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Community Composition and Phytochemical Constituents of *Rheum australe* D. Don in Rasuwa District, Nepal

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ABSTRACT

Rheum australe (Himalayan Rhubarb), a medically significant perennial herb, is endemic to the Himalayan region. This paper evaluates the population status and phytochemical composition of *R. australe* in the Rasuwa District of Nepal, along an elevation range from 3,300 m asl to 3,800 m asl. For the study, sixty quadrats of size 2 m × 2 m were studied to assess the community characteristics such as density, frequency, and importance value index (IVI). Also, hexane, ethyl acetate and methanol extracts of the rhizome from the plant was taken and the phytochemical screening was performed. Phenolics and flavonoids content, antioxidant activity, and antibacterial properties were evaluated. The results for population status indicated that *R. australe* flourishes in east-facing slopes in between 40-50° at higher altitudes ranging from 3,700m-3,800 m asl. Also, the population status was significantly influenced by altitude and disturbance levels ($p < 0.05$). The result indicated that the methanolic extract showed higher amounts of phenolic content and antioxidant activity, while ethyl acetate extract exhibited superior flavonoid content and antibacterial efficacy against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Staphylococcus aureus*.

Key words: Antibacterial activity, antioxidant activity, environmental factors, species composition

INTRODUCTION

Medicinal plants have played a very important role in pharmaceutical development over the centuries, and their usage has increased tremendously in recent times due to their negligible side effects, affordability, and accessibility, particularly in underprivileged areas of the world (Acharya *et al.*, 2009; Ekor, 2014; Sen and Chakraborty, 2017). About 80% of people from Global South countries get their primary medical treatment from herbal sources (WHO, 2018). Rural communities in Nepal rely heavily on traditional knowledge and practices to utilize these plants for their basic needs, with community forests providing nearly half of the collected medicinal plant products (Joshi *et al.*, 2011; Kunwar *et al.*, 2013). In Nepal, particularly in the rural, mountainous, and mid-hill areas medicinal plants remain the primary form of treatment for most of the illnesses where the access of modern medicinal services is limited (Kunwar *et al.*, 2010).

The plants having medicinal value are primarily due to their phytochemical constituents, which exert specific physiological effects on the human body (Bandiola, 2018). The components present in medicinal plants are not limited to phenol, flavonoids, quinine, and terpenoids that offers therapeutic benefits, but also including anti-inflammatory, anti-mutagenic, antioxidant, and anti-carcinogenic properties (Batiha *et al.*, 2020). Numerous antioxidants present in medicinal plants act as a defense mechanism against diseases (Lawal *et al.*, 2016). Medicinal plants also offer antimicrobial properties and extensive research has been conducted on this though the resistance of various bacterial and fungal strains that is increasing to a broad spectrum of antibiotics (Zargar, 2011).

Rheum australe (Himalayan Rhubarb), commonly referred to as Padhamchal in Sanskrit, is a robust perennial medicinal herb endemic to the Himalayan region, and belongs to Polygonaceae family (Figure 1). It is distributed generally in the temperate and subalpine zones of the Himalaya at an altitude between 3,200 and 4,300 meters above sea level (m asl) (Shrestha *et al.*, 2022). *R. australe* was recently evaluated for the IUCN Red List of Threatened Species in 2022 and is classified as "Data Deficient" i.e. comprehensive information on its native distribution, habitat, population size and trends, conservation status, and potential threats is urgently needed (Chauhan, 2023).

Although several studies have been conducted regarding the population status and phytochemical composition of various medicinal plants, including those by Bhattarai *et al.* (2014), Khadka *et al.* (2016), Khanal *et al.* (2020), Lamichhane *et al.* (2023), Rokaya *et al.* (2012; 2012a), and Semwal *et al.* (2007), a study on the same-itemized population status and phytochemical study for *R. australe*, collected from the same site, has not yet been done. This paper focuses on studying the population status, and biochemical (antioxidant, and antibacterial) properties of *R. australe* in Nepal Himalaya.

