PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF RHEUM WEBBIANUM ROYLE FROM NALTER VALLEY, GILGIT-BALTISTAN, PAKISTAN

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Abstract

This study investigated the phytochemical composition and antioxidant activity of *Rheum webbianum* Royle, an important medicinal plant from Naltar Valley, Gilgit-Baltistan, Pakistan. The roots and aerial parts were subjected to methanol extraction followed by sequential fractionation with ethyl acetate, dichloromethane, and n-hexane. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, and phenolic compounds in both plant parts, while carbohydrates were exclusively detected in roots. Steroids were absent in root extracts. The antioxidant potential was evaluated using DPPH free radical scavenging assay, with all extracts demonstrating significant activity (70.9-94.2% inhibition). The ethyl acetate root fraction exhibited the highest antioxidant activity (94.2%), followed by dichloromethane (91.3%) and n-hexane (90.4%) root fractions. The strong correlation between phenolic, flavonoid content and antioxidant capacity suggests these compounds as major contributors to the observed bioactivity. These findings validate the traditional medicinal uses of *R. webbianum* and highlight its potential as a natural source of antioxidants for pharmaceutical applications. The superior activity of root extracts, particularly the ethyl acetate fraction, warrants further investigation into isolation of active compounds and their mechanism of action. This study contributes to the scientific validation of traditional knowledge while emphasizing the conservation value of medicinal plants in the Himalayan region.

Keywords: *Rheum webbianum*, phytochemicals, antioxidant activity, DPPH assay, medicinal plants, Gilgit-Baltistan

Introduction

Nature has bestowed upon mankind countless gifts, many of which remain beyond human comprehension. As inherently curious beings, humans are deeply fascinated by the natural world and its diverse creations. Since the dawn of civilization, humans have relied on nature for their fundamental needs, utilizing natural resources for food production, shelter, clothing, fragrances, transportation, and medicine (Mekonnen et al. 2022). Among these, medicinal plants have played a pivotal role in healthcare, serving as the richest source of therapeutic compounds for treating various ailments across cultures and historical periods (Hussain et al. 2024)

Plants constitute one of the most remarkable components of the natural world, serving as the foundation of life on Earth. They not only sustain themselves through photosynthesis but also form the basis of food chains and energy flow within ecosystems (Gondim and Rondon 2020). Beyond ecological benefits, plants hold immense value for human survival, contributing to biodiversity conservation and providing essential resources for sustenance and health (Heywood, 2023). Their medicinal properties, both preventive and curative, have been harnessed for

centuries in traditional and modern medicine (Niazi and Monib 2024).

The relationship between humans and plants dates back to ancient times, with early civilizations gradually discovering the uses of local flora for food, shelter, and healing (Tańczuk 2022). Indigenous communities, in particular, possess extensive knowledge of native medicinal plants, utilizing them to combat diseases attributed to environmental factors (Aziz et al. 2018). The therapeutic efficacy of these plants is largely due to phytochemicals naturally occurring bioactive compounds that exhibit defensive and protective properties against pathogens and chronic conditions (Riaz et al. 2023).

Oxidative stress, driven by reactive oxygen species (ROS) and free radicals, is a key contributor to the pathogenesis of chronic diseases such as asthma, diabetes, neurodegenerative disorders (e.g., Alzheimer's and Parkinson's), cancer, and cardiovascular ailments (Jomova et al. 2023). Antioxidants play a critical role in mitigating oxidative damage by neutralizing free radicals and maintaining redox balance (Chaudhary et al. 2023). These compounds are broadly classified into enzymatic antioxidants (e.g., superoxide dismutase, catalase) and non-enzymatic antioxidants (e.g., vitamins C and E, flavonoids, phenolics, and carotenoids) (Ezema et al. 2024). Phenolic compounds, alkaloids and flavonoids are particularly notable for their ability to scavenge peroxides and lipid radicals, thereby inhibiting oxidative degradation (Shi et al. 2022).

Rheum webbianum Royle, a member of the Polygonaceae family, is a medicinally significant plant native to Asia, distributed across regions such as China, India, Nepal, Kashmir, and Pakistan's Gilgit-Baltistan (Tabin et al. 2022). Known by various vernacular names Himalayan Rhubarb (English), Ravanchini (Hindi), and Chontal in Gilgit (Pakistan). This species is renowned for its anthraquinone derivatives, including rhein, emodin, aloe-emodin, physcion, and chrysophanol, which underpin its pharmacological value (Maqbool et al. 2014).

