tannins, and terpenoids. These compounds enhance the medicinal properties of the plants. (Wahab *et. al*.,2018, Gourlay *et. al*., 2018, Lambers *et.al*., 2019, Elkelish *et.al*., 2019). In mountainous regions, elevation gradients cause significant environmental changes over relatively short distances, impacting factors such as temperature, humidity, exposure, and atmospheric gas concentrations (Hovenden & Vander Schoor, 2004; Rawat *et al*., 2015). These unique conditions make mountainous areas ideal for studying plant responses to diverse climatic variations, which reflect broader latitudinal patterns (Zidorn *et al*., 2001). As elevation rises, precipitation and light intensity generally increase, accompanied by variations in UV-A (315–400 nm) and UV-B (280–315 nm) radiation, while temperature, carbon dioxide, and oxygen levels tend to decrease (Rozema *et al*., 1997; Bilger *et al*., 2001). These environmental shifts influence plant morphology and physiology, enabling them to adapt to the stresses associated with varying elevations (Hovenden & Brodribb, 2000; Körner, 2007; Ncube *et al*., 2012).

Soil elemental content is a vital factor influencing nutrient uptake by plants and plays a vital role in their growth (Wang Y. *et al*., 2023). Carbon, nitrogen and phosphorus are particularly essential for plant growth, development, and nutrient cycling (Parveen *et al*., 2022a &b). Carbon acts as a structural component and contributes to the ecosystem's carbon cycle, while nitrogen and phosphorus serve as limiting nutrients necessary for plant development (Güsewell and Freeman, 2005). Due to variations in individual traits, different plant species and life forms adopt distinct strategies to sustain growth and reproduction, resulting in varying leaf carbon, nitrogen, and phosphorus contents in response to soil nutrients and altitude (Cheng et al., 2021). Altitudinal variations frequently influence plant reproduction, survival, metabolism, and structural as well as morphological characteristics (Zhang *et al.*, 2023).

Potentilla fructicosa is a medicinal plant found in mountainous areas of Pir Panjal range of Jammu and Kashmir, India. This hardy deciduous shrub, a member of the *Potentilla* genus in the Rosaceae family, is native to the cool temperate and subarctic regions of the Northern Hemisphere and typically flourishes at high altitudes (Miliauskas *et al.*, 2007; Wang *et al.*, 2013**).** In traditional Chinese medicine, extracts from *Potentilla* plants have been utilized to treat a range of conditions, including diarrhea, hepatitis, rheumatism, and scabies, as well as for detoxification purposes (Miliauskas *et al.*,2009; Xue *et al.*, 2006: Khramova *et al.*,2019). The plant's extracts are known for their high levels of phenolic acids and flavonoids, which contribute to their strong radical scavenging abilities (Miliauskas *et al.*, 2007; Tomczyk *et al.*, 2010; Liu *et al.*,2016). Certain extracts have shown antioxidant activity surpassing that of the synthetic antioxidant BHT (butylated hydroxytoluene) and extracts from *Salvia officinalis* L., which is known for its potent antioxidants (Miliauskas *et al.*, 2007: Liu *et al.*,2016: Khramova *et al.*,2019). Keeping in view the variations in different elevations, the current study is designed to investigates how a narrow range of elevational gradients impacts the morphology and physiology of *Potentilla fructicosa.*

Methodology

2.1 Study Area

The present study was Carried out in the three different places of Pir Panjal range of Jammu and Kashmir, India. Particularly, in the highland region of Tandwal (Altitude 930m) and Dhanore (Altitude 1183m) areas of district Rajouri and Mandhar (Altitude 1843m) area of district Poonch. The geographical coordinates and the elevations of the study sites are presented in Table 1

Table 1: Different altitude of the study sites with longitude and latitude

S.NO.	SITE CODE	ALTITUDE	LATITUDE	LONGITUDE
01	A1	930m	33.3800233	74.2830426
02	A2	1183m	33.402633	74.335836
03	A3	1843m	33.8192287	74.1728223

2.2. Sample collection and measurement of plant growth parameters

The field sampling was conducted in the month of August to September (flowering season) 2021 for the plants. Sampling was conducted randomly using the approach outlined by Paudel *et al.* (2019). At each sampling plot, a 100-meter horizontal transect was established, and twenty plants were randomly selected along the transect, ensuring a minimum distance of 5 meters between nearby sampled plants (Paudel *et al.*, 2019).