MATERIAL AND METHODS

Study area

The current study was conducted in the Rasuwa District in Central Nepal, specifically in the Noje of Aamachodingmo Rural Municipality -3 (Figure 2). The altitude of the district ranges from 792 to 7,245 meters above sea level, and its global positions are between 27° 2' and 27° 10' N and 84° 45' and 85° 88' E. The climate varies from subtropical to temperate and alpine, but most of the area has temperate and alpine climate. The average annual precipitation is 1605 millimeters, and the average annual temperature is 15.6°C (Shrestha *et al.*, 2017). The study site was located at the subalpine region of the Southwest part approximately 4 km from Gatlang Village. *Tsuga dumosa*, *Larix potaninii var. himalaica*, and *Abies spectabilis* are the dominant tree species interspersed with *Rhododendron arboreum, R. barbatum*, and *R. campanulatum* in the study sites. Herbaceous vegetation includes *Rumex acetosa*, *Elsholtzia blanda*, *Anaphalis busua*, *Sambucus sp.*, and *Hemiphragma heterophyllum*, indicating the slower pace of climax community regeneration in burned sites (Dhungana *et al.*, 2024).

Figure 1: Individuals of *Rheum australe* in Langtang

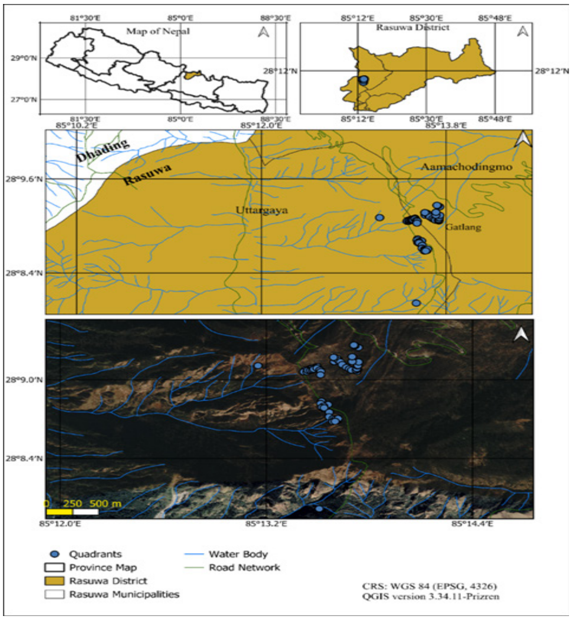

Figure 2 : Study Sites

Field methods

Field work was carried out in June 2023. A stratified random sampling technique was applied at initial reference point which was extended 20m vertically and 20m horizontally for the further sampling. A total of 60 quadrats of size 2m×2m was established to study the population status of *R. australe*.

A compass (Sunto) was used to record physiographic features such as aspect, and slope, and a Global Positioning System (GPS) device (Garmin Oregon 650) was employed to record latitude, longitude, and altitude of the studied quadrats.

All the vascular plant species present in the studied quadrat were noted. The anthropogenic disturbance was visually recorded on a scale of 0 (no disturbance) to 3 (high disturbance).

Quantitative analysis

The abundance, density, and frequency of the vegetation data were quantitatively analyzed using the formulas provided by Curtis and Mcintosh (1950) and Misra (1968). Calculating the Importance Value Index (IVI) by adding the relative values (Curtis, 1959). Density and abundance were expressed in units of plants/m^2, whereas frequency was given as a percentage of frequency. Using Kershaw (1973) criterion, an A/F value of less than 0.025 indicates a regular distribution, 0.026 to 0.050 indicates a random distribution, and > 0.050 indicates a contiguous distribution.

