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The elevational effect on soil parameters and biochemical constituents of *Rheum emodi* Wall. exMeisn: an important medicinal plant of Garhwal Himalaya.

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Abstract

In developing nations, the healthcare sector is mainly dependent on medicinal plants which are the major source of curative molecules to treat various diseases. Rheum emodi Wall. The therapeutic benefit of *Rheum emodi* exMeisn is well known in the Indian system of medicine, the modern pharmaceutical industry, and the local system of medicine, leading to excessive and illegal extraction and habitat loss. The species is now limited to a few pockets and faces extinction. Keeping this in view, there is needed to study the different sites of *R. emodi* to get more information to take up their conservational strategies. The present study deals with the variation observed in the number of phyto-constituents and soil NPK content in the plant *R. emodi* at two different altitudes; Baniyakund (2582 m) and Tungnath (3460 m) representing lower and higher altitudes respectively. The sample result reveals that total nitrogen content and total organic carbon content increase with altitude while available nitrogen content and potassium content decrease with increasing altitude. The result reveals that the amount of total phenolic content, and overall alkaloid content increase with increasing altitudes. To a certain level, this study gives proof that altitude is one of the environmental factors that can influence the number of phytochemicals.

Keywords: *R. emodi*, Ethnobotany, Phytochemistry, soil nutrient analysis, secondary metabolites

1. Introduction:

The vast Himalayan area is home to thousands of plants that are unquestionably beneficial to human health. Medicinal plants are plants and components that have therapeutic value or pharmacological effects on the human or animal body. These medicinal plants synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, and volatile oils that possess medicinal properties. Medicinal plants have assumed a fundamental part from the old time frame as they are utilized in customary medication and as conventional however egotistical human exercises lead to the destruction of such plants because of this a lot of plants become extinct from their regular environment and others come in endangered conditions. Environmental factor like altitudinal gradient keys affects the plant's growth, physiology, morphology, and biochemistry (Hultine and Marshall, 2000). A broad drift in the conditions like temperature, humidity, concentration, and exposure of the climatic gases have been observed and recorded in the environment due to the change in the elevational gradient in the alpine region. (Hovenden and Vander Schoor, 2004). Altitude is one such factor that has been reported to bring about changes in the number of phytoconstituents. Certain metabolites are only synthesized their contents significantly increase/decrease under specific environments (Dong et al., 2011). Furthermore, earlier reports have suggested that plants and herbs grown in diverse environmental altitudes produce variation in secondary metabolites resulting, in differences in their healing properties **(**Penuelas and Llusia, 1997).