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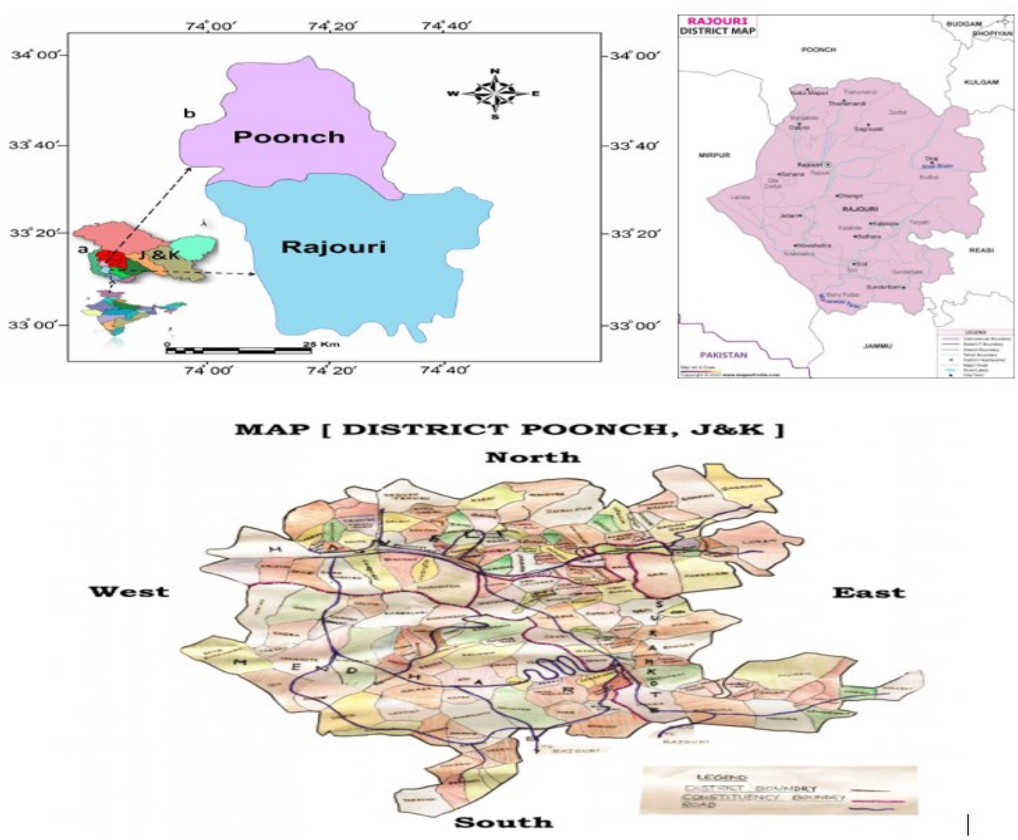

Fig. 1. Map of the study area

Mature leaves were collected from four orientations (north, south, east, and west) and from different positions (upper, middle, and lower parts) of each plant. These leaves were then combined evenly into a single test sample for each region, resulting in a total of three test samples. All samples were vacuum-dried at 40°C and then pulverized into powders, and stored in the dark at −20°C for further analysis. Plant height, measured using a standard metric ruler as the distance from the ground to the top of the stem, was recorded as a morphological variable. Physiological variables included leaf area and photosynthetic pigment content was analyzed by following (Paudel *et al.*, 2019). Freshly collected leaves of medicinal plant were separated after being rinsed under running tap water to eliminate soil and dust. The Image J software was used to collect uniform leaf area from each plant (Parveen *et al.*, 2023).