$$\text{Frequency}(\%) = \frac{Number\ of\ quardats\ in\ which\ an\ individual\ species\ occured}{Total\ number\ of\ quadrats\ sampled} \times 100$$

$$\text{Relative frequency (RF,\%)} = \frac{Frequency\ of\ individual\ species}{Sum\ of\ the\ frequencies\ of\ all\ species} \times 100$$

$$\text{Density}(\text{ind/m}^2) = \frac{Total\ no.of\ individual\ species\ \ in\ all\ quadrats}{Total\ no.of\ quadrats\ studied \times Size\ of\ the\ quadrat}$$

$$\text{Relative density}(\text{RD},\%) = \frac{Density\ of\ individual\ species}{Total\ density\ of\ all\ species} \times 100$$

$$\text{Abundance} = \frac{Total\ no.of\ individuals\ of\ a\ species\ in\ all\ quadrats}{Total\ no.of\ quadrats\ in\ which\ species\ occurred}$$

$$\text{Relative abundance} = \frac{Abundance\ of\ the\ individual\ species}{Total\ abundance\ of\ all\ species} \times 100$$

$$\text{A/F} = \frac{Abundance}{Frequency}$$

IVI=RD+RF+RA

Extraction process for phytochemical analysis

The rhizomes were chopped into little pieces, cleaned with distilled water, and allowed to dry for ten weeks under the shade. The dried plant components were finely ground into powder form by using mortar and pestle followed by an electric grinder. 50g powdered sample was extracted with 250 ml of hexane followed by ethyl acetate, and then with methanol using Soxhlet apparatus. A rotary evaporator was used to evaporate the extract, producing solid mass that was then kept for additional analysis.

Phytochemical Screening

The usual protocol was followed to evaluate the extracts for the presence of different types of phytochemical constituents such as reducing sugars, polyphenols, basic alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins and quinines (Culie, 1982). The total phenolic and flavonoid content of the plant extract was evaluated by following the method of Waterhouse (2002) and Woisky and Salatino (1998) respectively. DPPH radical scavenging activity method was used to determine the antioxidant activity (Giri and Rajbhandari, 2020).

Antibacterial test

Preparation of bacterial culture media

Thirteen grams of liquid broth (LB) powder was dissolved in one liter of water to make the LB medium (Sisco Research Laboratories Pvt. Ltd, India). And then, the prepared mixture was placed in the autoclave for 25 minutes at 121 °C and 15 psi pressure. After cooling to 40–50 °C, the sterilized medium was dispensed into pre-sterilized 15 mL falcon tubes at 5 mL each. Media for co-culturing a separate bacterial seed culture in each of the tubes was prepared, and incubation was allowed for a whole day.

MH media plates preparation and antibacterial assay

For the preparation of MHA plates, thirty nine grams of MH agar powder (Sisco Research Laboratories Pvt. Ltd, India) was dissolved in 1 L of water. Further, the mixture was autoclaved for 25 minutes at 121°C and 15 psi of pressure. After the temperature of the sterilized medium was reduced to 40–50 °C, it was transferred into 25 mL Petri dishes. On the agar surface, wells were created and an aliquot of each sample was introduced into the well with standard kanamycin 5 mg/mL, 10 µL. The plates with the media were then kept for 24 hours into the incubator at 37°C. Before being used, the prepared media plates were kept in the refrigerator. Using a sterile cotton swab, 150 µL of liquid bacterial seed was placed on the surface of the medium plates, which were labeled with sample names A.

Statistical analysis

The vegetation and environmental data were analyzed using different statistical tools. ANOVA was carried out to establish the relationship among populations of *R. australe* distributed in different altitudes, slope, and aspect. First order generalized linear model (GLM) regression analysis was employed for testing the association between the various disturbance factors and *R. australe* population. All statistical analyses were done in the R statistical software (R Development Core Team, 2023).

RESULTS

Community composition

Altogether 26 species of vascular plants belonging to 19 families and 24 genera were reported in association with *R. australe* (Table 1). Majority of plant species were herbs followed by shrubs and subshrubs. Polygonaceae was the dominant family representing six species.

