

EVALUATION OF ANTIMICROBIAL, CYTOTOXIC AND PHYTOTOXIC ACTIVITIES OF *RIBES HIMALENSE* DECNE. COLLECTED FROM THANDIANI, ABBOTTABAD, PAKISTAN

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Abstract

A comprehensive study was carried out to demonstrate the antibacterial, cytotoxic and phytotoxic potentials of *Ribes himalense* Decne. collected from Thandiani, Pakistan. Ethanolic extract from the leaves inhibited the growth of *Escherichia coli*, *Proteus mirabilis*, *Streptococcus mutans* and *Bacillus subtilis*, with inhibition zones from 14 to 17 mm. Similarly, the stem extract showed inhibition against the same bacterial strains with inhibition zone between 13 and 18 mm. Phytotoxic studies revealed that leaf extract of 10 µL, caused no growth inhibition, while stem extract showed 10% growth inhibition. Growth inhibition reached 70% for leaves and 100% for stem at 1000 µL. Cytotoxic analysis using lethality of brine shrimp larvae showed that stem extract was more potent than leaf extract at 2000 µg/mL concentration and below, and the cytotoxicity was 90%. Statistical analysis (ANOVA, $p \leq 0.05$) confirmed the influence of extract type and concentration across assays. The extracts of *R. himalense* exhibited significant antibacterial, cytotoxic and phytotoxic activities, which highlight its potential as a source of bioactive compounds for pharmaceuticals applications.

Keywords: Antimicrobial activity, bioactive compounds, cytotoxicity, phytotoxicity

INTRODUCTION

Abbottabad, located in the Hazara division, is a region of diverse ecological significance. The area predominantly lies on the Eurasian land plate, with its eastern part adjacent to the Indian subcontinent (Abbasi *et al.*, 2010). Geographically, the area is located at 73° 13' E longitude and 34° 09' N latitude, with an altitude of 1,255 meters. The region experiences pleasant summers accompanied by a significant monsoon season, followed by snowfall during winter (Ullah and Ullah, 2023). Thandiani, classified within Pakistan's moist temperate forest zone, is known for its rich plant diversity (Khan *et al.*, 2018). Medicinal plants continue to be a rich source of bioactive compounds with potential therapeutic and agro-biological applications. Within the genus *Ribes* (Grossulariaceae), various species have

demonstrated significant pharmacological activities, including antibacterial, antioxidant, anti-inflammatory, and antitumor effects, attributed primarily to phenolic glycosides, flavonoids, and proanthocyanidins in their extracts.

However, *Ribes himalense* Decne., an endemic species reported from montane regions of South Asia, remains poorly characterized in terms of its biological properties (Ivanova, D. A., *et al.*, 2024). Plants are known to have bioactive compounds, such as alkaloids, polyphenols, and volatile oils, which exhibit antimicrobial properties (Shah *et al.*, 2012; Ferdosi *et al.*, 2023). These natural compounds inhibit the growth of many bacterial strains without adverse effects on human health, which make them important in developing new antibiotics in the face of rising drug resistance (Javaid *et al.*, 2022). Phytotoxic activities,

characterized by the inhibition of plant growth and germination, also hold potential for sustainable weed management through the application of plant's allelochemicals (Abd-ElGawad *et al.*, 2019). Allelopathy, the study of such interactions, has greatly identified a number of plants as effective source of organic herbicides (Pan *et al.*, 2015; Javaid *et al.*, 2020). Currently, pharmacological research primarily focuses on utilizing plant extracts for the treatment of chronic diseases (Aye *et al.*, 2019; Oppedisano *et al.*, 2020).

Numerous in vitro and in vivo studies have demonstrated the antioxidant and anti-cancer potential of bioactive compounds derived from plants (Tabassum and Ahmad, 2011; Khan *et al.*, 2019). Additionally, it is estimated that 70% of anti-cancer drugs originate from plant sources, which highlight their importance in modern pharmacology (Alonso *et al.*, 2011). Despite the ecological and pharmacological importance of *R. himalense*, there is a lack of research on its antimicrobial, phytotoxic, and cytotoxic activities. Therefore, the present study aimed to bridge the knowledge gap by exploring the bioactive potential of the plant from Thandiani region of Abbottabad, Pakistan.

