Ecological Vulnerability and Phytochemical Richness of Rheum australe in the Indian Himalayan Region: Insights for Conservation

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Abstract

The Indian Himalayan Region, a globally recognised biodiversity hotspot, supports over 10,000 plant species, including a rich repository of medicinal flora (over 1,700 medicinal plants) integral to ecosystem services and traditional healthcare. Among them, *Rheum australe*, a high-value medicinal species currently listed as "Data Deficient" on the IUCN Red List, facing escalating threats from overharvesting, climate change, and habitat fragmentation. This study assessed the population status, phytochemical diversity, and habitat suitability to inform targeted conservation strategies. Extensive field surveys were conducted across altitudinal gradients (3200-4200 m amsl) in Uttarakhand using a stratified random sampling. Population data revealed an aggregated distribution pattern, with significant site-wise variation in population density (0.73–1.82 ind./m^2) and relative density (0.93-2.48%). A clear increase in population density was observed at higher altitudes, supporting higher density, particularly in rocky crevice habitats. Phytochemical analysis showed notable site-specific variations in total phenolic (2.35-12.70 mg GAE/g DW), flavonoid (6.13-15.30 mg QE/g DW), tannin (1.18-10.60 mg TAE/g DW), and antioxidant activity (DPPH: 2.51- 6.67 mM AAE/100g DW; FRAP: 1.31 - 3.73 mM AAE/100g DW). HPLC profiling identified gallic acid as a predominant polyphenolic compound. A significant positive correlation (p<0.01) between altitude and phytochemical parameters suggested, high-altitude stressors, such as UV radiation and temperature extremes, on secondary metabolite accumulation. Habitat suitability modelling, performed using R Studio software, identified a high habitat probability zone (0.7–0.9) above 3000 m asl, particularly in Uttarakhand, Jammu & Kashmir, and Himachal Pradesh. These findings highlight both ecological vulnerability and phytochemical richness of *R. australe*, emphasising the urgent need for integrated conservation. In situ conservation should prioritise habitat protection and regulated harvesting, while ex-situ strategies are essential for safeguarding genetic resources.

Keywords: Conservation, Habitat Modelling, Medicinal plant, Population density, Phytochemical

Abbreviations:

IHR: Indian Himalayan Region, MPs: Medicinal Plants, CAMP: Conservation Assessment and Management Prioritizations, IUCN: International Union for Conservation of Nature, SDM: Species distribution modelling, IVI: Importance Value Index, TPC: Total Phenolic Content, TFC: Total Flavonoid Content, TTC: Total Tannin Content, DPPH: 2,2-Diphenyl-1-picrylhydrazyl; DW: Dry Weight; FRAP: Ferric Reducing Antioxidant Power, HPLC: High Performance Liquid Chromatography, GLM: Generalized Linear Models, SVM: Support Vector Machines, RF: Random Forest, DMRT: Duncan's multiple range test, AUC: Area under the curve, TSS: True Skill Statistic, ANOVA: Analysis of Variance

1 Introduction

The Indian Himalayan region (IHR), known as a global biodiversity 'hotspot', harbours distinctive plant diversity due to a range of micro-climatic conditions. Medicinal plants are a vital component of biodiversity in IHR, recognized for their active constituents and traditional healthcare systems, due to their therapeutic properties and widespread acceptance, largely attributed to their perceived minimal side effects (Tiwari et al. 2023). However, rising demand, unsustainable harvesting practices, and habitat degradation have severely impacted the natural populations of several medicinal plants. India ranks as the world's second-largest exporter of MPs after China, contributing approximately 13% of the global exports, with a rapidly growing pharmaceutical market (Bhat et al. 2018). Climate change, species invasions, overgrazing and trampling, habitat fragmentation, and improper use of natural resources further exacerbate the threat to these species (Wudu et al. 2023). A considerable amount (approximately 90%) of medicinal plant raw materials is still harvested from the wild, which places several species in threatened, endangered, and rare categories, thereby requiring urgent attention from conservationists for the long-term viability of their genetic pool.

Rheum australe D. Don (synonym: *R. emodi*), commonly known as 'Revand-chini' is a high-value medicinal herb of the family Polygonaceae (Kumar et al. 2023). Traditionally used in the treatment of various disorders, including stomach disorders, muscular swelling, tonsillitis, mumps, and wound healing, the species also has industrial applications in dyeing due to its antimicrobial properties (Khan et al., 2012; Kumar et al., 2025). *R. australe* is spread across the IHR, extending from Jammu & Kashmir to Arunachal Pradesh in the east, with a higher concentration dispersed in the western Himalaya and typically inhabits the alpine zone between 2,800 m and 4,500 m asl, especially in rocky terrain, moraines, and streamside slopes (Hooker et al. 1897; Kumar et al. 2025). It prefers moist, well-drained, heavy (loam) to medium (clay) texture soil and typically favours fully sunny to semi-sunny locations (Pandith et al. 2018). Due to illegal trade for commercial extraction and habitat degradation, the species was categorized as "Vulnerable" by CAMP (Ved et al. 2003), and more recently as 'Data Deficient' on the IUCN Red List due to limited ecological data (Chauhan, 2023).

The widening gap between growing demand and limited supply has positioned *R. australe* among the most extensively exploited in the IHR (Singh et al. 2021; Kumar et al. 2025). Its populations are highly fragmented and often restricted to specific microhabitats. Despite its ecological and pharmacological importance, comprehensive studies on its population ecology, phytochemical variation, and habitat modelling are lacking. While a few preliminary studies have analysed phytochemical profiling (Aslam et al. 2012; Gupta et al. 2014), systematic investigations across altitude gradients and habitats remain absent. This knowledge gap hinders the identification of genetically and chemically superior populations for sustainable utilization and cultivation.

Species distribution models (SDMs) have been extensively used in conservation biology to predict the current and potential geographical range of species based on environmental variables (Wani et al. 2021; Kumar et al. 2024; Chahuan et al. 2025). They enable the prediction areas with favorable environmental conditions for a species, including regions where the species is not currently reported, referred to as its fundamental niche or potential distribution niche (Gong et al. 2023). Additionally, SDMs quantify the relationship between environmental factors and species distribution (Schwager and Berg 2021). Therefore, ensemble modelling in SDMs is gaining traction due to its effectiveness in assessing the effects of climate change on plant species, identifying vulnerable areas, and highlighting regions that require immediate conservation efforts (Sharma et al. 2023). Moreover, SDMs have been instrumental in guiding field surveys, evaluating climate change effects, and enhancing conservation planning (Gebrewahid et al. 2020).

The above-mentioned context highlights the urgent need to assess population status, phytochemical diversity and potential distribution of this poorly studied species. This study focuses on Uttarakhand, a key stronghold for *R. australe* in the western Himalaya, due to its rich topographic and altitudinal diversity. Accordingly, the aims of the current investigation are: (i) to characterize the ecological status and distribution pattern of *Rheum australe* in Uttarakhand, (ii) to evaluate the phytochemical potential of *R. australe*, and (iii) to identify potential distribution areas of *R. australe* using the SDM tool, with implications for conservation strategies.

2 Materials and methods

2.1 Target area, field sampling, and plant material collection

The proposed research was conducted in the Indian state of Uttarakhand (28°43ˈ to 31°8ˈN latitude and 77°35ˈto 81°2ˈE longitude), part of the northwest Himalayan biodiversity hotspot. Potential sites for *Rheum australe* were selected through an extensive literature review, herbarium records from BSI (Botanical Survey of India), Dehradun, regional flora (Naithani 1985; Gaur 1999), and an online database (GBIF 2022). Following secondary data analysis, extensive field surveys were undertaken from 2021 to 2024 across thirteen (13) representatives' geographical regions of Uttarakhand, western Himalaya (Fig. 1). These sites were chosen to encompass variation in altitude (3200-4200 m asl), habitat types (Moist grassy slope, Alpine rocky slope near streams, Open rocky slope with boulder and Rocky cervices) and geographical distribution, allowing for a comprehensive ecological assessment. Site characteristics, including altitude, Latitude, longitude, and habitat of each population, were noted using a Global Positioning System (GPS) device (Garmin eTrex 30xs, United States), as presented in Table S1 for reproducibility. The plant specimens of *R. australe* were initially authenticated by Dr. K. C. Sekar, an alpine plant taxonomist at the Govind Ballabh Pant National Institute of Himalayan Environment. An herbarium voucher number (1417) was assigned to the species, and the specimen was submitted to the BSI, Dehradun herbarium.