Table 1: Community composition and quantitative characteristics of *R. australe*

S. N.	Name of Species	Family	Life form	RF (%)	RD (%)	RA (%)	A/F ratio	**IVI**
1	*Rheum australe* D.Don	Polygonaceae	Herb	16.02	8.95	2.93	0.13	27.89
2	*Polygonum aviculare* L.	Polygonaceae	Herb	12.55	22.24	9.29	0.51	44.08
3	*Elsholtzia pilosa* (Benth.) Benth.	Lamiaceae	Herb	10.39	15.89	8.02	0.54	34.29

4	*Anaphalis busua* (Buch. Ham. ex D.Don) DC.	Asteraceae	Herb	5.19	8.95	9.03	1.21	23.17
5	*Potentilla nepalensis* Hook.	Rosaceae	Herb	6.06	8.24	7.13	0.82	21.42
6	*Bistorta amplexicaulis* (D.Don) Greene	Polygonaceae	Herb	7.36	7.99	5.69	0.54	21.04
7	*Persicaria capitata* (Buch. Ham. ex D.Don) H.Gross	Polygonaceae	Herb	3.03	5.49	9.50	2.18	18.02
8	*Hydrocotyle nepalensis* Hook.	Araliaceae	Herb	7.36	6.14	4.37	0.41	17.87
9	*Fragaria nubicola* (Hook.f.) Lindl. ex Lacaita	Rosaceae	Herb	3.46	4.87	7.38	1.48	15.72
10	*Rumex acetosa* L.	Polygonaceae	Herb	8.66	3.95	2.39	0.19	15.00
11	*Impatiens bicornuta* Wall.	Balsaminaceae	Herb	3.03	2.31	4.00	0.92	9.35
12	*Pteridium* sp.	Dennstaedtiaceae	Herb	6.49	1.33	1.07	0.11	8.89
13	*Primula primulina* (Spreng.) H.Hara	Primulaceae	Herb	3.03	1.11	1.92	0.44	6.06
14	*Cynoglossum furcatum* Wall.	Boraginaceae	Herb	0.43	0.37	4.48	7.20	5.29
15	*Geranium pratense* L.	Geraniaceae	Herb	0.43	0.31	3.74	6.00	4.48
16	*Trifolium repens* L.	Fabaceae	Herb	0.43	0.28	3.36	5.40	4.07
17	*Silene caespitella* F.N.Williams	Caryophyllaceae	Herb	0.43	0.28	3.36	5.40	4.07
18	*Rhododendron anthopogon* D.Don	Ericaceae	Shrub	0.87	0.34	2.06	1.65	3.26
19	*Saxifraga brachypoda* D.Don	Saxifragaceae	Herb	0.43	0.19	2.24	3.60	2.86
20	*Viola biflora* L	Violaceae	Herb	0.87	0.22	1.31	1.05	2.39
21	*Juncus* sp.	Juncaceae	Herb	0.43	0.12	1.49	2.40	2.05
22	*Imperata* sp.	Poaceae	Herb	0.43	0.12	1.49	2.40	2.05
23	*Bistorta vacciniifolia* (Wall. ex Meisn.) Greene	Polygonaceae	Subshrub	0.43	0.12	1.49	2.40	2.05
24	*Himalayacalamus asper* Stapleton	Poaceae	Shrub	0.87	0.06	0.37	0.30	1.30
25	*Aster diplostephioides* (DC.) Benth. ex C.B.Clarke	Compositae	Herb	0.43	0.06	0.75	1.20	1.24
26	*Rhododendron campanulatum* D.Don	Ericaceae	Shrub	0.43	0.06	0.75	1.20	1.24
27	*Arisaema consanguineum* Schott	Araceae	Herb	0.43	0.03	0.37	0.60	0.84

Effect of environmental factors on population status of *R. australe*

The results indicated the maximum frequency of *R. australe* was reported in the elevation ranging from 3,400 to 3,500 m asl (Table 2). However, the highest density and abundance were recorded in the upper elevation range, i.e. between 3,700 - 3,800 m asl represents the relative distribution of *R. australe* in different physiographic factors.