Plant species: *Potentilla fruticosa* (Fig. A)
Family: Rosaceae
Altitude: 930m, 1183m and 1843m.

Fig. 2. Photos of Selected plant species. **A.** *Potentilla fruticosa*

2.3. Phytochemical screening

2.3.1. Measurement of total chlorophyll and carotenoid levels

Total chlorophyll and carotenoid content was estimated in leaves of selected plant. Chlorophyll estimation was done by following the method of Arnon, (1949), and carotenoids content by Duxbury and Yentsch, (1956). Freshly collected leaf samples (0.5 g) were crushed with 10 ml of acetone (80% v/v acetone/water chilled) in a mortar pestle and centrifuged at 10°C at 5000 rpm for 15 minutes. Using a spectrophotometer, the optical density of the supernatant was measured at 663, 645 and 480 nm. Acetone was used as blank. The chlorophyll and carotenoids content were measured by using the following formula:

Chlorophyll a= $= \frac{[12.7(A663)-2.69(A645)]\times V}{1000\times W}$

Chlorophyll b= $\frac{[22.9(A645)-4.6(A663)]\times V}{1000\times W}$

Total chlorophyll= $\frac{[20.2(A645)+8.02(A663)]\times V}{1000\times W}$

Carotenoids= $\frac{[7.6(A480)-2.63(A663)]\times V}{1000\times W}$

Where, V=volume of acetone
W=weight of sample
A=absorbance

2.4. Soil biochemical parameters

In this study, three different altitudes of *Potentilla fruticosa* were selected to evaluate soil nutrient status and its impact on species composition and structure. Soil samples were collected from each altitudinal profile at a depth of 0–50 cm. The samples were manually homogenized and sieved through a 2 mm mesh to remove concrete and other debris. The soil was oven-dried at 65°C for 24 hours for physico-chemical analysis. Soil pH was measured using a digital pH meter. The soil organic carbon percentage was determined using the rapid titration method of Walkley (1947) as described by Parveen *et al.*, 2024. The soil organic matter percentage was calculated by multiplying the organic carbon percentage by a factor of 1.724. Available phosphorus was estimated following the method of Olsen *et al.*, (1954). Potassium was extracted using the ammonium acetate method (Morwin and Peach, 1951) and quantified using a flame photometer. Total nitrogen content was measured using the Kjeldahl procedure as described by Bremner and Mulvaney (1983).

2.5. **Statistical analysis**: Data was analyzed statistically by MS excel, metaboanalyst 5.0 and IBM SPSS statistics 20. One way analysis of variance was done to compare the mean values by Duncan's multivariate range test ($P<0.05$).

3. RESULTS and DISCUSSION

3.1. Variations of plant growth parameter with elevation

The elevational gradient is a crucial environmental factor influencing the development, structure, and function of plants. In this study, it was found that the height of *Potentilla fruticosa* reduced markedly with raising elevation and a comparable pattern was observed for leaf area, which also significantly reduced as elevation increased.(fig 3) It might be due to structural Change to harsher environmental conditions including lower carbon dioxide levels, cooler temperatures, increased solar radiation, and/or reduced water availability, which is in lineation with the study of Guerin, Wen & Lowe, (2012) and Cao *et al.*, (2020).