MATERIAL AND METHODS

Plant Collection

Ribes himalense Decne., a medium shrub, was collected from Thandiani, Abbottabad, Pakistan and identified with help of available literature (Ali and Nasir, 1989-1991; Ali and Qaiser, 1993-2022; Nasir and Ali, 1970-1989). Leaves, roots and stems were sampled during field surveys conducted in August-September 2023. The leaves and stems were carefully washed and then air-dried in the shade at room temperature. It was finely ground using a laboratory grinder and the powders were stored in moisture-free containers until further analysis.

Biological assays

All biological assays were conducted using a completely randomized design (CRD). Each treatment was performed in triplicate unless otherwise stated. Appropriate positive and negative controls were included in all experiments. Data were recorded as mean values and subjected to statistical analysis to ensure reproducibility and reliability of results.

Extraction Procedure

Ethanol extract was prepared following the standard protocols by Ahmad (Ahmad *et al.*, 2022). A 255.8 g of leaf powder and 363.2 g of stem powder were incubated with ethanol for 48 hours with periodic shaking. The mixture was agitated and then passed through a single-layer paper, followed by the addition of more ethanol to residue for re-extraction. Using rotary evaporator, the combined filtrates were evaporated at 40°C to obtain the crude extracts and were stored at 2-8°C for subsequent testing. Dimethyl sulfoxide (DMSO, 0.1%) was used as the solvent for stock solutions.

Antibacterial Assay

The antibacterial activity of ethanolic leaf and stem extracts was evaluated using the agar well diffusion method following standard procedures described by CLSI (2018) and Ali *et al.* (2019). The experiment was conducted using a completely randomized design with three independent replicates per treatment. Mueller-Hinton agar plates were inoculated with freshly prepared bacterial suspensions adjusted to 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL). Wells of 5 mm diameter were aseptically punched into the agar and filled with 100 μ L of plant extract (100 mg/mL). Ciprofloxacin (25 μ g) and azithromycin (25 μ g) were used as positive controls, while 0.1% DMSO served as the negative control. Plates were incubated at 37 ± 1 °C for 24

hours under aerobic conditions. Zones of inhibition were measured in millimeters using a digital Vernier caliper, and mean values were calculated from three replicates.

Phytotoxic activity

Phytotoxic activity was assessed using *Lemna minor* L. following the standard protocol described by Meyer *et al.* (1982) and Gilani *et al.* (2010). The experiment was conducted under a completely randomized design with three replicates per treatment. Plant extracts were dissolved in ethanol and tested at concentrations of 10, 100, and 1000 μ L per Petri dish. Each treatment consisted of ten *Lemna minor* plants, each bearing three fronds, placed in 20 mL of E-medium. Paraquat was used as a positive control, while 0.1% DMSO served as the negative control. Petri dishes were maintained in a controlled growth environment at 25 ± 2 °C under a 16 h light / 8 h dark photoperiod with a light intensity of approximately 3000 lux for seven days. Frond numbers were recorded after the incubation period, and percentage growth inhibition was calculated relative to the control using the following formula:

$$\text{Inhibition (\%)} = [100 - (\text{Number of fronds in treated samples} / \text{Number of fronds in control samples}) \times 100]$$

Cytotoxic Activity

Cytotoxic activity was evaluated using the brine shrimp (*Artemia salina*) lethality bioassay according to the standard method described by Meyer *et al.* (1982). The experiment was arranged in a completely randomized design with three replicates per concentration. Brine shrimp eggs were hatched in artificial seawater (38 g sea salt per liter of distilled water) under continuous aeration and illumination at 28 ± 1 °C for 48 hours. Active nauplii were collected and transferred into test tubes

containing different concentrations of plant extracts (62.5–2000 μ g/mL). Each test tube contained ten nauplii, and mortality was recorded after 24 hours of incubation under constant light conditions. Artificial seawater with 0.1% DMSO served as the negative control. Percent mortality was calculated, and LC₅₀ values were estimated.

Statistical Analysis

Data obtained from all assays were expressed as mean \pm standard deviation (SD) of three independent replicates. Statistical analysis was performed using analysis of variance (ANOVA), and treatment means were compared at a significant level of $\alpha = 0.05$. All analyses were conducted using SPSS statistical software.