Table 2: Frequency, density, and abundance of *R. australe* according to altitude, slope and aspect

		Frequency (%)	Density(ind/m²)	Abundance
Altitude	3300-3400	50.00	0.44	3.50
	3400-3500	73.33	0.78	4.09
	3500-3600	45.45	0.80	7.00
	3600-3700	63.16	1.17	7.42
	3700-3800	54.55	2.55	18.67
Slope	10-20	50.00	0.91	7.25
	20-30	68.75	1.42	8.27
	30-40	65.22	1.26	7.73
	40-50	66.67	2.00	12.00
	50-60	50.00	0.13	0.50
Aspect	E	50.00	2.23	17.80
	N	71.43	1.71	9.60
	NE	64.10	0.94	5.84
	S	50.00	0.31	2.50

The effect of different physiographic factors on population of *R. australe* is presented in table 3. The results indicated that altitude showed significant relation with the population status, while aspect and slope were insignificant.

Table 3: Effect of physiographicfactors on number of individuals of *R. australe*

Physiographic factors	Df	F-value	P-value
Altitude	4	2.646	0.0430
Slope	4	0.562	0.6110
Aspect	3	2.279	0.0894

Anthropogenic disturbances are more important in shaping composition of vascular plant species in a particular vegetation community. The result of GLM to know the effect of anthropogenic disturbance in the number of individuals of *R. australe* is given in table 4.

Table 4: Effect of different disturbance factor on the number of individuals of *R. australe*

Parameters	Estimate	z-value	P-value
Intercept	1.15067	10.16	2e-16
Grazing	-0.50387	-5.841	5.18e-09
Trample	0.55238	4.711	2.47e-06
Lopping	-0.30956	-1.948	0.0514
Fire	0.21520	2.159	0.0309
Harvesting	0.29714	4.052	5.07e-05

Phytochemical screening

The phytochemical constituents of *R. austale* revealed that both methanolic and ethyl acetate extracts were composed of polyphenols, tannins, terpenoids, and flavonoids while only the latter exhibited the presence of glycosides and quinones, and absence of active chemical compounds from hexane extracts (Table 5).

Table 5: Phytochemical screening of rhizome of *R. australe*

Extract	Hexane	Ethyl acetate	Methanol
Reducing sugar	-	-	-
Polyphenol	-	+	+
Alkaloids	-	-	-
Flavonoids	-	+	+
Saponins	-	-	-
Terpenoids	-	+	+
Glycosides	-	+	-
Tannins	-	+	+
Quinones	-	+	-

Total phenolic content (TPC)

The total phenolic content present in ethyl acetate and methanol extracts of *R. australe* rhizome was 92±1.74 mg GAE/g extract and 151.25±2.99 mg GAE/g respectively, with methanol extracts exhibiting higher phenolic content than ethyl acetate extracts.

Total flavonoid content (TFC)

In this study, the total flavonoid content in the ethyl acetate and methanol extracts of *R. australe* was 541±6.07mgCE/g extract and 175±7.39mgCE/g respectively. Neupane & Lamichhane (2020) reported flavonoid content as 480.84 ± 8.81 µg/mg Rutin equivalent in the methanolic extract of *R. australe*.

Antioxidant activity

The results indicated that the rhizome of *R. australe* is a rich source of natural antioxidants, with the methanolic extract showing high efficacy.

Antibacterial activity

The antibacterial activity of *R. australe* rhizome extracts obtained from methanol, ethyl acetate and hexane are presented in table 6. The results indicated that methanolic and ethyl acetate extracts exhibit stronger antibacterial properties than hexane extracts, particularly against Gram-negative bacteria. However, the hexane extract demonstrated significant activity against *S. aureus*, a Gram-positive bacterium, highlighting the importance of selecting the appropriate solvent for extracting antibacterial compounds based on the target microorganism.

Table 6: Antibacterial activity in different extracts

Bacterial strain	Type	Positive control	Methanol	Ethyl acetate	Hexane
Escherchia coli	-ve	2.3	2.1	2.2	1.8
Klebsellia pnuemoniae	-ve	1.6	1.7	1.3	0.0
Bacillus subitilis	+ve	2.3	1.5	1.2	1.2
Staphylococcus aureus	+ve	2.3	1.6	1.3	2.0