2.2 Ecological status and distribution patterns

A phytosociological analysis was conducted using a random quadrate sampling method. At each site, three 20 × 20-meter plots were randomly established. Within each plot, twenty 1 x 1-meter quadrats were laid out for vegetation data collection. Given the patchy and restricted distribution of *R. australe,* sampling was confined to areas with confirmed occurrence. Quantitative parameters, such as frequency, density, and abundance, were calculated using regular methodologies by Curtis and McIntosh (1950) and Misra (1968). The species distribution pattern was evaluated through the abundance-frequency (A/F) ratio, wherein an A/F value of less than 0.025 indicates regular dispersal, values between 0.026 to 0.050 suggest random distribution, and values higher than 0.050 signify a contiguous/aggregated distribution pattern (Kershaw, 1973). Further, for identification of the community structure and importance of the target species in that area, the importance value index (IVI) was calculated (Singh et al. 2021). Habitat type, dominant associated species, or other active threats at the site were also recorded (Fig. 2). The following formulas were used to calculate diverse ecological parameters investigated in the present investigation.

$$\text{Density (D)} = \frac{\text{Total number of individuals of a species in all the quadrats}}{\text{Total number of quadrats studied}}$$

$$\text{Frequency (F\%)} = \frac{\text{Total number of quadrats in which the species occurs}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Abundance (A)} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats in which the species occured}}$$

$$\text{Relative Density (RD)} = \frac{\text{Total number of individuals of a species in all the quadrats}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Relative Frequency (RF)} = \frac{\text{Number of occurrence of the species}}{\text{Numberof occurrence of all the species}} \times 100$$

$$\text{Relative Abundance (RA)} = \frac{\text{Abundance of species}}{\text{Total abundance}} \times 100$$

Importance Value Index (IVI) = RD + RF+ RA

2.3 Phytochemical assessment

Extract preparation

Aerial and root tissue of target species (n=5 individuals per site) were gathered and rinsed thoroughly with double-distilled water, and dried in a hot-air oven at 40°C. The dried powdered material was then sieved through a <150 μM mesh and stored at -20°C in airtight containers for subsequent analyses. For the preparation of methanolic extraction, 1 g of powdered sample was mixed with 50 milliliters of 80% (v/v) methanol, and the suspension was stirred at 120 rpm using a magnetic stirrer for 24 h. The extract was passed using filter paper (Whatman No. 1), and the resulting supernatant was subsequently collected and preserved at 4ºC for further analytical procedures.

Estimation of total phenol, flavonoids, and tannin content

TPC (total phenol content), TFC (total flavonoid content), and TTC (total tannin content) were determined following an established protocol with slight modification (Jugran et al. 2016; Kumar et al. 2024). For phytochemical quantification, standard calibration curves were prepared. TPC was quantified using gallic acid, quercetin for TFC, and tannic acid for TTC, all dissolved in methanol (80% v/v). The result was reported as milligrams of gallic acid equivalent per gram dry weight (mg GAE/g DW), milligrams of quercetin equivalent per gram dry weight (mg QE/g DW), and milligrams of tannic acid equivalent per gram dry weight (mg TAE/g DW).

Antioxidant activity

Antioxidant activity was assessed using two antioxidant methods, like DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method and FRAP (Ferric Reducing Antioxidant Power) method, using standard protocols (Cai et al. 2004). Ascorbic acid served as the calibration standard, and the antioxidant activity of the sample was evaluated as millimoles of ascorbic acid equivalents per 100 grams of dry weight (mM AAE/100g DW).

High-pressure liquid chromatography (HPLC) assessment

HPLC assessment of the samples was conducted following the protocol of Joshi et al. (2023) with minor alterations. The methanolic extract of samples was filtered by using a nylon acrodisc filter (0.22 μM) earlier than the injected into the HPLC instrument (Thermo Scientific Ultimate 3000, USA). Phenolic constituents were separated by a reversed-phase chromatography setup equipped with a photodiode array (PDA) detector, and operated by Chromeleon 7 software. Separation of individual constituents was achieved on a Plus C18 column (5-μm particle size, 4.6 x 250 mm) of Agilent Eclipse at 30°C under isocratic conditions, with a 20 μL injection volume. The mobile phase comprised a 30:70 proportion of acetonitrile (solvent A) and 0.1% formic acid in water (solvent B), delivered at a constant flow rate (1 mL/min) throughout a 35 min run time. To quantify phenolic constituents, standard solutions of gallic acid ($C_7H_6O_5$), naringin ($C_{27}H_{32}O_{14}$), catechin ($C_{15}H_{14}O_6$), ascorbic acid ($C_6H_8O_6$), (-)-epigallocatechin gallate (EGCG) ($C_{22}H_{11}O_{11}$), and phloridzin ($C_{21}H_{24}O_{10}$) were prepared in 80% methanol. Chromatographic detection was carried out for salicylic acid, gallic acid, and naringin at 220 nm; for catechin at 280 nm; and for *p*-coumaric acid at 310 nm. R^2 values for all standard calibration curves exceeded 0.998, ensuring quantification accuracy. Compound concentrations were quantified in mg/g DW (milligrams per gram of dry weight), using a calibration curve generated from standard solutions.

2.4 Habitat distribution modelling

Occurrence data of *R. australe* were obtained from field surveys and secondary sources (GBIF and peer-reviewed literature). All data entries were carefully reviewed to ensure they met the quality standard appropriate for species distribution modelling (SDM) (Kumar et al. 2024). Records lacking precise geographic coordinates or containing duplicates were excluded. After validation, a total of 48 unique and accurate occurrence points were retained for modelling purposes and saved in a CSV file, structured by plant species name, longitude, and latitude. To predict the probable dispersal of the species, 19 bioclimatic variables encompassing precipitation and temperature metrics were acquired from the WorldClim database (http://www.worldclim.org). These climate layers represent interpolated monthly averages for the period 1950-2000, derived from global meteorological station data and mapped at a spatial resolution of 30 arc seconds (~1 km^2) (Kumar et al. 2024). Since high collinearity among predictor variables can negatively affect model performance, Pearson correlation analysis was conducted. Variables with correlation coefficients exceeding >0.8 were excluded to minimize redundancy. The curated occurrence data and selected environment variables were processed in RStudio using various packages, including SSDM, ShinyFiles, Sp, raster, and rJava (Araújo and New 2007). An ensemble modelling approach was employed, integrating three widely used machine learning algorithms known for robust predictive accuracy, such as Generalized Linear Model (GLM) (McCullagh 2019), Random Forest (RF) (Breiman 2001), and Support Vector Machine (SVM) (Cortes and Vapnik 1995). The data was partitioned into training (70%) and testing (30%) subsets to assess the influence of environmental predictors on habitat suitability. Model performance was assessed using the Area under the curve (AUC) and True Skill Statistic (TSS) of the receiver operating characteristic curve. Only models with TSS values greater than 0.8 and AUC scores exceeding 0.95 were retained for final analysis (Chauhan et al. 2025).

2.5 Data analysis

Significant differences among the measured ecological parameters were analysed using one-way ANOVA, followed by DMRT (Duncan's multiple range test) for post hoc comparison, using a statistical software (SPSS version 22, USA). The chord diagram was utilized to visualize and examine the associations between plant species and the sampling sites of *R. australe*. It allowed us to assess the assessment of species richness present at each location and determine the relative diversity of the sites based on the thickness of the connecting bars. This visualization of data was generated using the "circlize" package in R software (version 4.3.0). The phytochemical analysis of the sampling sites was expressed as the mean value ± standard error (SE). To evaluate remarkable differences among the phytochemical attributes, one-way ANOVA was performed, by DMRT for post hoc analysis. Additionally, phytochemical variability across altitudinal zones and habitat types, as well as the relationships among studied parameters, were assessed using Pearson correlation coefficient (r) analysis. A correlogram illustrating the correlations was constructed in the 'CORRPLOT' package using R Studio (version 4.3).