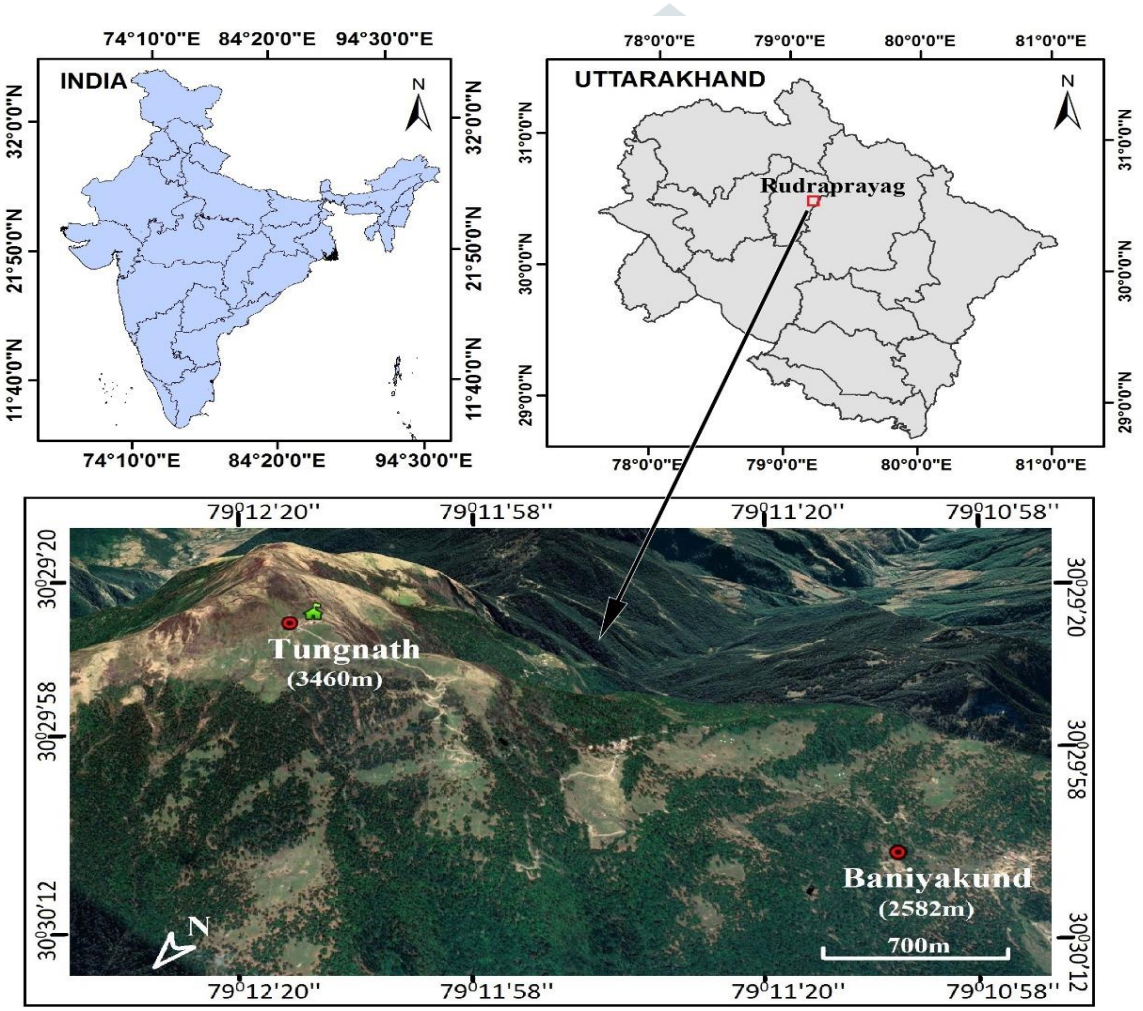
Bromley discovered a connection between height and soil chemistry (1995). Soil is the foundation upon which all constructions are built. Soil converts dead organic materials into various nutrient forms which are readily available to plants. It is a critical component of any ecosystem and carries a lot of weight. It is a medium for the growth and development of various micro, macro, and avian flora and fauna. The pH of the soil influences biological variables such as biomass composition and microbial activity. Changes in altitudinal gradients affect soil erosion, soil water balance, soil organic matter, and biomass output of indigenous vegetation and cultivated plants. (Tan et al., 2004). In developing nations, the healthcare sector is mainly dependent on medicinal plants which are the major source of curative molecules to treat various diseases. The Himalayas is considered the largest and most accessible source of the gathering of wild plants including wild fruits and medicines. Many of the plant species are confined to small areas and the size of the population of medicinally important species is declining at a dangerous rate (Nayar and Shastri 1987; 88; 89). *Rheum emodi* is one of the valuable medicinal plant species. It is the large group of the perennial stout herbs in the alpine and sub-alpine regions of the world, chiefly in Asia. It is a Perennial stout herb, 1.0-3.0m in height, between 2800 and 3600 meters above sea level, they may be found throughout the temperate and sub-tropical regions of the planet. Exceedingly strong roots; leaves radical, long petiole, very large, 30-40cm; flower small, dark purple or pale red in tall axilla panicles (Naithani, 1985). Emodin, Chrysophanol, Rhein, Rutin, and Chrysophenic are the active ingredients in rheum. *Rheum* is an anthraquinone purgative with a moderate effect. It is used as an astringent tonic; its stimulating effect combined with aperients properties render it especially useful in tonic dyspepsia ***(Chopra et al., 1958).***

The oil is also applied to ulcers to help them heal, and powdered roots are used to brush teeth. The paste produced from the root is traditionally combined with water and used to heal boils, wounds, and cuts in Garhwal (Maikhuri et al., 2000). The goal of this study was to see how elevation affected soil characteristics, as well as the quantity of biochemical and secondary metabolites in *R. emodi*.

2. Material and Methods

2.1. Study sites

Two study sites were selected from the Rudraprayag District of Uttarakhand i.e., Baniyakund (2582 m) located between latitude 30.4843º North; longitude 79.2170º East and Tungnath (3460 m) located between latitude 30.4887ºN; longitude 79.2170ºE according to the species natural distribution in Garhwal Himalaya (Fig. 1).