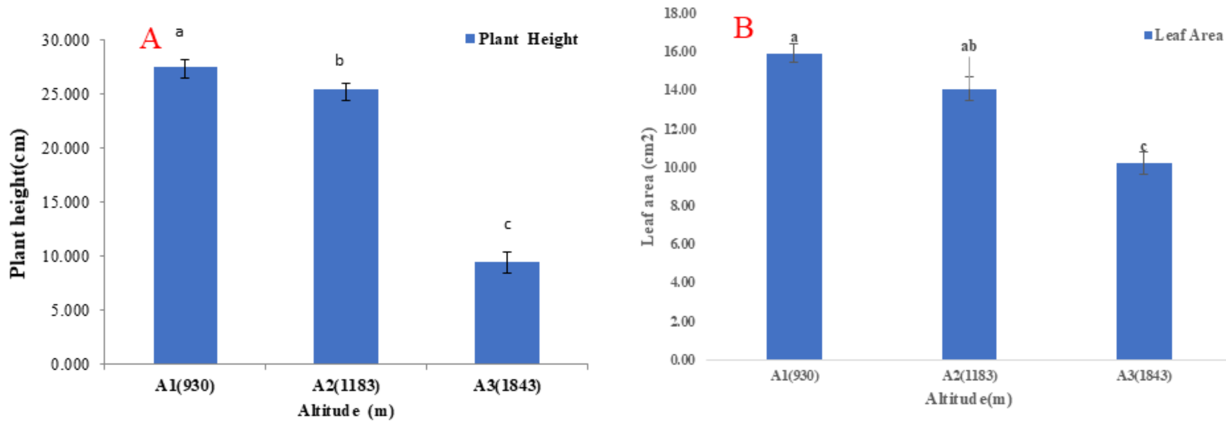

Fig. 3: Plant Height and leaf area of *Potentilla fruticosa* from three different altitude (A1930, A21183 and A31843m ASL.) *Data are means of three replicas (n=3) ± standard error of mean. Means followed by the same letter in a column / bar are not significantly different by Duncan's multivariate test (DMRT)*

The smaller size and shorter leaves of these plants at higher elevations may indicate a local adaptation to minimize transpiration and optimize water use (Peppe *et al.*, 2011: Guerin, Wen & Lowe, 2012: Sun, *et al.*, 2017). The decrease in plant height and leaf area with increasing altitude is an adaptive strategy to cope with cold temperatures, strong winds, intense UV radiation, short growing seasons, limited water and nutrients, and other environmental stresses. These adaptations help plants conserve resources, reduce physical damage, and survive in challenging high-altitude ecosystems (Hirano *et al.*, 2017; Cao *et al.*, 2020)

3.2. Change in the Photosynthetic Pigments

In the current studies it was observed that, Chlorophyll a increased significantly with higher altitude, and chlorophyll b also showed an increase as the elevation rises (fig 4). Similarly, as we move upward there is increasing trend in carotenoids content in the leaf of the selected plant species. Our study is in lineation with the study of (Hashim, *et al.*, 2020) who observed that the combination of high light intensity, UV radiation, cooler temperatures, oxidative stress, and adaptive pressures at higher altitudes drives the increased synthesis of chlorophyll and carotenoids in plants. This might help them to maximize photosynthetic efficiency and survive in challenging conditions. At higher altitudes, light intensity, particularly ultraviolet (UV) radiation, is much stronger due to thinner atmospheric layers. Enhanced chlorophyll production ensures efficient light harvesting for photosynthesis under intense sunlight. High altitudes typically have cooler temperatures, which can slow down enzymatic degradation of chlorophyll and carotenoids. (Soliman *et al.*, 2018; Gong *et al.*, 2018). Carotenoids not only serve as light-harvesting pigments but also act as scavengers of singlet oxygen and helps to quench the triplet state of chlorophyll molecules. (Deepti *et al.*, 2021).