3 Results

3.1 Population status and Ecological assessment

A total of thirteen sites were identified, ranging from 3200 to 4200 m amsl, encompassing four dominant microhabitat conditions. The detailed site-specific characteristics of *R. australe*, including dominant associated species and perceived threats, are provided in (Table S2)**.** The diversity and species richness of *R. australe* indicated significant differences ($p < 0.05$) across different sites. Maximum density and relative density were observed at Kuari pass site (1.82 ind/m^2 and 2.48%, respectively), while minimum density (0.73 ind/m^2) and relative density were recorded at Maiktoli site (0.93%). The maximum frequency was recorded in the Kuari Pass (60.00%) and Kafni sites (60.00%), followed by the Dayara site (58.33%), while it was the minimum in the Maiktoli site (35.00%). Likewise, maximum relative frequency (8.68%) was observed in the Kedarnath population (8.68%), while it was minimum in the Maiktoli population (3.79%). A significantly ($p<0.05$) higher abundance (3.02%) of the species was found in the Bedni population, followed by Kuari Pass (2.97%) sites, and minimum abundance was recorded in the Maiktoli population (1.75%) (Table 1).

In the majority of the study sites (9 Sites), *R. australe* had an aggregated distribution pattern (69.23%), while the Dayara, Furkiya, Kafni, and Kedarnath sites exhibited a random distribution (30.77%). Further, the highest (12.93) IVI of the species was obtained from the Dayara sites (12.93) and the minimum (6.48) from the Maiktoli site (Table 1). *Danthonia cachemyriana* Jaub. & Spach, *Cassiope fastigiate* (Wall.) D.Don, *Plantago major* var. asiatica, *Geranium wallichianum* D.Don ex Sweet, *Bistorta affinis* D. Don*, Felicia rosulate* Yeo, *Potentilla atrosanguinea* Raf., *Anemone tetrasepala* (Royle) Holub, and *Potentilla acuminata* H.M.Hall were identified as major dominant associated species of *R. australe* (Fig. S1; Table S2). A few major threats, like habitat degradation and fragmentation, unsustainable harvesting practices, anthropogenic disturbance, and grazing pressure, were consistently observed across all the studied sites. In addition, the high ethnomedicinal value of *R. australe* makes overharvesting a significant threat to its survival in alpine meadows.

3.2 Population Status of *R. australe* across different altitudinal zones and Habitat types

The population status of *R. australe* across four altitudinal zones revealed slight differences among the studied ecological parameters (Table S3). The maximum population frequency (57.50%), RF (8.68%), and IVI (12.29) were reported at 3951-4200 m amsl, while the maximum density (1.23 ind/m^2), abundance (2.34), and RD value (1.87%) of *R. australe* were recorded between 3451 and 3700 m asl. In contrast, a decreasing trend in population relative density and abundance was observed with increasing altitudinal zones (Fig. S2).

Among the four examined habitats (moist grassy slopes, alpine rocky slopes near streams, rocky crevices, and open rocky slopes with boulders), rocky crevices documented the highest plant density (1.43 ind/m^2), abundance (2.75), and relative abundance (2.63%) for *R. australe* (Fig.S2; Table S3). The maximum frequency (53.33%), RD (2.12%), and RF (6.74%) of *R. australe* were observed in alpine rocky slope near stream habitat types, while minimum density (1.01 ind/m^2) and abundance (1.92) were recorded from similar habitats. However, minimum relative density (1.61%), relative frequency (5.53%), relative abundance (2.16%), and IVI (9.12) were observed in open rocky slopes with boulder habitat type.

3.3 Phytochemical assessment

The Total phenol content (TPC) exhibited statistically significant (p<0.05) variations in aerial and root parts across the thirteen studied sites of *R. australe* (Table 2). A higher TPC was observed in the aerial part of the Kedarnath population (9.71 ± 0.031 mg GAE/g DW) and root part of Baluni top population (12.71 ± 0.03 mg GAE/g DW). However, the lower TPC level was observed in the aerial part (2.35 ± 0.07 mg GAE/g DW) of Dayara and the root (3.78 ± 0.01 mg GAE/g DW) of Himtoli populations. Considerable variations (p<0.05) in TFC were detected in aerial and root parts of *R. australe* populations. TFC was higher recorded in aerial (10.11 ± 0.01 mg QE/g DW) and root part (15.26 ± 0.01 mg QE/g DW) of Baluni top and Kafni populations and lower in aerial (6.13 ± 0.010 mg QE/g DW) and root part (10.17 ± 0.010 mg QE/g DW) of Bedni and Himtoli populations, correspondingly. Similarly, TTC significantly varied (p < 0.05) between the plant parts of *R. australe* populations. Higher TTC was recorded in aerial (5.65 ± 0.26 mg TAE/g DW) and root part (9.32 ± 0.01 mg TAE/g DW) of Baluni top population, while a lower TTC was found in aerial part (1.65 ± 0.02 mg TAE/g

DW) of Tungnath and root part (1.85 ± 0.05 mg TAE/g DW) of Himtoli populations.

A remarkable (p<0.05) variation in antioxidant activities, as detected using DPPH and FRAP assays, was observed in aerial and root parts of *R. australe* across the thirteen sites (Table 2). Kedarnath population exhibited significantly maximum (6.48 ± 0.03 mM AAE/100g DW) DPPH activity in the aerial parts, followed by Baluni top population (5.33 ± 0.06 mM AAE/100g DW), while the minimum (2.51 ± 0.02 mM AAE/100g DW) was recorded in Maiktoli population. The root part of Baluni top population recorded maximum (6.67 ± 0.02 mM AAE/100g DW) DPPH activity, while minimum (4.15 ± 0.06 mM AAE/100g DW) was observed in Himtoli population. The FRAP assay demonstrated significant (p<0.05) variations between the aerial and root parts across *R. australe* populations. Notably, the Rudranath population exhibited the maximum (3.63 ± 0.01 mM AAE/100g DW) activity, and the Bedni population showed the minimum (1.31 ± 0.02 mM AAE/100g DW) activity in aerial parts. In root, Kuari Pass population exhibited the maximum (3.73 ± 0.01 mM AAE/100g DW) antioxidant property, while Bedni population showed the minimum value (1.98 ± 0.010 mM AAE/100g DW), then the other studied sites of *R. australe*.

High-performance liquid chromatography (HPLC) analysis revealed significant (p<0.05) variations in polyphenolic content among the studied populations and between aerial and root parts (Fig. 3). Different compounds were measured separately through HPLC in both aerial and root parts of the populations (Table S4). In aerial part significantly (p<0.05) higher gallic acid (1.51 ± 0.12 mg/g DW) and naringin (0.45 ± 0.01 mg/g DW) content were recorded in Baluni top population, while ascorbic acid (2.28 ± 0.021 mg/g DW), E-GCG (0.45 ± 0.01 mg/g DW), phloridzin (0.75 ± 0.12 mg/g DW) and catechin (0.70 ± 0.02 mg/g DW) was recorded higher in Kedarnath site as compared to other sites. However, the lowest ascorbic acid (0.13 ± 0.01 mg/g DW), phloridzin (0.11 ± 0.01 mg/g DW), and catechin (0.13 ± 0.01 mg/g DW) were recorded from the Dayara population. Among different populations, root samples showed considerable variation (p< 0.05) in gallic acid (1.54 ± 0.03 mg/g DW), phloridzin (0.89 ± 0.03 mg/g DW), and catechin (1.64 ± 0.02 mg/g DW) in Baluni top population, while naringin (0.67 ± 0.04 mg/g DW) and E-GCG (1.18 ± 0.03 mg/g DW) was recoreded higher in Kedarnath population. However, the lowest amounts of gallic acid (0.32 ± 0.04 mg/g DW), naringin (0.28 ± 0.03 mg/g DW), and catechin (0.70 ± 0.02 mg/g DW) were found in the Tungnath population. E-GCG (0.27 ± 0.011 mg/g DW) and phloridzin (0.23 ± 0.02 mg/g DW) content were detected at the lowest value in the Dayara population. The chromatogram of gallic acid of the active bioactive compounds is presented.

3.4 Variation in phytochemical content across altitudinal zones and habitat types

Phytochemical diversity across the altitudinal zones showed higher variation in *R. australe* (Table S5). In the aerial part maximum amount of TPC (9.71 ± 0.02 mg GAE/g DW), DPPH (6.48 ± 0.010 mM AAE/100g DW), FRAP (3.56 ± 0.01 mM AAE/100g DW), AA (2.28 ± 0.021 mg/g DW), E-GCG (0.45 ± 0.011 mg/g DW), PD (0.75 ± 0.12 mg/g DW) and CC (0.70 ± 0.02 mg/g DW) was recorded highest between the elevation 3951 and 4200 m asl, while highest TFC (10.11 ± 0.011 mg QE/g DW), TTC (5.65 ± 0.26 mg TAE/g DW), GA (1.51 ± 0.12 mg/g DW) and NG (0.45 ± 0.01 mg/g DW) was recorded at 3701-3950 m asl. Similarly, in root part, maximum TPC (12.71 ± 0.02 mg GAE/g DW), TTC (9.32 ± 0.01 mg TAE/g DW), DPPH (6.67 ± 0.01 mM AAE/100g DW), GA (1.54 ± 0.03 mg/g DW), PD (0.89 ± 0.03 mg/g DW), and CC (1.64 ± 0.02 mg/g DW) was found between in the altitudinal zone of 3701-3950 m asl. However, minimum phytochemical content was detected at 3200 and 3450 m asl in both parts (aerial and root). In this case, no positive trends were observed in total phenol content or antioxidant activity across the increasing altitudinal zones.