Picture 1: study site Tungnath and Baniyakund

2.2. Sample collection

Studied material was collected during June 2022 from both selected sites. The upper part of the plant was collected and marked properly. For soil analysis, to reduce mistakes, 6 composite soil samples were randomly taken from each location at a depth of 0-20cm and properly mixed. Thereafter, the samples were packed and carried out to the laboratory for future work.

2.3. Laboratory Analysis

The soil samples were collected, dried in the shade, and then crushed to pass through a 2 mm filter. A pH meter was used to determine the pH of the soil (Mclean, 1982). The available nitrogen in the soil was estimated using the alkaline permanganate method (Sahrawat and Burford, 1981). According to the approach given, the micro Kjeldahl method was employed to determine total nitrogen in the soil **(**AOAC, 1995). With the assistance of a Spectrophotometer the quantitative determination of phosphorus was measured (Olsen et al., 1954). Potassium contents in digested samples were determined by a flame photometer. The organic Carbon of soil was measured by the method given by Walkley and Black, 1934.

2.4. Preparation of *R. emodi* plant extract

The collected plant materials were cleaned with water to get rid of soil, dirt, and other adhering materials and cut into small pieces. The plant material then shadows dried for one month at room temperature. The dried samples were ground in the electric grinder to get a coarse powder. The dried and coarse plant materials were extracted with 100% Distilled water (100℃) and 100% methanol (62℃).

A rotary evaporator was used to evaporate the methanol and water, leaving a tiny quantity of extract (2-3 mL).

2.5 Quantitative Estimation

Estimation of Pigment Content: Arnon's formulae were used to estimate and compute the chlorophyll content in the fresh sample (1949).

$$\text{Chlorophyll a} = \frac{[9.78 \times A662] - [0.99 \times A644] \times \text{volume}}{1000 \times \text{fresh weight (gm)}}$$

$$\text{Chlorophyll b} = \frac{[21.14 \times A669] - [4.65 \times A662] \times \text{volume}}{1000 \times \text{fresh weight (gm)}}$$

Total chlorophyll = chlorophyll a + chlorophyll b

$$\text{Carotenoids} = \frac{[4.69 \times A440.5] - (\text{chl a} + \text{chl b}) \times 0.26 \times \text{volume}}{1000 \times \text{fresh weight (gm)}}$$

Under primary metabolite parameters, the soluble protein was estimated using the Bradford technique (1976), carbohydrates were estimated using the McCready et al (1950) method, and total amino acid was estimated using the Moore and Stein method (1954) from the fresh samples.

2.6 Estimation of secondary metabolites:

As per normal protocols, the extracts were submitted to qualitative analysis for phytoconstituents such as alkaloids, phenolics, tannins, and flavonoids. Total phenolic acid content was calculated using the Folin-Ciocalteu technique (Bahukhandi et al., 2018). The phenolic content was calculated using the Gallic acid standard curve and represented as Gallic acid equivalent. The absorbance was recorded at 750 nm in the UV spectrometer. Using conventional methods, the total flavonoid content of dry extracts was estimated by using the aluminium chloride colorimetric method (Chang et al., 2002). Total flavonoid content was calculated as quercetin equivalent. Quercetin was used as the standard and the absorbance was measured at 415 nm. The Folin-Ciocalteu technique was modified to determine the total tannin concentration **(**Makkar et al., 1993)**.** Total tannin content was calculated as milligram tannic acid equivalent and the absorbance was measured at 700 nm. The total alkaloid content was calculated using the technique proposed by Ajanal et al., 2020. The alkaloid content was obtained from the standard curve of Atropine and absorbance was measured at 470 nm in the UV Spectrophotometer.

2.7 Statistics analysis:

Multiple Analysis of Variance (MANOVA) and Karl-Pearson Correlation analysis was done by using IBM SPSS software (SPSS version 25) and R Studio software (version 3.6.2). MS Excel 2007 was also used for statistical analysis.

3. Results:

3.1 Soil parameter analysis:

The soil of both Tungnath and Baniyakund was found to be acidic. In Tungnath average soil pH was 5.67 whereas in Baniyakund total pH of the soil was 6.14 which was more inclined toward the neutral value of pH.

Significantly, the most accessible nitrogen was found in the soil of Baniyakund (538.94 kg ha-1-1), followed by Tungnath (225.62 kg ha-1-1), while the total nitrogen concentration was found to be highest in the soil of Tungnath (2.79 percent) (1.59 percent). Total potassium in soil was determined to be highest in Baniyakund (50.94 kg ha-1-1) and lowest in Tungnath (44.56 kg ha-1) Site Tungnath (0.22 percent) had the greatest organic carbon concentration, whereas site Tungnath had the lowest Baniyakund (0.18 percent).