DISCUSSION

Community composition and population status of *Rheum australe*

R. australe prefers to grow on the habitat ranges from alpine rocky slopes, grassy slopes, near streams and open area (Pandith *et al.*, 2018). Thus, it grows in association with mostly herbs and few shrubs. The IVI is a useful marker for assessing the distribution and availability status under various environmental and biotic situations (Negi *et al.*, 1992; Ram and Arya, 1991). The IVI value of *R. australe*. indicates its contagious distribution. The contagious distribution may be caused by the plant's abundance in natural vegetation (Greig-Smith, 1983; Kershaw, 1973) as well as by notable shifts in environmental conditions (Odum, 1971). Ghimire *et al.* (1999)

reported 23.81% frequency and 0.38 ind/m^2 density of and respectively in Ponger and 71.42% and 1.38 ind/m^2 in Changle, Manang District, Nepal. Ranpal (2009) documented *R. australe* in Paplekharka, Mustang District at a frequency of 65%, density of 0.1788 ind/m^2, and abundance of 0.2764 ind/m^2. Though the frequency values are almost similar, the density and abundance in Paplekharka were very low. This might be due to the number of species in those plots was lesser and *R. australe* was an associated species dominated by *Dactylorhiza hatagirea*. Further, Khadka *et al.* (2016) reported *R. australe* in Lete village, Mustang at an altitude range of 3,200-3,600m with 8% frequency, 0.0156 ind/m^2 density and 0.223 ind/m^2 abundance. These values are indicative of its lower occurrence in those regions possibly due to overharvesting, heavy grazing pressure, and its status as an associated species. The variation in occurrence may also be attributed to ecological disturbances and habitat suitability (Ghimire *et al.*, 1999).

Effect of environmental factors on population status of *Rheum australe*

The results found that *R. australe* prefers the quite steep slope as it is a fairly widespread plant on moist subalpine meadows and may thrive in a variety of habitats (Chhetri and Gupta, 2007). Additionally, *R. australe* preferred east facing slope to vigorously grow. Corresponding to this study, Wani *et al.* (2021) reported that the species of *R. webbianum* predominantly prefer facing toward the Northeast (NE) and East (E) directions in Zanskar Mountain. A site's aspect and slope change the amount of solar radiation that the surface receives, which has a significant impact on the ecological conditions there (Acharya *et al.*, 2009). According to Yanyan *et al.* (2017), the slope aspect affects both the relative abundance and composition of plant communities. The significant relation of altitude with population status of *R. australe* and insignificant relation with aspect and slope in the present study also corroborates with the findings of Tiwari *et al.* (2020) and Hussain *et al.* (2024). They also found altitude as one of the most critical factors affecting the species diversity and structure, while the aspect and slope have less or negligible effect.

The disturbance factors, such as, grazing, trampling, looping, fire, and harvesting significantly affect the population of *R. australe*. In accordance with this result Chapagain *et al.* (2021) reported that the environmental factor and human disturbance, such as harvesting and livestock grazing, have significant impacts on population structure. Gajurel *et al.* (2015) also found that grazing and trampling are major factors in most herbaceous medicinal plant species in the Indian eastern Himalaya.