Phytochemical attributes in aerial and root parts of *R. australe* showed variations across the different habitat types (Table S6). In the aerial parts, highest TPC (5.85 ± 1.62 mg GAE/g DW), TFC (8.33 ± 0.81 mg QE/g DW), TTC (3.56 ± 0.95 mg TAE/g DW), DPPH (4.64 ± 0.78 mM AAE/100g DW), GA (0.86 ± 0.31 mg/g DW), NG (0.34 ± 0.04 mg/g DW), PG (0.42 ± 0.13 mg/g DW), and CC (0.54 ± 0.06 mg/g DW) was observed in moist grassy slope habitat, while highest FRAP (3.06 ± 0.20 mM AAE/100g DW) and AA (1.66 ± 0.29 mg/g DW) were found in open rocky slope with boulder habitat. However, the lowest TPC (3.28 ± 0.75 mg GAE/g DW), TFC (6.99 ± 0.60 mg QE/g DW), TTC (1.70 ± 0.25 mg TAE/g DW), DPPH (3.26 ± 0.18 mM AAE/100g DW), FRAP (1.98 ± 0.40 mM AAE/100g DW) and NG (0.22 ± 0.02 mg/g DW) were observed in alpine rocky slope near stream habitat. Similarly, in root part, TPC (7.86 ± 2.17 mg GAE/g DW) and PD (0.68 ± 0.12 mg/g DW) were recorded highest in moist grassy slope habitat and TFC (14.28 ± 0.09 mg QE/g DW), DPPH (6.19 ± 0.19 mM AAE/100g DW), FRAP (3.73 ± 0.01 mM AAE/100g DW), GA (1.09 ± 0.01 mg/g DW), AA (1.19 ± 0.07 mg/g DW), and E-GCG (0.90 ± 0.02 mg/g DW) was reported highest in rocky crevices habitat. Moreover, the alpine rocky slope near stream habitat type exhibited the lowest polyphenolic content in the root part.

3.5 Relationship between altitude, ecological, and phytochemical parameters

While analysing a Pearson's correlation coefficient analysis among different studied parameters, no significant relationship was observed between altitude and ecological attributes (Fig. 4). Species density exhibited a significantly positive ($p<0.05$) relation with abundance (r = 0.903), RD (r = 0.777), and RA (r = 0.770). While frequency exhibited a negative, significant relationship with abundance (r = -0.581) and a positive relationship with RA (r = 0.748). Similarly, abundance exhibited a positive and significant relationship with RD (r = 0.713) and RA (r = 0.875) and a negative relationship with RF (r = -0.553). IVI demonstrated a significant positive relationship with RF (r = 0.764; $p<0.05$). Similarly, Pearson's correlation coefficient analysis among phytochemical attributes and altitude displayed a significant ($p<0.01$) relationship between the studied parameters of *R. australe*. However, there was no significant correlation observed between population density (DEN) and phytochemical attributes (Fig. 4).

3.6 Habitat distribution model performance

The current distribution range of *R. australe* within the Indian Himalayan Region was projected using an Ensemble Species Distribution Model (Fig. 5). The model established strong predictive accuracy, with area AUC values >0.90, indicating a high level of reliability in forecasting suitable habitats for species. The individual and ensemble model presentations were assessed through multiple metrics. The TSS of the model achieved a satisfactory value (≥ 0.55), confirming its robustness, except for CART (TSS < 0.51). The relative importance of environmental factors was assessed using jackknife test, which identified Bio15 (precipitation seasonality), Bio19 (precipitation of the driest quarter), and Bio8 (mean temperature of the wettest quarter) as the most influential bioclimatic variables, contributing 38%, 30%, and 10% respectively, accounting for a cumulative contribution of 78%. The correlation coefficients (*r*) among the environmental predictors remained below the threshold of 0.8, minimizing multicollinearity. Bio15 and Bio19 were recognized as the principal drivers of *R. australe* present distribution. The final habitat suitability map highlighted areas with high to very high probability (0.7 to 0.90) of species occurrence in the sub-alpine and alpine zones (mainly >3000m asl), particularly across the western Himalaya (J&K, HP, and UK). Conversely, low suitability (0.01 to 0.5) was noted in the eastern Himalayan regions. The pronounced discrepancy in documented occurrence data and modelled distribution map suggests that the species' potential habitat range is considerably broader than its actual distribution range, indicating a capacity for the species to expand and occupy these suitable but uncolonized areas.

4 Discussion

The population status and distribution of endemic Himalayan plants remain poorly understood due to harsh climates, geographical remoteness, and limited accessibility (Shaheen et al. 2011; Ahmed et al. 2020; Singh et al. 2021). In this study, reconnaissance surveys and herbarium records documented *R. australe* across habitats between 3200–4200 m asl. Mean density (0.73–1.82 ind/m²) was lower than earlier reports from Uttarakhand (1.93–4.24 ind/m²) (Rawat et al. 2016) and Himachal Pradesh (0.5–5.3 ind/m²) (Uniyal and Shiva, 2005; Singh et al. 2005). Discrepancies likely reflect differences in sampling design, habitat accessibility, and survey timing, with earlier work often focused on accessible or species-rich plots, possibly overestimating density.

Low densities across sites suggest pressures from overharvesting, poor seed germination, weak regeneration, and competition (Negi et al. 2018; Singh et al. 2021). Comparable values were reported in Niti Valley (0.4–0.8 ind/m²; Kandari et al. 2011) and Jammu & Kashmir (0.1–1.23 ind/m²; Wani et al. 2021), pointing to a fragmented and declining population structure. Frequency ranged from 35% (Baluni Top) to 60% (Kuari Pass, Kafni), consistent with 10–80% across other regions (Kandari et al. 2011; Wani et al. 2021). However, high frequency does not ensure dominance, as seen in Maiktoli (density 0.73 ind/m²; IVI 6.48). Higher IVI at Dayara, Kafni, Kedarnath, and Kuari Pass highlights these as important populations. Similar studies from Nepal recorded densities of 0.67–4.06 ind/m² (Ghimire et al. 1999; Bhattarai et al. 2014; Singh et al. 2021). Lack of data from Afghanistan, Pakistan, and Tibet limits broader biogeographic insights, underscoring the need for standardized monitoring across the species' range.

Across altitudes and habitats, *R. australe* predominantly showed aggregated distribution (69.23%), with random distribution (30.77%) only in uniform environments, confirming patchy growth (Odum and Barrett 1971). Aggregation suggests microhabitat specificity, particularly rocky crevices, consistent with Kandari et al. (2011) and Wani et al. (2021). Such patterns are common in nature, shaped by subtle environmental variations (Odum and Barrett 1971; Kershaw 1973; Suyal et al. 2019; Singh et al. 2021). Our findings confirm preference for rocky habitats over open boulder slopes (Uniyal et al. 2002; Kandari et al. 2011; Bisht et al. 2015; Wani et al. 2021). Alpine species often show narrow habitat specificity, rendering them vulnerable to natural and anthropogenic stressors (Shaheen et al. 2011; Singh et al. 2021). Past ecological assessments often emphasized density alone, neglecting habitat quality, regeneration, or biotic interactions. Yet ecological status varies along gradients due to vegetation composition, altitude, soil, microbial activity, and other biotic–abiotic factors (Bargali et al. 2018; Manral et al. 2020; Pandey et al. 2024). Mountain ecosystems exhibit sharp microclimatic and edaphic heterogeneity (Bäumler et al. 2015; Fartyal et al. 2025), influencing vegetation cover, litter decomposition, nutrient cycling, and overall ecosystem function (Manral et al. 2022; Karki et al. 2022; Pandey et al. 2023).