3.2 Biochemical analysis:

Environmental factors strongly affect the metabolism and accumulation of phytoconstituents. Most plants control the quantity and quality of active components in response to their surroundings. Significant variations in the amounts of biochemical and active phytoconstituents in *R.emodi* were discovered in this investigation.

Total chlorophyll content was recorded highest at Tungnath with increasing quantities of chlorophyll a (1.29 mg/gm), chlorophyll b (1.63 mg/gm), and carotenoid (2.92 mg/gm) whereas, at Baniyakund, decreasing quantities of chlorophyll a (1.08 mg/gm), chlorophyll b (1.59 mg/gm), and carotenoids (1.59 mg/gm) were recorded. Tungnath had the greatest total chlorophyll content (2.92 mg/gm), whereas Baniyakund had the lowest (2.67 mg/gm).

Carbohydrate content was calculated in two parts: first for soluble protein, and then for total starch content. The total soluble sugar content increases as the elevation rise, but the overall sugar content decreases. Total soluble sugar was recorded highest at Tungnath (167.3 ± 7.03 mg/gm) whereas at Baniyakund it is recorded low (39± 12.12 mg/gm). The starch content with 46 maximum at Baniyakund (165±41.6 mg/gm) and the minimum value was recorded at Tungnath (88±19.92 mg/gm). The variation in the protein content of the leaf is negatively correlated with the altitude. The maximum value was recorded at Baniyakund (38 mg/gm) and the minimum value was recorded at Tungnath (14.62 mg/gm). Total free amino acid decreased with the increasing elevation. The highest value was recorded at Baniyakund (108.33 mg/gm) and the minimum at site Tungnath (101.66 mg/gm) **(Table 2)**.

Table 2: *R. emodi* biochemical components were studied quantitatively.

Sites	**Total chlorophyll content**	**Total carotenoid content**	**Total soluble protein content**	**Total soluble sugar content**	**Total starch content**	**total free amino acid content**	**Total phenolic content (fresh sample)**
Baniyakund	2.67 mg/gm	1.59±0.16 mg/gm	38±6.8 mg/gm	39±12.12 mg/gm	165.3± 41.6 mg/gm	108.33± 9.29 mg/gm	64.40±3.45 mg/gm
Tungnath	2.92 mg/gm	2.92±0.4 mg/gm	14.62±3.12 mg/gm	167.3±7.03 mg/gm	88±19.92 mg/gm	101.66± 5.15 mg/gm	88.29±9.69 mg/gm

According to Karl-Pearson correlation coefficients between different biochemical variables and altitude, carotenoid content ($r = -0.936$, $p<0.01$), soluble sugar ($r = -0.929$, $p<0.01$), and phenolic content from fresh sample ($r = -0.895$, $p<0.05$) were all negatively correlated with altitude, whereas protein ($r =0.936$, $p<0.01$) and starch ($r =0.823$, $p<0.05$) were positively correlated **(Table 4).**

3.3. Detection of secondary metabolites

Significant variations in the number of active components in the entire plant of *R. emodi* growing at two different elevations were discovered in this investigation. Total flavonoid content and total alkaloid content were greater in plants gathered at higher altitudes, whereas total tannin content and total flavonoid content were higher in plants collected at lower altitudes **(Table 3).**

Table 3: Quantitative analysis of secondary metabolite in the aqueous and methanolic extract of *R. emodi* at both elevations.

Sites	**TPC (mg GAE/g)**	**TFC (mg QEC/g)**	**TTC (mg TAC/g)**	**TAC (mg ATC/g)**

	Aqu	Met	Aqu	Met	Aqu	Met	Aqu	Met
Baniyakund	41.7±1.4	56.47±1.5	6.24±0.27	4.18±0.30	5.07±0.32	17.41±1.6	78.99±14.3	45.55±4.4
Tungnath	68.44±7.5	76.06±3.08	5.02±0.33	4.6±0.1	5.90±0.40	13.24±0.9	95.77±4.2	56.88±4.5

Aq: aqueous; Met: methanol

Karl-Pearson correlation coefficients among secondary metabolites and altitude indicated that aqueous extract ($r = -0.946$, $p<0.01$), and methanolic extract (($r = -0.980$, $p<0.01$) of total phenolic content, and aqueous extract of total alkaloid content ($r = -0.841$, $p<0.05$) were negatively correlated with altitude however aqueous extract of total tannin content ($r = 0.974$, $p<0.01$) and methanolic extract of total tannin content ($r = 0.882$, $p<0.05$) were positively correlated with altitude **(Table 4)**