RESULTS

All quantitative data are presented as mean \pm standard deviation (SD) of three independent replicates. Statistical significance among treatments was determined using analysis of variance (ANOVA), and differences were considered significant at $P < 0.05$.

Antibacterial assay

ANOVA revealed a significant effect of extract type (leaf vs. stem) on antibacterial activity ($P < 0.05$), whereas differences among bacterial strains were not statistically significant. Ethanolic extracts derived from the leaves and stems of the plant exhibited antimicrobial activity against four bacterial strains. *E. Coli*, *P. mirabilis*, *S. mutans*, and *B. subtilis*. Maximum inhibition zones were observed against *Bacillus subtilis* (leaves) and *Proteus mirabilis* (stem). Comparative analysis with standard antibiotics revealed moderate efficacy (table 1, Fig. 2, 3, 4). The bar chart and heatmap clearly showed that *Bacillus subtilis* and *Proteus mirabilis* have highest inhibition zones 17 mm and 16 mm, respectively, while *Streptococcus mutans*

showed the lowest (14 mm). ANOVA indicated significant differences in inhibition between extracts but not among microbial strains (Table 2).

Table 1: Antibacterial activity of ethanolic leaf and stem extracts of *Ribes himalense* against selected bacterial strains, expressed as zone of inhibition (mm)

S.No.	Strains used	Zone of inhibition(mm) at concentration (100mg/ml)		Standards		
		Leaves	Stem	Azithr.	Ciprof.	Clotr.
				25 µg	25 µg	30 µg
1.	<i>E. coli</i> ,	16	15	31	42	NA
2.	<i>P. mirabilis</i>	16	18	40	41	NA
3.	<i>S. mutans</i>	14	13	32	42	NA
4.	<i>B. subtilis</i>	17	16	21	41	NA
5.	<i>C. albicans</i>	15	12	31	52	32.5

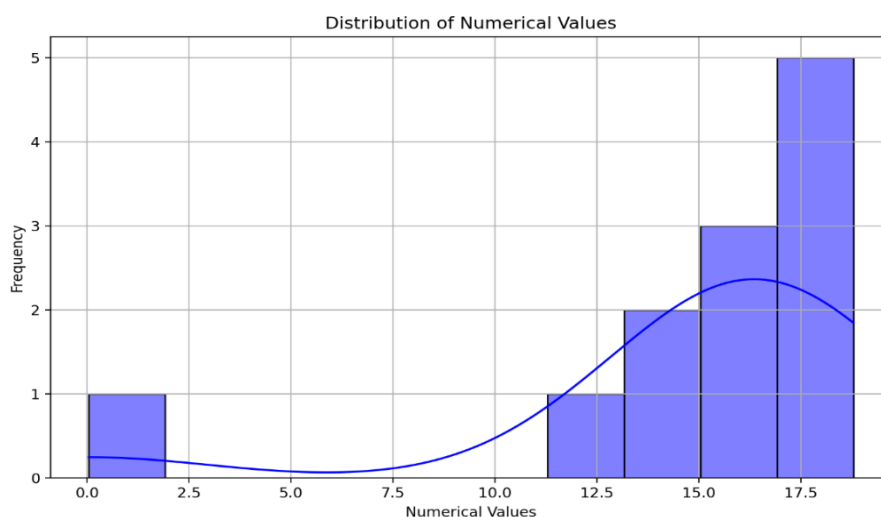


Figure 1: Distribution of inhibition zones (mm) produced by ethanolic leaf and stem extracts of *Ribes himalense* against selected bacterial strains.