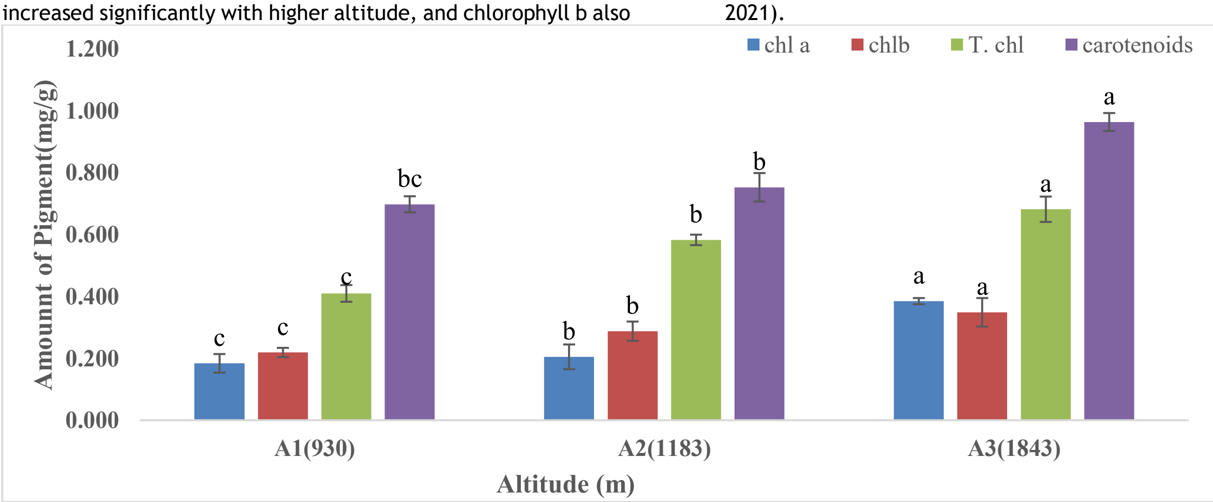

Fig. 4: Chlorophyll a, b, total chlorophyll, and carotenoid in methanolic extract of ***Potentilla fruticosa*** at different altitudes. Data of mean of three replicas (n=3) ± standard error of mean. *Means followed by the same letter in a column / bar are not significantly different by Duncan's multivariate test (DMRT)*

3.3. Soil biochemical parameters

Soil samples collected from three different altitudes in the Pir Panjal range revealed that the soils were slightly alkaline, with pH values decreasing as altitude increased. Both soil organic carbon and organic matter showed an increase with higher altitudes. Nitrogen also exhibited a rising trend with increasing elevation (fig 5). However, no clear trend was observed for phosphorus and potassium with altitude. Previous study revealed that as we go lower to higher altitude pH of the soil decreases primarily due to organic matter accumulation, enhanced leaching of base cations, cold temperatures, acidic plant litter, and limited soil development. These factors combine to create an environment where acidic components dominate, resulting in more acidic soils at higher altitudes (Sheikh *et al.*, 2010; He *et al.*, 2023). The increase in soil organic carbon with higher altitudes may be attributed to the greater moisture content, which is a strong indicator of higher soil organic carbon (SOC) levels.