Phytochemical screening

A variety of phytochemicals are present in plant extracts. The extracts from rhizome of *R. australe* also contain numerous phytochemical classes of compounds. Kumai *et al.* (2023) also determined the presence of polyphenols, flavonoids, quinones, saponins, and tannins in the methanolic extract of the rhizome of *R. australe*. Basnet and Kalauni (2020) investigated eight species of medicinal plants and in most of the methanolic extracts of plants have identified the presence of quinones, polyphenols, terpenoids, reducing sugars, and glycosides. Alemu *et al.* (2024) carried out a similar study on five medicinal plants and found that steroids, alkaloids, flavonoids, saponins, and terpenoids in ethanol and aqueous extracts. The ethyl acetate and methanolic extracts of *R. australe* from the Rolpa district contains glycosides, reducing sugars, alkaloids, sterols, and terpenes (Pokhrel and Lamichhane, 2021). The quantity of different constituents may vary due to the different climatic conditions as the environmental variables play a crucial role in the biosynthesis and variation of plant secondary metabolites (Verma and Shukla, 2015). Singh and Chaturvedi (2018) have reported total phenolic content as 92.82 ± 0.23 µg GAE/mg in rhizome extracts of *R. emodi*. On the other hand, the methanolic extracts of *R. australe* contained 249.58 ± 7.73 µg GAE/mg phenolic contents as determined by Neupane and Lamichhane (2020). Gupta *et al.* (2014) reported methanolic and aqueous phenolic contents for *R. australe* by 6.85 and 14.51 g GAE/100g dried weight correspondingly. On the other side, Rolta *et al.* (2018) described methanolic extract of *R. emodi* rhizome indicated a total phenolic content to be 258±6.87 mg/g GAE. These variation in phenolic yields may result from using different solvents for extraction process (Park and Lee, 2021). According to Kumai *et al.* (2023), 24.97±2.857 mg QE/gram of flavonoid content was found in the root extract of *R. australe*. A study by Rolta *et al.* (2018) found a total flavonoid content of 50±2.6 mg/g RE in the methanolic extract of the rhizome of *R. emodi*. The variation in the quantity of flavonoid content in the present study and other studies mainly due to the differences in the variation in the harvesting period (Mehrabani *et al.*, 2023).

Antioxidant activity

Different phytochemicals such as phenolic compounds and flavonoids present in the plant extracts exhibit antioxidant activity. The findings of present study corroborate with the findings Gupta *et al.* (2014), who reported that the methanolic extract of *R. australe* possesses more potent DPPH free radical scavengers. High antioxidant activities also reported in the methanolic extracts of *R. emodi* rhizome (Rahman *et al.*, 2006; Singh & Chaturvedi, 2018; Tanigawa *et al.*, 2007; Tsao and Deng, 2004). Numerous research show that plants antioxidant abilities are directly related to the quantity of phenolic substances, and flavonoids that function by transferring hydrogen from phenolic hydroxyl groups present (Lu and Foo, 2000; Miliauskas *et al.*, 2004). However, different antioxidant molecules have varying chemical properties and polarity and may or may not be soluble in a given solvent, the type of the extracting solvent has a significant impact on the extraction yield and subsequent antioxidant activities of plant materials (Sultana *et al.,* 2009).

Antibacterial activity

The phytochemicals present in medicinal plants have inhibitory effects on the growth of some pathogens. The extracts of rhizome of *R. australe* showed antibacterial activity. The methanolic extract of *R. emodi* exhibited significant antibacterial activity compared to the aqueous extract (Malik *et al.*, 2018). Rehman *et al.* (2014) also assessed the antibacterial activity of *R. emodi* in contrast to *B. subtilis* and *P. aeruginosa.* Gupta *et al.* (2014) observed that methanolic and ethyl acetate extracts of *R. australe* rhizome effectively subdued the growth of *S. aureus, E. coli*, *B. subtilis* and *K. pneumonia*, while hexane extract inhibited only *E. coli.* Hassan *et al.* (2021) found that 90% ethanol extract of *R. emodi* rhizome significantly inhibited the growth of *B. subtilis* and *S. aureus*. Additionally, Pokhrel and Lamichhane (2021) reported that methanol extracted from tuber of *R. australe* effectively subdued the growth of, *B. subtilis, E. coli, S. aureus* and *K. pneumoniae.*

CONCLUSIONS

The population status of *R. australe* varies notably with altitude, peaking in frequency at 3,400–3,500m and reaching maximum density and abundance at 3,700–3,800m, while slope and aspect have minimal influence.

Grazing, trampling, fire, and harvesting significantly affect its population, though lopping has marginal impact, and the species exhibits a contagious distribution pattern. The Phytochemical screenings showed the presence of abundant bioactive compounds such as flavonoids, glycosides, tannins, terpenoids, quinones, and phenols in *R. australe* which is responsible for its medicinal properties. Also, The DPPH free radical assay confirmed that strong antioxidant activity was occurring, especially from methanolic rhizome extracts which gave the highest and consistent activities against bacterial strains. Further studies can be undertaken for population status at multiple sites, phytochemical variations in relation to different stages of growth, and analysis for all plant parts regarding bioactive compounds.

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