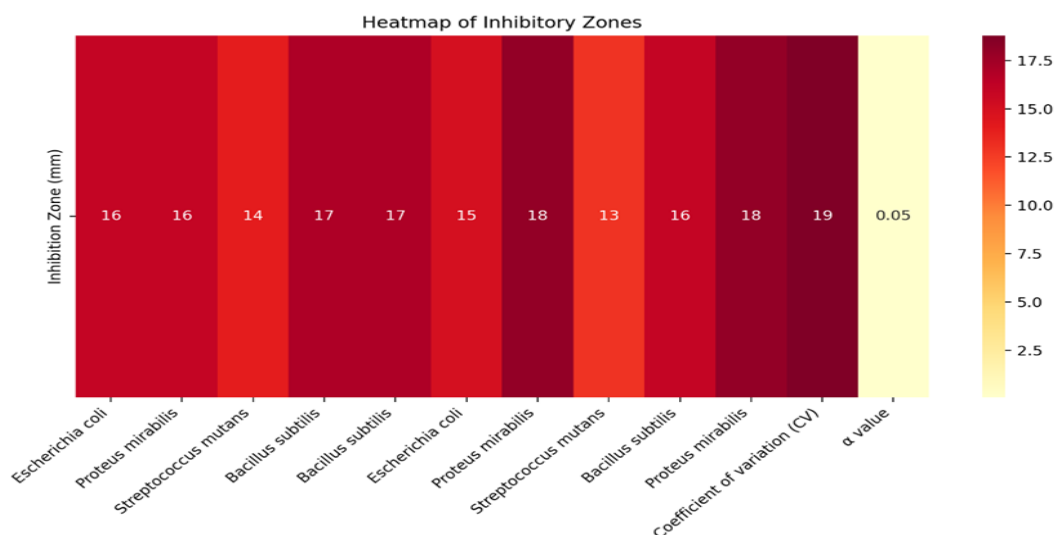


Figure 2: Heatmap of inhibitory zones

Table 2: ANOVA Analysis of Antimicrobial Activities of Leaf and Stem Extracts

SOV	d.f.	S.S.	M.S.	F-Value	P-Value
	4	96.889	24.222	1.3490	0.2750
Extracts	2	4874.178	2437.089	135.7290	0.0000
Interaction	8	197.378	24.672	1.3741	0.2476
Error	30	538.667	17.956		
Total	44	5707.111			

Coefficient of variation (CV) = 18.79%, α value @ 0.05

Phytotoxic activity

Statistical analysis showed a significant concentration-dependent effect of both leaf and stem extracts on *Lemna minor* growth inhibition (P < 0.05). Phytotoxic activity was assessed against *Lemna minor* L. at extract concentrations of 10 µl, 100 µl, and 1000 µl. Leaf extract showed growth inhibition of 0%, 30%, and 70%, while stem extract exhibited 10%, 10%, and 100%, respectively. Positive control (Paraquat solution) showed 100% inhibition, whereas the negative control (DMSO) displayed no activity. Results confirmed the concentration-dependent phytotoxic potential of *Ribes himalense* extracts (Fig. 3 and 4).

Cytotoxic activity

ANOVA indicated significant effects of extract type, concentration, and their interaction on brine shrimp mortality (P < 0.05). The brine shrimp lethality assay revealed that stem extracts were more cytotoxic than that of leaves extracts. Ethanolic leaf extracts showed 90% mortality at 500, 1000, and 2000 µg/mL concentrations, while lower concentrations (250, 125, and 62.5 µg/mL) exhibited 70%, 50%, and 30% mortality. Ethanolic leaf extracts displayed 90% mortality at Stem extracts maintained 90% cytotoxicity across concentrations from 125 to 2000 µg/mL, with 80% mortality at 62.5 µg/mL (Tables 3-4, Fig. 5, 6). The statistically significant effects of extract type, concentration, and their interaction observed in

ANOVA (Table 4) are visually represented in Figure 6.

The heatmap highlights the cytotoxic effects for both extracts, while the bar chart provides a direct comparison at each concentration.