Plants serve as major sources of secondary metabolites vital for medicine, food, and pharmaceuticals (Tiwari et al. 2023). Active compounds such as phenolics, flavonoids, tannins, anthocyanins, and vitamins provide health benefits with minimal side effects (Jugran et al. 2016; Joshi et al. 2022). Enriched extracts act as antioxidants and antimicrobials with drug discovery potential (Ndezo et al. 2022). Phenolics, for example, are induced by environmental cues like light, cold, and pollution, aiding plant defense and growth (Valentines et al. 2005). Flavonoids accumulate under high radiation and cold stress (Tattini et al. 2005). Environmental conditions thus shape antioxidant build-up and stress adaptation. Alonso-Amelot et al. (2007) showed phenolic content rose in sun-exposed plants with altitude. Our study revealed marked variation in phytochemicals between aerial and root parts of *R. australe* across populations, habitats, and altitudes. Roots consistently accumulated higher TPC, TFC, TTC, DPPH, FRAP, and polyphenolics. Similar part-specific differences have been documented in Himalayan medicinal plants (Jugran et al. 2016; Joshi et al. 2022, 2023). For example, *Platanthera edgeworthii* roots contained high flavonoids (3.051 mg QE/g DW) and tannins (2.241 mg TAE/g DW) (Rawat et al. 2013), while *Astavarga* plants showed comparable TPC, TFC, TTC, and antioxidant activity (Giri et al. 2017; Suyal et al. 2019). Similar patterns are observed in *V. jatamansi* (Jugran et al. 2015), *R. procera* (Rawat et al. 2018), *P. verticillatum* (Suyal et al. 2020), and *H. spicatum* (Rawat et al. 2020).

HPLC analysis confirmed high polyphenolic content (gallic acid, ascorbic acid, E-GCG, naringin, phloridzin, catechin) in Kedarnath (4115 m) and Baluni Top (3794 m). These findings align with Joshi et al. (2022), who reported increased gallic acid at higher Himalayan elevations. Altitude significantly influences dispersal and gene flow (Singh et al. 2021; Negi et al. 2023; Fartyal et al. 2024). Environmental conditions, including soil and climate, enhance antioxidant capacity, supporting stress tolerance (Musial et al. 2020). Conversely, lower antioxidant levels at Tungnath (3259 m) and Bedni (3325 m) contrast with Bahukhandi et al. (2019), who reported uniformly low values. Thus, Kedarnath and Baluni Top are promising sources for phenolic extraction. These compounds enhance nutritional security and stress resilience, echoing reports on western Himalayan herbs (Jugran et al. 2016; Joshi et al. 2023; Kumar et al. 2024).

Correlation analyses revealed that altitude significantly influenced density and phytochemicals. Density showed a curvilinear trend, with greater species diversity between 3451–3700 m asl (Singh et al. 2021), while diversity declined at higher altitudes due to harsh conditions and short growing seasons (Ahmed et al. 2020). Phytochemical content positively correlated with altitude, with optimal accumulation between 3700–4200 m asl. Similar altitudinal increases in phenolics and antioxidant activity have been reported in alpine species (Suyal et al. 2019; Singh et al. 2021; Joshi et al. 2022; Kumar et al. 2024). Such variation reflects genetic, habitat, and location-specific effects (Marrelli et al. 2014). The low frequency and sparse distribution observed suggest adaptation to specific microhabitats (Uniyal and Shiva, 2005; Kandari et al. 2011; Singh et al. 2021), a pattern also noted in other endangered Himalayan medicinal plants (Suyal et al. 2020; Mehta et al. 2020). Ecological and evolutionary constraints shape phytochemical diversity, with altitude, habitat, and microclimate key predictors (Suyal et al. 2020; Kumar et al. 2024). Rising altitude intensifies UV radiation and environmental stress (Zlatev et al. 2012; Kumar et al. 2024), triggering structural and functional adjustments that enhance phenolic production. These contrasts must be integrated into conservation and sustainable use planning.

Species distribution modelling (SDM) is critical for projecting responses of Himalayan endemics to climate change, given their restricted ranges and habitat specificity (Wani et al. 2021; Rawat et al. 2025; Chahuan et al. 2025). Ensemble modelling predicted high habitat suitability (0.7–0.9) for *R. australe* in sub-alpine and alpine zones above 3000 m in Jammu & Kashmir, Himachal Pradesh, and Uttarakhand, but low suitability (0.01–0.5) in the eastern Himalaya. Temperature-dependent variables were key, with Bio19 (precipitation of coldest quarter) and Bio15 (precipitation seasonality) major determinants. These results align with earlier work showing precipitation–temperature interactions govern Himalayan flora distributions (Rawat et al. 2022; Kumar et al. 2024). Biological traits, including physiological and phenological characteristics, also influence climate responses. Species with narrow ranges and specialized habitats face greater risks than generalists (Abolmaali et al. 2018). It is important to recognize that local-scale factors, including habitat fragmentation, grazing, biotic interactions, and illegal harvesting, could further influence the actual distribution and persistence of *R. australe*.

5 Conclusion

Overall, the findings highlight that R. australe populations in the western Himalaya are fragmented, sparsely distributed, and ecologically constrained to rocky alpine habitats. Low densities, combined with poor regeneration and anthropogenic harvesting, underscore the vulnerability of natural populations. Phytochemical analyses revealed higher concentrations of phenolics and antioxidants in roots and in high-altitude populations, suggesting altitude-driven adaptive responses that enhance medicinal value but also increase harvesting pressure on limited habitats. Correlation analyses confirmed altitude as a critical driver of both ecological and phytochemical variation. Furthermore, SDM predictions identified potential habitat "hotspots" in Jammu & Kashmir, Himachal Pradesh, and Uttarakhand, where conservation efforts should be prioritized. Integrating population monitoring, habitat restoration, and community-based sustainable harvesting practices is essential to prevent further decline. Targeted cultivation in identified suitable habitats, along with the promotion of phytochemical-rich populations, could balance conservation with medicinal utilization. Collectively, the ecological, phytochemical, and modelling evidence provides a scientific basis for formulating site-specific management and conservation strategies for *R. australe* in the Indian Himalayan Region.

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Authorship contributions:

A.K. contributed to sample & data collection, laboratory experiment, data analysis & writing - original draft preparation. N.T. contributed to species modelling, data analysis & writing the draft. A.K.J. Conceptualized, methodology, Writing- original draft, review & editing & funding acquisition. S.S.B. and I.D.B. contributed to the Writing- original draft. review & editing.

Declaration of competing interests

The authors declare that they have no conflict of interest. All authors have read, understood, and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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Data availability

Data will be made available on request

Reference

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Tables

Table 1: Population status and distribution pattern of *R. australe* species in different studied sites in Uttarakhand, Western Himalaya