	Altitude	chl a	chl b	carotenoid	protein	soluble sugar	starch	AA	PC	TPC (AQ)	TPC (M)	TFC (AQ)	TFC (M)	TTC (AQ)	TTC (M)	TAC (AQ)	TAC (M)
Altitude	1																
Chl a	-0.615	1															
Chl b	-0.371	0.436	1														
Carotenoid	-.936**	0.751	0.219	1													
Protein	.937**	-0.673	-0.317	-.920**	1												
Soluble sugar	-.929**	0.783	0.229	.998**	-.916*	1											
Starch	.823*	-0.675	0.001	-.845*	.831*	-.859*	1										
AA	0.478	-0.487	-0.740	-0.486	0.360	-0.479	0.021	1									
PC	-.895*	.856*	0.554	.876*	-.833*	.892*	-0.799	-0.568	1								
TPC(Aq)	-.949**	0.798	0.338	.978**	-.893*	.983**	-.860*	-0.525	.952**	1							
TPC(M)	-.980**	0.487	0.292	.870*	-.901*	.859*	-.833*	-0.340	.831*	.888*	1						
TFC(Aq)	.930**	-0.624	-0.131	-.979**	.863*	-.970**	0.809	0.474	-.814*	-.950**	-.883*	1					
TFC(M)	-0.708	0.249	-0.173	0.618	-0.743	0.613	-.857*	0.279	0.501	0.595	0.800	-0.621	1				
TTC(Aq)	.974**	-0.717	-0.386	-.960**	.982**	-.956**	.822*	0.492	-.899*	-.952**	-.924**	.917*	-0.671	1			
TTC(M)	.882*	-0.517	-0.604	-0.810	.821*	-0.792	0.478	0.773	-0.775	-0.808	-.815*	0.804	-0.362	.881*	1		
TAC(Aq)	-.841*	0.209	0.158	0.674	-0.647	0.659	-0.697	-0.226	0.659	0.722	.913*	-0.756	0.731	-0.701	-0.646	1	
TAC(M)	-0.697	0.225	0.695	0.533	-0.538	0.508	-0.176	-.812*	0.583	0.570	0.659	-0.573	0.133	-0.631	-.907*	0.626	1

Table 1: Correlation between different primary metabolites, secondary metabolites, and altitudes of *R. emodi*.

***significant at 0.05 level.**

****significant at 0.01 level.**

AA: amino acid; PC: phenolic content; TPC: total phenolic content; TFC: total flavonoid content; TTC: total tannic content; TAC: total alkaloid content; Aq: aqueous; M: methanol

4. Discussion

Soil has a close relationship with geomorphology and the vegetational type of the area. However, fluctuation in soil characteristics even within the same geomorphic location has also been reported. At the alpine sites of *R.emodi*, the soil was acidic. Common soil at high altitudes is known to be acidic (Körner C. 2003).

Soil mineralogical information is important for soil fertility evaluation. The major source of soil nitrogen is organic materials. Alpine plants are often adapted to low nitrogen content and it has long been admitted that chilly regions are inadequate in nutrients (Bliss 1971, 1985). Available nitrogen was recorded as highest at Baniyakund and lowest at Tungnath. Total nitrogen was recorded highest at Tungnath and minimum at Baniykund. Similar trends were recorded for phosphorus content in all the populations of both species. Phosphorus content was observed at maximum at Tungnath and minimum at Baniyakund it was supported by the research done by Panthi, 2010. The determination of the type of vegetation of the area or plant growth in a particular region may be due to the low amount of phosphorus in the soil. Phosphorus permits the plant to grow in a specific geographical location (Smeek, 1972). Due to decreasing temperature, respiration rates decreases in the higher altitude which leads to the enhancement of carbon and nitrogen in the soil (Vieira et al. 2011). Dar and Sundarapandian 2015 also mentioned that altitude is one of the main factor that influences the soil organic carbon.

The current study was designed with the idea that changing altitude might influence the quantity of various biochemical and phytochemicals contained in *R. emodi*, as well as their activity. Plants that thrive in a variety of environments experience varying degrees of stress, so evolve a defense mechanism by collecting a variety of secondary metabolites to alleviate unpleasant circumstances. Plants that thrive in a variety of environments are subjected to varying degrees of stress, so they evolve a defense mechanism by collecting diverse secondary metabolites to alleviate unpleasant circumstances.