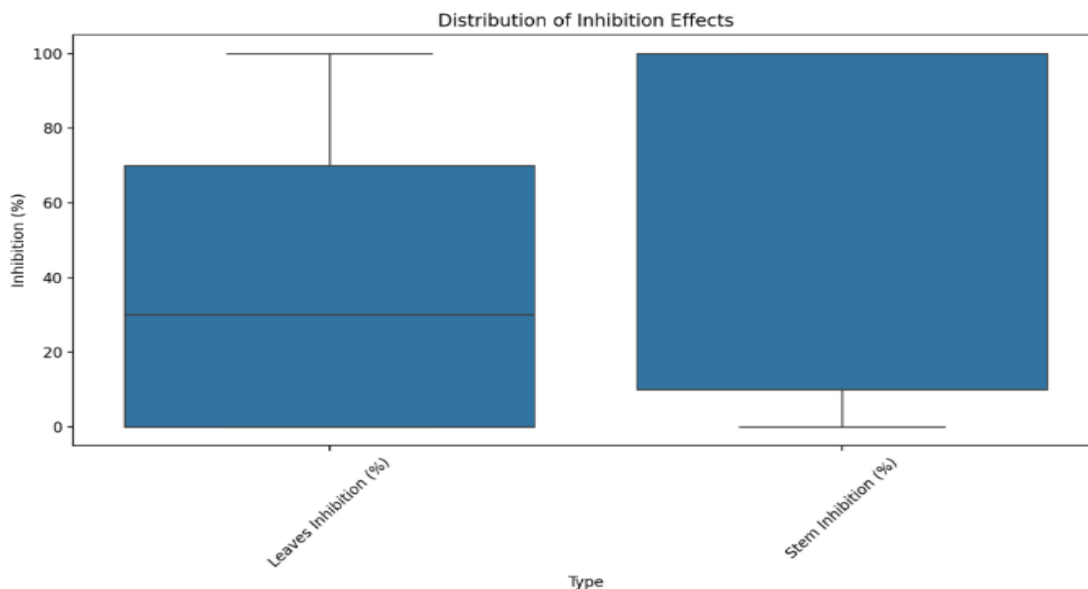


Fig.3: Distribution of inhibition effects of leaves and stem

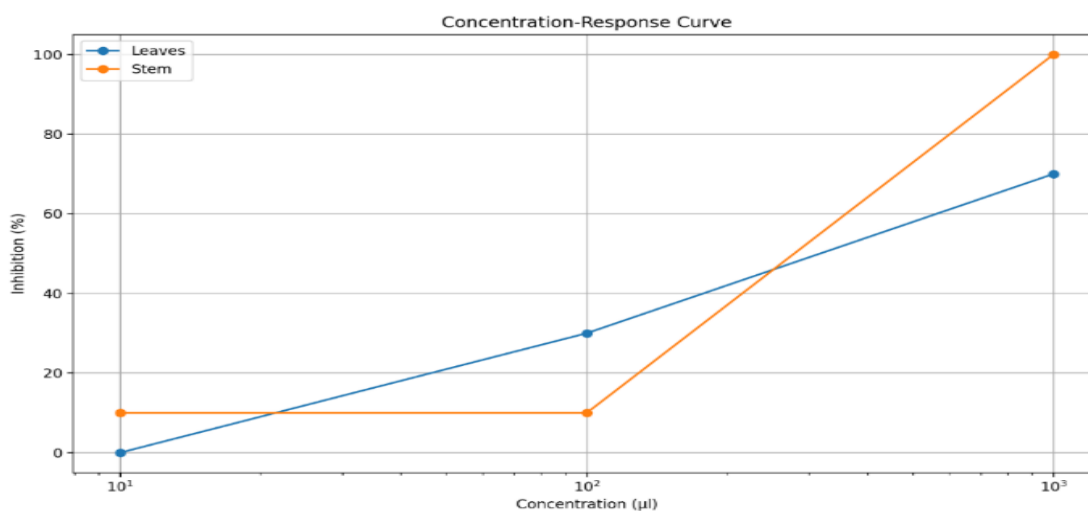


Fig.4: Concentration-Response Curve of leaves and stem

Table 3: Brine Shrimp Mortality (%) After 24 Hours at Different Concentration

Sample	2000 µg/mL	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL
Leaves	90	90	90	70	50	30
Stem	90	90	90	90	90	80

Table 4: ANOVA Analysis of Cytotoxic Activity at Various Concentrations of Leaf and Stem Extracts

Source of Variation (SOV)	d.f.	S.S.	M.S.	F-Value	P-Value
Concentration	5	6125.00	1225.00	639.130	0.000
Extracts	1	3025.00	3025.00	1578.260	0.000
Interaction	5	3725.00	745.00	388.695	0.000
Error	24	46.00	1.917		
Total	35	12921.00			