Sites	D (ind/m^2)	RD (%)	F (%)	RF (%)	A	RA (%)	IVI	A/F ratio
S1	$1.25^{ab} \pm 0.40$	$1.80^{ab} \pm 0.53$	$48.33^{abc} \pm 8.83$	$7.27^{abc} \pm 1.51$	$2.46^{bc} \pm 0.35$	$2.24^{abcd} \pm 0.25$	$11.31^{ab} \pm 2.28$	0.052
S2	$1.22^{ab} \pm 0.31$	$2.28^{a} \pm 0.62$	$45.00^{bc} \pm 7.65$	$6.33^{abcd} \pm 1.09$	$2.62^{ab} \pm 0.27$	$3.02^{a} \pm 0.39$	$11.63^{ab} \pm 1.89$	0.060
S3	$0.83^{b} \pm 0.12$	$1.37^{ab} \pm 0.21$	$40.00^{bc} \pm 2.89$	$4.92^{bcd} \pm 0.37$	$2.19^{bcd} \pm 0.15$	$2.49^{abcd} \pm 0.18$	$8.78^{abc} \pm 0.76$	0.052
S4	$0.95^{b} \pm 0.15$	$2.45^{a} \pm 0.17$	$58.33^{ab} \pm 6.02$	$7.66^{ab} \pm 0.52$	$1.62^{de} \pm 0.03$	$2.79^{abc} \pm 0.18$	$12.93^{a} \pm 0.63$	0.026
S5	$0.73^{b} \pm 0.15$	$0.93^{b} \pm 0.16$	$35.00^{c} \pm 2.89$	$3.79^{d} \pm 0.17$	$2.06^{bcd} \pm 0.24$	$1.75^{e} \pm 0.18$	$6.48^{c} \pm 0.50$	0.059
S6	$0.87^{b} \pm 0.17$	$1.60^{ab} \pm 0.35$	$56.67^{ab} \pm 4.41$	$6.22^{abcd} \pm 0.49$	$1.51^{e} \pm 0.18$	$2.01^{cd} \pm 0.29$	$9.83^{abc} \pm 1.04$	0.026
S7	$1.03^{b} \pm 0.22$	$1.67^{ab} \pm 0.42$	$43.33^{bc} \pm 6.02$	$6.67^{abc} \pm 1.01$	$2.34^{bc} \pm 0.18$	$2.29^{abcd} \pm 0.22$	$10.63^{abc} \pm 1.64$	0.055
S8	$1.30^{ab} \pm 0.25$	$1.71^{ab} \pm 0.29$	$50.00^{abc} \pm 8.17$	$4.85^{cd} \pm 0.55$	$2.65^{ab} \pm 0.18$	$2.35^{abcd} \pm 0.11$	$08.92^{abc} \pm 0.93$	0.056
S9	$1.82^{a} \pm 0.30$	$2.48^{a} \pm 0.38$	$60.00^{ab} \pm 4.09$	$6.76^{abc} \pm 0.37$	$3.16^{a} \pm 0.30$	$2.97^{ab} \pm 0.25$	$12.17^{ab} \pm 0.98$	0.056
S10	$0.98^{b} \pm 0.12$	$1.65^{ab} \pm 0.24$	$45.00^{bc} \pm 0.00$	$5.04^{bcd} \pm 0.64$	$2.34^{bc} \pm 0.12$	$2.46^{abcd} \pm 0.28$	$08.41^{bc} \pm 0.91$	0.057
S11	$1.40^{ab} \pm 0.30$	$2.13^{ab} \pm 0.49$	$60.00^{a} \pm 8.17$	$8.43^{a} \pm 1.09$	$2.05^{a} \pm 0.23$	$2.09^{bcd} \pm 0.35$	$12.66^{ab} \pm 1.84$	0.031
S12	$0.92^{b} \pm 0.08$	$1.78^{ab} \pm 0.22$	$37.50^{bc} \pm 2.04$	$5.67^{bcd} \pm 0.66$	$2.29^{bcd} \pm 0.11$	$2.86^{abc} \pm 0.43$	$10.30^{abc} \pm 0.86$	0.058
S13	$1.08^{ab} \pm 0.14$	$1.65^{ab} \pm 0.22$	$57.50^{ab} \pm 0.22$	$8.68^{a} \pm 1.05$	$1.85^{cde} \pm 0.11$	$1.97^{cd} \pm 0.10$	$12.29^{ab} \pm 1.25$	0.033
Mean ± SD	1.11 ± 0.21	1.81 ± 0.33	48.97 ± 5.49	6.33 ±0.73	2.24 ± 0.19	2.41 ±0.25	10.49 ± 1.19	0.050

Note: All data are reported as the mean of three replicates (each replicate consists of 20 quadrates) ± standard error (SE). SE with different letters (a-d) indicates significant differences (p<0.05) in the column according to Duncan's multiple range test (DMRT). D- density (ind/m^2); F- Frequency A- abundance; RD- relative density; RF- relative frequency; RA- relative abundance; IVI- importance value index

Table 2: Variation in phytochemical content and antioxidant activities in the aerial and root parts of *R. australe* populations in Uttarakhand, Western Himalaya

S. No.	Populations	Plant parts									
		Aerial part					Root part				
		TPC	TFC	TTC	DPPH	FRAP	TPC	TFC	TTC	DPPH	FRAP
1.	S1	$2.65^{j} \pm 0.02$	$6.65^{g} \pm 0.01$	$1.65^{hi} \pm 0.02$	$3.06^{g} \pm 0.01$	$1.32^{l} \pm 0.01$	$4.65^{i} \pm 0.02$	$10.4^{k} \pm 0.01$	$2.41^{i} \pm 0.02$	$4.21^{k} \pm 0.01$	$2.12^{h} \pm 0.02$
2.	S2	$2.73^{j} \pm 0.03$	$6.13^{h} \pm 0.01$	$1.99^{g} \pm 0.04$	$3.09^{g} \pm 0.01$	$1.31^{l} \pm 0.02$	$4.06^{k} \pm 0.02$	$11.1^{j} \pm 0.01$	$2.48^{i} \pm 0.01$	$4.19^{l} \pm 0.01$	$1.98^{i} \pm 0.01$
3.	S3	$3.78^{h} \pm 0.01$	$7.30^{ef} \pm 0.01$	$2.31^{f} \pm 0.06$	$3.69^{e} \pm 0.06$	$2.04^{j} \pm 0.02$	$3.78^{l} \pm 0.01$	$10.2^{l} \pm 0.01$	$1.85^{j} \pm 0.05$	$4.15^{l} \pm 0.01$	$2.59^{g} \pm 0.03$
4.	S4	$2.35^{k} \pm 0.07$	$6.67^{g} \pm 0.01$	$1.91^{gh} \pm 0.06$	$3.07^{g} \pm 0.03$	$1.95^{k} \pm 0.01$	$4.35^{j} \pm 0.07$	$12.1^{i} \pm 0.01$	$2.38^{i} \pm 0.01$	$4.68^{j} \pm 0.01$	$2.62^{g} \pm 0.01$
5.	S5	$3.51^{i} \pm 0.04$	$7.12^{f} \pm 0.01$	$1.59^{i} \pm 0.07$	$2.51^{h} \pm 0.02$	$2.83^{g} \pm 0.01$	$4.86^{h} \pm 0.01$	$13.2^{g} \pm 0.01$	$3.29^{h} \pm 0.01$	$4.92^{i} \pm 0.02$	$2.97^{f} \pm 0.01$
6.	S6	$4.77^{f} \pm 0.04$	$8.16^{d} \pm 0.01$	$1.18^{j} \pm 0.01$	$3.62^{f} \pm 0.02$	$2.67^{h} \pm 0.01$	$5.77^{g} \pm 0.04$	$12.3^{h} \pm 0.01$	$4.62^{f} \pm 0.04$	$5.26^{h} \pm 0.01$	$3.29^{c} \pm 0.01$
7.	S7	$4.19^{g} \pm 0.01$	$7.11^{f} \pm 0.01$	$2.76^{d} \pm 0.06$	$4.23^{d} \pm 0.01$	$3.63^{a} \pm 0.01$	$6.19^{f} \pm 0.01$	$14.2^{c} \pm 0.01$	$5.66^{e} \pm 0.02$	$5.99^{d} \pm 0.02$	$3.72^{a} \pm 0.03$
8.	S8	$5.54^{e} \pm 0.01$	$9.15^{b} \pm 0.01$	$3.62^{c} \pm 0.06$	$4.78^{c} \pm 0.01$	$2.90^{f} \pm 0.01$	$6.54^{e} \pm 0.01$	$13.4^{f} \pm 0.01$	$5.77^{e} \pm 0.01$	$5.81^{f} \pm 0.01$	$3.29^{c} \pm 0.02$
9.	S9	$4.85^{f} \pm 0.01$	$7.20^{ef} \pm 0.04$	$2.37^{ef} \pm 0.04$	$4.20^{d} \pm 0.01$	$2.47^{i} \pm 0.07$	$5.85^{g} \pm 0.01$	$14.4^{b} \pm 0.01$	$4.38^{g} \pm 0.01$	$6.38^{c} \pm 0.01$	$3.73^{a} \pm 0.01$
10.	S10	$5.79^{d} \pm 0.03$	$8.63^{c} \pm 0.26$	$2.62^{de} \pm 0.05$	$3.65^{ef} \pm 0.01$	$3.04^{e} \pm 0.03$	$7.79^{d} \pm 0.03$	$14.2^{d} \pm 0.01$	$10.6^{a} \pm 0.03$	$5.86^{e} \pm 0.01$	$3.08^{e} \pm 0.03$
11.	S11	$6.39^{c} \pm 0.02$	$7.38^{e} \pm 0.02$	$1.87^{ghi} \pm 0.08$	$4.18^{d} \pm 0.01$	$3.50^{c} \pm 0.01$	$8.39^{c} \pm 0.02$	$15.3^{a} \pm 0.01$	$7.83^{c} \pm 0.04$	$6.61^{b} \pm 0.01$	$3.58^{b} \pm 0.03$
12.	S12	$7.29^{b} \pm 0.03$	$10.1^{a} \pm 0.01$	$5.65^{a} \pm 0.26$	$5.33^{b} \pm 0.01$	$3.15^{d} \pm 0.01$	$12.7^{a} \pm 0.03$	$13.4^{e} \pm 0.01$	$9.32^{b} \pm 0.01$	$6.67^{a} \pm 0.02$	$3.22^{d} \pm 0.01$
13.	S13	$9.71^{a} \pm 0.03$	$9.25^{b} \pm 0.01$	4.63 ± 0.12^{b}	$6.48^{a} \pm 0.01$	$3.56^{b} \pm 0.01$	$10.3^{b} \pm 0.03$	$14.2^{c} \pm 0.02$	$6.61^{d} \pm 0.28$	$5.77^{g} \pm 0.01$	$3.16^{d} \pm 0.03$
	Mean ± SD	4.89 ± 0.58	7.76 ± 0.33	2.62 ± 0.36	3.99 ± 0.30	2.64 ± 0.22	6.55± 0.73	12.9 ± 0.44	5.17 ± 0.78	5.42 ± 0.26	3.02 ± 0.15