The overall chlorophyll concentration rose as the altitude climbed. In both species, the concentrations of chlorophyll a and chlorophyll b rose when the altitude was raised. Carotenoid content seemed to increase with altitude. This may be due to the reasons reported by Simmkin et al., (2003) that light is necessary for carotenoid synthesis. Hence, the increase in the amount of carotenoid with increasing altitude may be associated with the protective function of the pigment in the scavenging of free radicals and dissipation of excess energy.

The amount of soluble sugar in *R.emodi* increases as the elevation rises. An increasing trend in the soluble sugar is observed along with the altitude as populations at high altitudes need to cope with persistent freezing temperatures so that they accumulate higher soluble sugar providing resistance to this low temperature (Nagele and Hayar 2013). Amino acid levels in *R. emodi* rise as elevation rises. The increase in total amino acid content along the altitudinal gradient is a common phenomenon of plant species at high altitudes, as these have a significant impact on dealing with various stresses as the accumulation of free amino acid and proline plays an important role in osmoregulation (Dedemo et al, 2013). In *R.emodi* dependent variable like leaf protein was not found significantly correlated with altitude. It means that some other factors other than altitude are responsible for these variations like the climatic variables that bring the changes at the microhabitat level

of the species by altering the available soil nutrient content **(**Kuniyal *et al.*, 2002) and photoassimilate investment pattern **(**Korner,1989).

The total phenolic content of fresh leaves, as well as extract, rose with altitude, according to the current study. In comparison to the methanolic extract of *R. emodi* in Baniyakund, the methanolic extract of *R. emodi* at Tungnath had the greatest TPC. This increase in phenolic content with an increase in altitude may be attributed to a response to plants to enhance UV- B radiation and decreased temperatures which elicit amplified biosynthesis of UV- absorbing and antioxidant phenolics in plants (Winkler 2011). In *R.emodi* TFC decreased with the increasing elevation. This result was supported by the study done by *Flippi et al.*, (2020). They observed that elevation was the main driver of *V. myrtillus* growth, having both direct and indirect effects on the leaf flavonoid content. In their observation, they noted that at higher elevation flavonoid content decrease, and at lower elevation it increases. In *R. emodi* total alkaloid content decreased with the increasing elevation. There is convincing evidence of increased herbivore pressures at lower altitudes; hence, a rise in alkaloid content may be considered an evident defense strategy chosen by plants at lower elevations. (Carey *et al.* 1994**,** Jugran *et al.*, 2016). Overall tannin content increases with the increasing elevation maybe avoid damage during unfavorable temperature conditions by adapting to the frost-resisting cells (Salaj and Karmutak, 1995). Upadhye et al., (2006**)** observed that increased tannin content in plants in the condition of the water stress may be due to the aftermath of low temperature.

5. Conclusion

The species *R.emodi* was taken into consideration across the specified location (Baniyakund and Tungnath) of Garhwal Himalaya and showed considerable variation in the soil parameters, and biochemical characteristics (primary and secondary metabolites). The results of this study show that the altitudinal shift has a substantial impact on several Physico-chemical properties of soil. Secondary metabolites like phenolics, tannins, flavonoids, alkaloids, and volatile oils are biosynthesized in plants. These secondary metabolites affect many physiological activities of plants. The presence of secondary metabolites enhanced the therapeutic properties of medicinal plants. It takes a lot of phytochemical research to identify pharmacologically active chemicals in medicinal plants. *Rheum* species is the source of secondary metabolites like phenolic, alkaloids, tannins, and flavonoids. Secondary metabolites enhance medicinal plants' therapeutic qualities, such as antibacterial, antifungal, anticancer, antiviral, and anti-inflammatory effects. For the manufacturing of new drug discovery, isolation, purification, and screening of phytochemically active compounds are required. The phytochemical analysis of medicinal plants is also important for research or commercial purpose and the manufacturing of new drugs. The *Rheum* species has the potential to increase the financial resources of people living in the Himalayan region; therefore, programs related to the breeding, planting, and conservation of *Rheum* species need to be started in the Himalayan region.

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7. Conflicts Of Interest

Writers do not have any conflict of interest to declare.

8. Authors' contribution

JT was involved in the study design, data collection, manuscript preparation, and manuscript writing and investigation. VS contributed to the statistical analysis, writing review, editing, and reviewing.

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