Coefficient of variation (CV) = 1.75%

Significance Level (α): 0.05

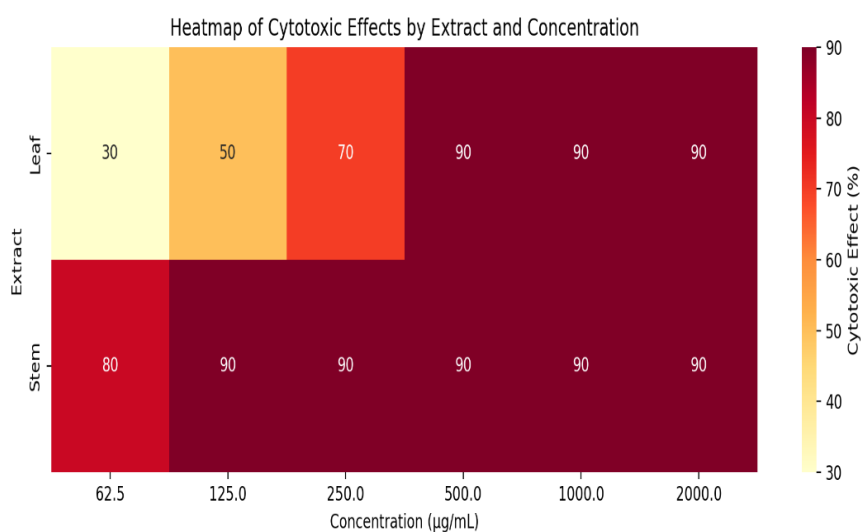


Fig.5: Heatmap of Cytotoxic Effects by Concentration and Extract

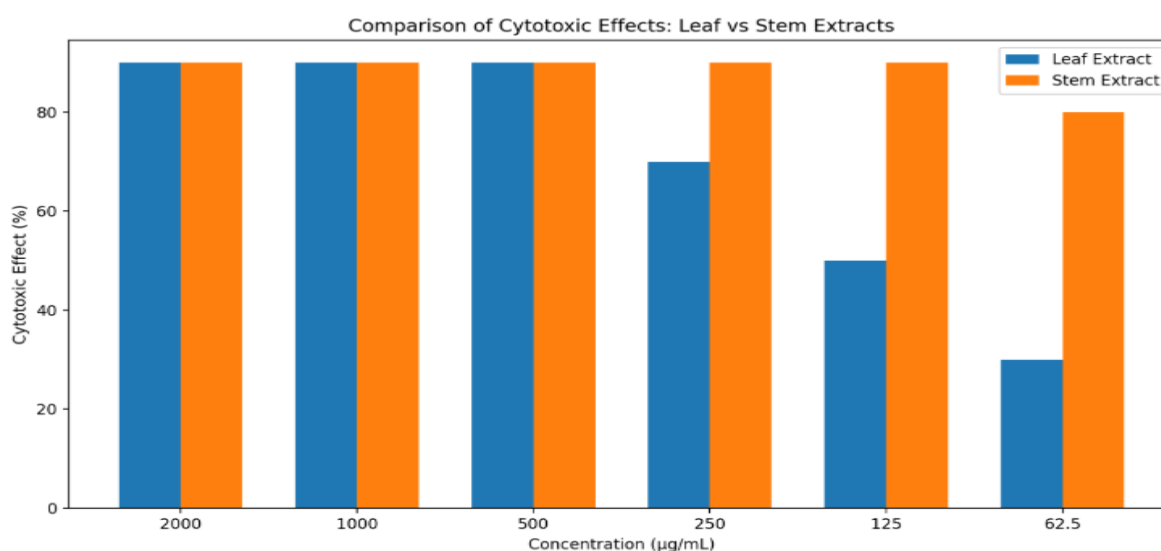


Figure 6: Comparison of cytotoxic activity of ethanolic leaf and stem extracts of *Ribes himalense* against brine shrimp nauplii at different concentrations. Values represent mean \pm SD of three independent replicates. Bars with different letters indicate statistically significant differences between treatments as determined by ANOVA (Table 4) at $P < 0.05$.

Discussions

The present study provides a comprehensive evaluation of the antibacterial, phytotoxic, and cytotoxic activities of ethanolic leaf and stem extracts of *Ribes himalense* Decne. collected from the Thandiani region of Pakistan. By integrating bioassays with statistical analysis, the findings contribute new evidence on the bioactive potential of this species and emphasize differences in bioactivity between plant parts and extract concentrations.