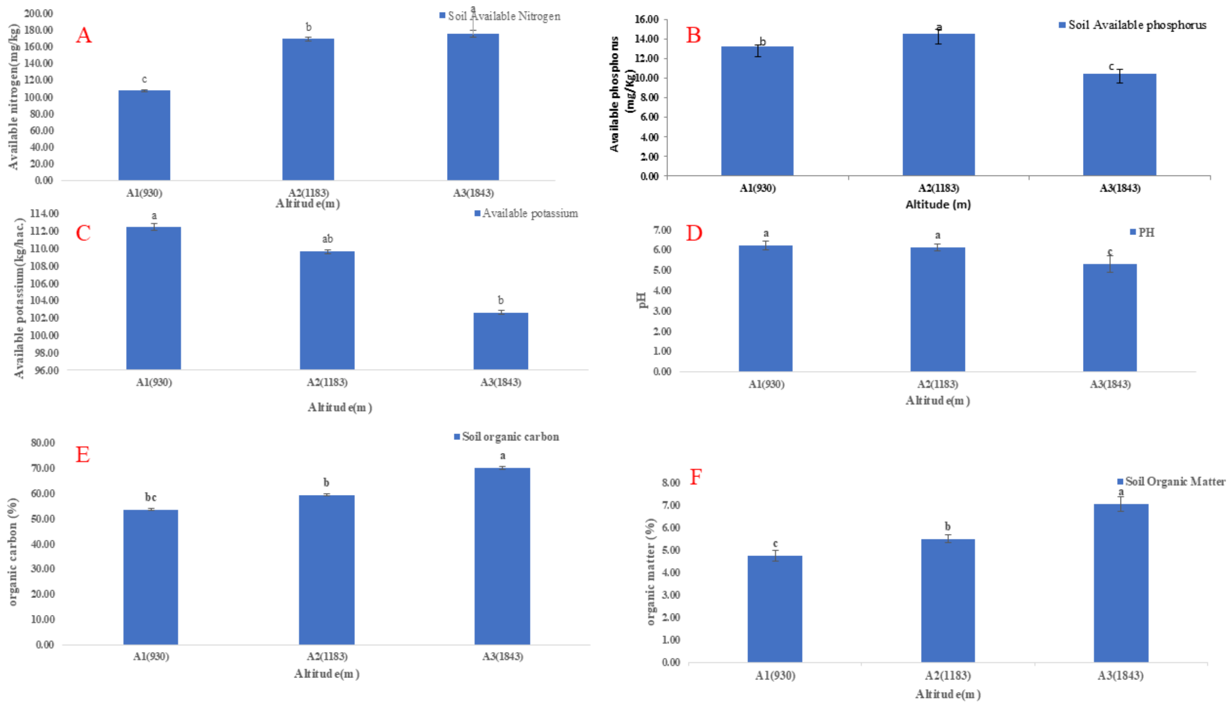

Fig. 5: Physio- chemical parameter of soil collected from three different altitudes. Data are means of three replicas/(n=3). *Means followed by the same letter in a column /bar are not significantly different by Duncan's multivariate test (DMRT).*

Similarly, nitrogen levels also rose with increasing altitude. Phosphorus and potassium availability in soil are governed by a combination of geological, biological, and climatic factors that do not change uniformly with altitude. This leads to localized variability without a clear or consistent trend. Instead, their availability at different altitudes is site-specific and depends on factors such as parent material, leaching, microbial activity, vegetation type, and soil development. (Kumar *et al.*, 2010; Rajput *et al.*, 2016; Singh *et al.*,2018; Tayir *et al.*, 2023).

3.4. Correlation analysis among chemical properties of soil with altitude

As altitude increases, organic carbon (OC) and organic matter (OM) content increase, but soil pH decreases. OC shows strong positive correlation (0.9962) and OM shows very strong positive correlation with altitude (0.9987) (Table 2). Whereas pH shows strong negative correlation with altitude (-0.9838). It is supported by the findings of Thakur and Bisht (2020). As pH decreases (soil becomes more acidic), altitude increases and organic matter, carbon, and nitrogen content increase. Acidic conditions may be linked to high-altitude environments. (Sheikh and Kumar 2010).

Table 2. Correlation coefficient among chemical properties of soil with altitude

	Altitude (m)	*N(Kg/ha)*	*P(Kg/ha)*	*K(KG/ha)*	*OC (%)*	*OM (%)*	*PH*
Altitude (m)	*1*						
N(Kg/ha)	*0.769665*	*1*					
P(Kg/ha)	*0.615027*	*-0.0300563*	*1*				
K(KG/ha)	*0.493271*	*0.9350238*	*-0.38253*	*1*			
OC (%)	*0.996162*	*0.8225924*	*0.54365*	*0.567515*	*1*		
OM (%)	*0.998672*	*0.8015402*	*0.573579*	*0.537438*	*0.999349*	*1*	
PH	*-0.98383*	*-0.6428529*	*-0.74632*	*-0.32947*	*-0.96437*	*-0.97329*	*1*

CONCLUSION

The growth, morphology, and physiology of *Potentilla fruticosa* show a significant relationship with altitude. These medicinal plants thrive best at the lower end of their distribution range, with their growth diminishing as elevation increases. The findings suggest that these species adapt to higher altitudes by exhibiting shorter heights, smaller leaves, and increased photosynthetic pigments to cope with the stressors associated with higher elevations. Variations in these traits across different altitudes likely reflect their response to a combination of abiotic and biotic selection pressures, creating distinct micro-environmental conditions. The reduction in growth forms and leaf area at higher elevations may help minimize water loss through transpiration, while the increase in photosynthetic pigments may be a strategy to adapt to lower carbon dioxide levels. These interconnected trait modifications at varying elevations are likely crucial for the local adaptation of *Potentilla fruticosa*.

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