Note: Alt- altitude; TPC- Total phenol content (mg GAE/g DW); TFC- Total flavonoid content (mg QE/g DW); TTC- Total tannin content (mg TAE/g DW); DPPH- 2, 2-Diphenyl-1-picryhydrazyl assay (mM AAE/100g DW); FRAP- Ferric reducing antioxidant power assay (mM AAE/100g DW). Values are means ± standard error (SE); Within columns, SE followed by different superscript letters (a-k) indicates significant differences ($p<0.05$) using One-way ANOVA Duncan multiple range test (DMRT).

Caption of Figures

Fig. 1. Geographical distribution map of 13 sampled sites of *Rheum australe* in Uttarakhand, Western Himalaya

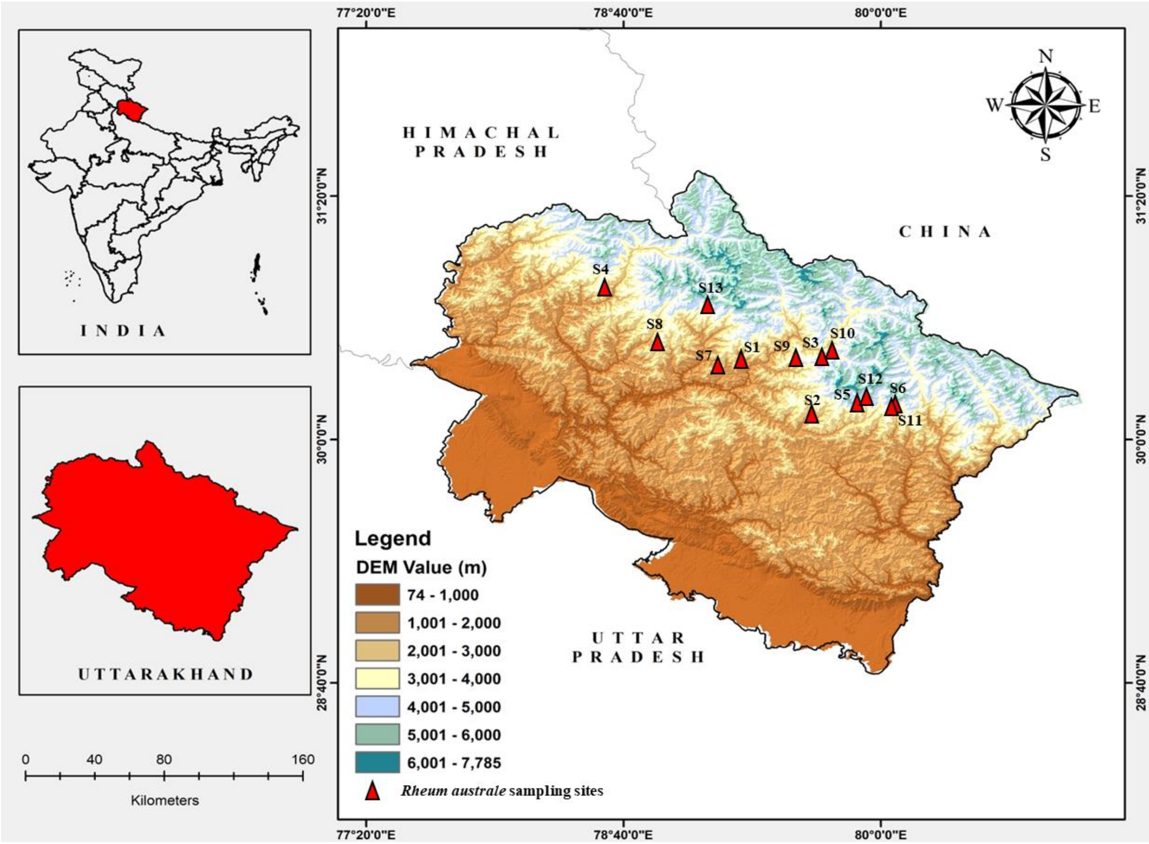


Fig. 2. Different representative habitat types of *R. australe* (a, b) Overview of Rheum australe habitat; (c) Phytosociological data collection and (d, e) Sample collection and identification.

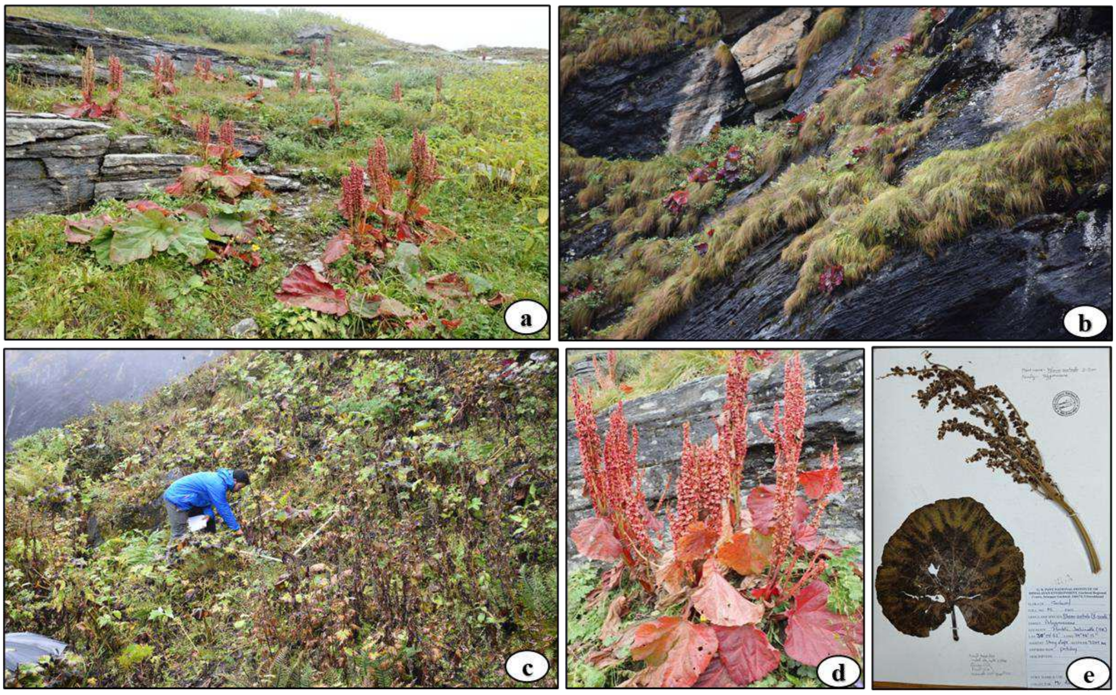


Fig. 3. High-performance liquid chromatography (HPLC) analysis (a) Chromatogram of standard, aerial and root parts from Tungnath site (b,c,d,f,g) polyphenolic content in *R. australe* species from different sites.