The antibacterial activity observed in both leaf and stem extracts likely reflects the presence of diverse secondary metabolites such as polyphenols, flavonoids, alkaloids, and tannins, which are widely recognized for their antimicrobial effects in medicinal plants (Riaz *et al.*, 2023). Such phytochemicals can disrupt bacterial cell walls, interfere with enzyme systems, and inhibit microbial growth (Breijyeh and Karaman, 2024; Riaz *et al.*, 2023) and have been reported in recent plant bioactivity screens where crude extracts exhibited moderate antibacterial efficacy in a concentration-dependent manner (Haq *et al.*, 2020). The comparatively higher activity of stem extracts may indicate a differential distribution of bioactive constituents between plant organs, a trend also documented in other species where stem fractions contained higher levels of phenolics correlated with stronger antibacterial responses (Wijerathna *et al.*, 2018). Although inhibition zones were lower than those of standard antibiotics, the consistent inhibition across all tested strains supports the presence of bioactive antibacterial agents and justifies further phytochemical characterization.

The phytotoxic effects of *R. himalense* extracts against *Lemna minor* exhibited a clear concentration-dependent pattern, with stem extracts inducing complete growth inhibition at the highest

concentration. This aligns with recent studies demonstrating concentration-dependent allelopathic effects of plant extracts on model species, attributed to phenolic acids, flavonoids, and other secondary metabolites that interfere with photosynthesis and cell division (Gilani *et al.*, 2010; Bereksi *et al.*, 2018; Maqbool *et al.*, 2022). Recent investigations in phytotoxic plant screens). Although few studies specifically target phytotoxicity in Himalayan taxa, similar dose-dependent inhibitory effects have been reported in other medicinal plants used as natural herbicide sources. While these findings suggest potential bioherbicidal activity, practical agricultural applications require further isolation of active compounds and environmental safety assessments.

In the brine shrimp lethality assay, cytotoxicity increased with concentration for both extracts, with stem extracts showing higher mortality. This conforms with the widespread use of the brine shrimp assay as an initial screen for cytotoxic and potentially anticancer compounds (Meyer *et al.*, 1982; Riaz *et al.*, 2023). The observed cytotoxic responses may be due to compounds such as alkaloids and phenolics, which have been implicated in cell membrane disruption and apoptosis in phytochemical studies (Riaz *et al.*, 2023). Although this assay does not directly predict efficacy against human cancer cells, the results support the need for further evaluation using mammalian cell lines and mechanistic studies to determine potential therapeutic relevance.

Overall, the significant effects of extract type and concentration across all assays emphasize the importance of plant part selection and dose in bioactivity screening studies. These patterns are consistent with recent research showing that extraction solvent, plant organ, and phytochemical composition critically influence observed biological

activities in medicinal plants (Riaz et al., 2023; Wijerathna et al., 2018). However, the present study is limited to crude extract assays and does not include compound isolation or detailed phytochemical profiling, which restricts definitive conclusions regarding specific active constituents.

Given the ecological sensitivity of Himalayan flora and the observed biological activities of *R. himalense*, future research should integrate phytochemical characterization, bioassay-guided fractionation, and ecological assessments to support both sustainable utilization and conservation strategies. Such interdisciplinary approaches will help clarify therapeutic and agricultural relevance while minimizing the potential impacts of overexploitation.

CONCLUSION

The present study demonstrates that ethanolic leaf and stem extracts of *Ribes himalense* exhibit measurable antibacterial, phytotoxic, and cytotoxic activities, with stem extracts showing comparatively stronger biological effects. The observed responses were concentration-dependent and statistically significant, indicating the presence of bioactive constituents in this species. Although the activities were moderate in comparison with standard controls, the findings highlight *R. himalense* as a promising species for further phytochemical and pharmacological investigations. Future studies focusing on compound isolation, mechanistic evaluation, and safety assessment are required to fully explore its potential applications.

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AUTHOR'S CONTRIBUTION

Nimra Zeb- Conceptualization, Research work, Experiments, collected and analyzing data, prepared initial draft. Asad Ullah- assisted experimental design, supervised research work, assisted draft. Safeer Ullah-Data interpretation, revising and editing manuscript. Ghulam Mujtaba Shah- Data analysis. Zulfiqar Ahmed- visual graphs for results, assisted in drafting. Maryam Shakir- Contributed literature review, editing manuscript. Khalil Ur Rehman- data analysis. Nazia Zeb- Editing and final approval of the article

CONFLICT OF INTREST

The authors declare that there is no conflict of interest regarding the publication of this study.

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