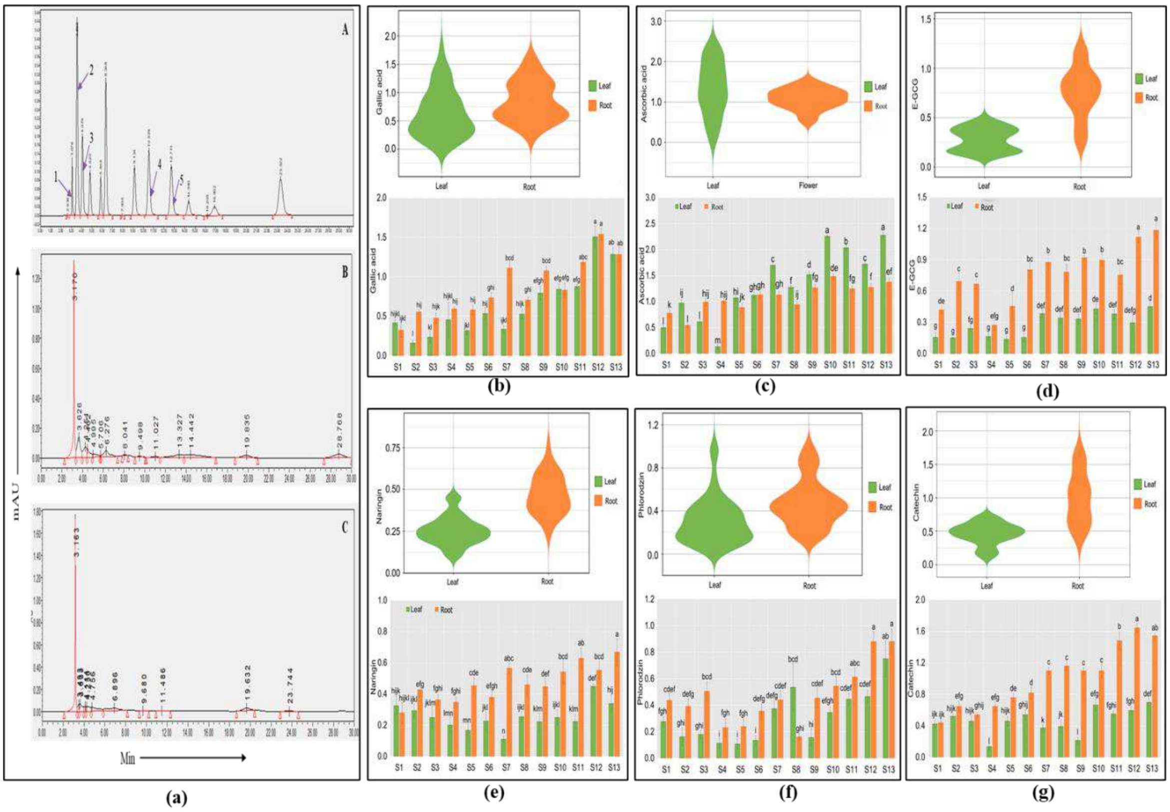


Fig. 4. Correlation analysis showing the relationship between all studied parameters of *R. australe*. Alt- - altitude, DEN- Density TPC-total phenol content, TFC-total flavonoid content, TTC-total tannin content, DPPH-2,2-diphenyl-1-picrylhydrazyl, FRAP-Ferric reducing antioxidant power, GA-gallic acid, AA-ascorbic acid, E-GCGA-epigallocatechin gallate, NG-naringin, PD- phloridzin, CC- Catechin

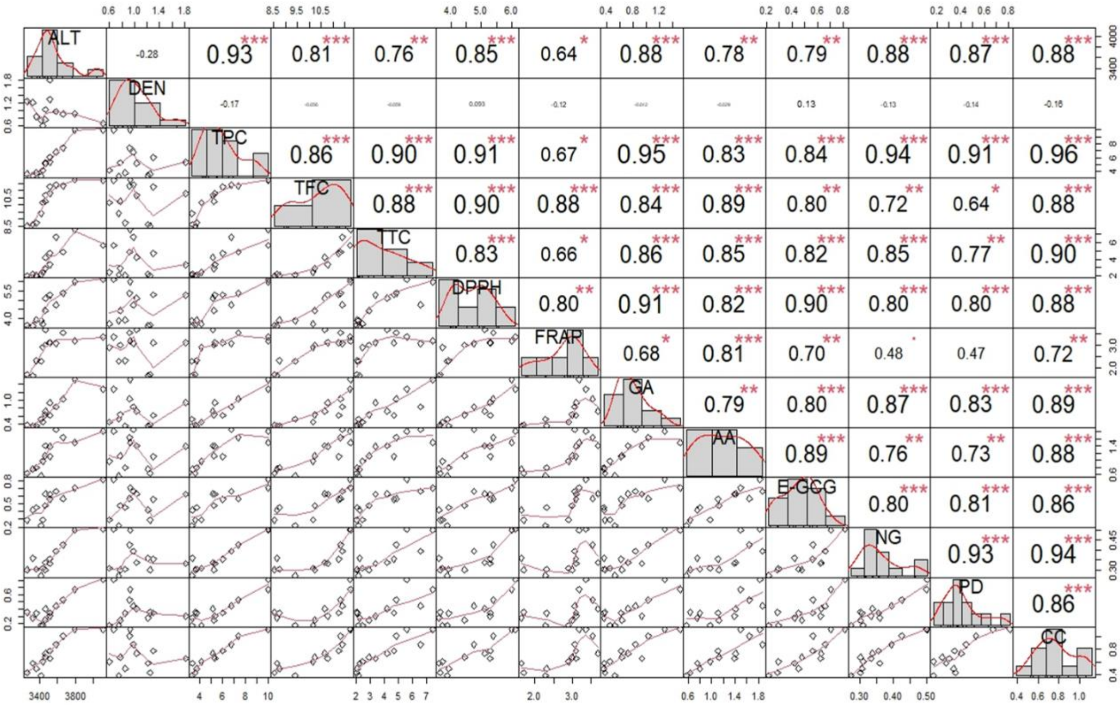
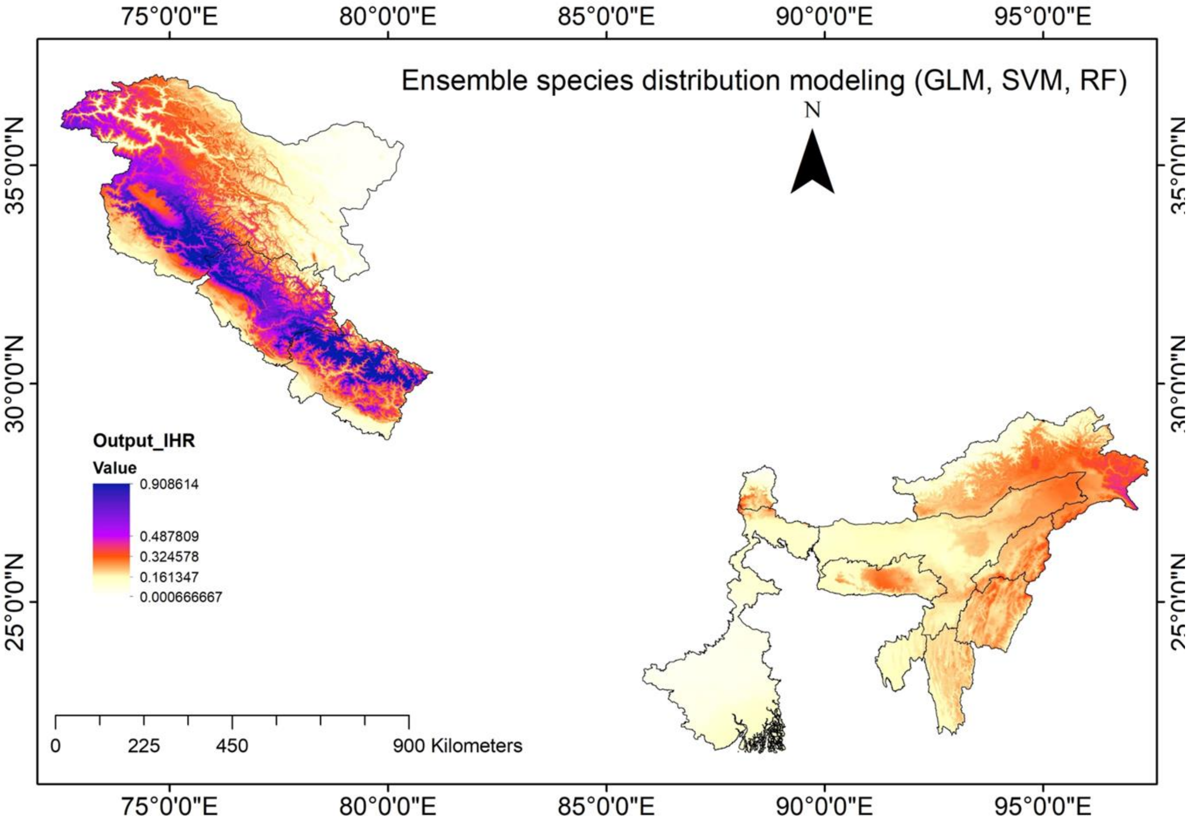


Fig. 5. Habitat suitable map of *Rheum australe* species in the India Himalayan region for the current climatic scenario.



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