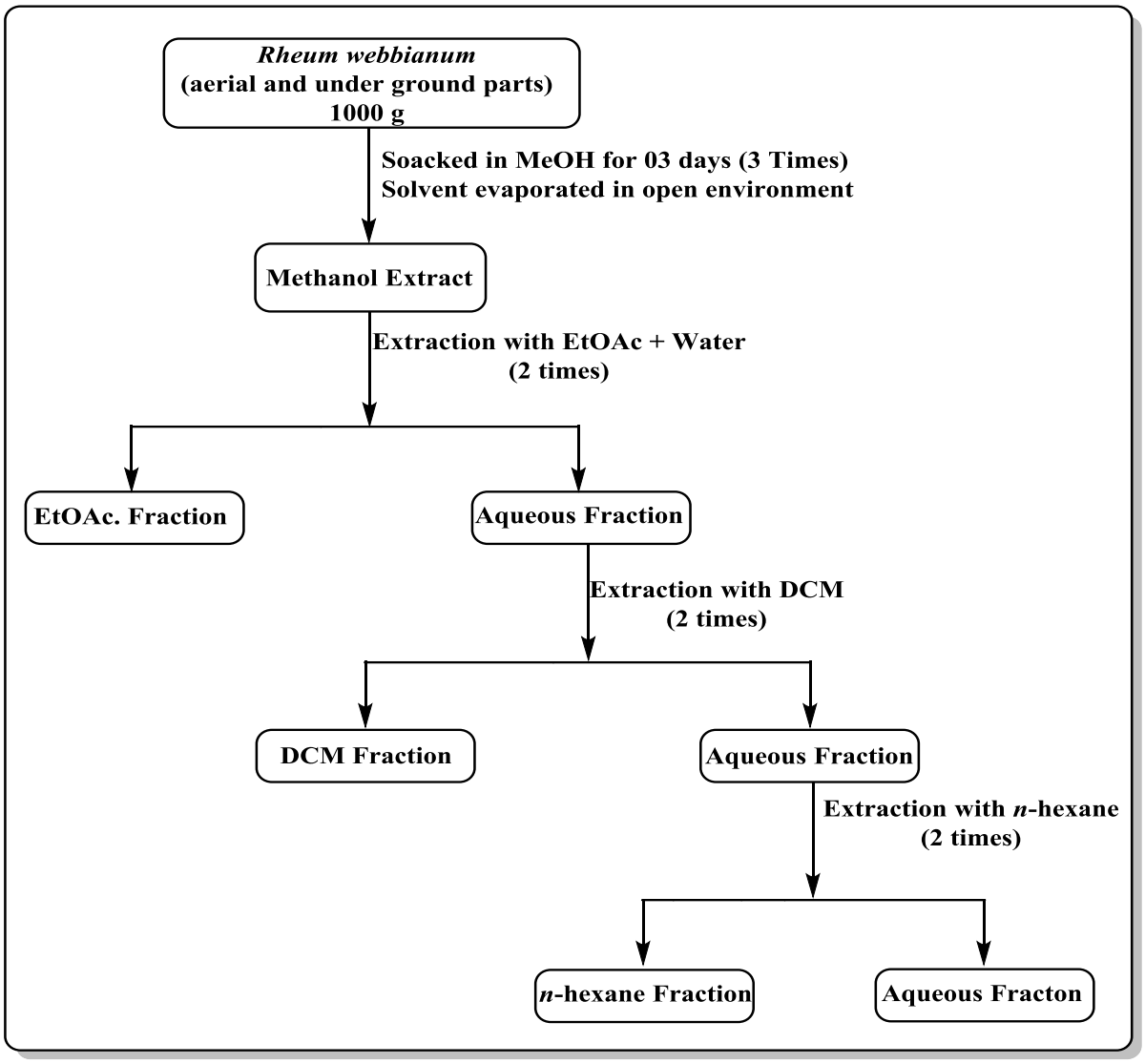
Materials and Methods

Plant Material Collection and Identification

Fresh specimens of *R. webbianum* were collected in September 2023 from Naltar Valley, located at an altitude of 3,800 meters in Gilgit-Baltistan, Pakistan. The collected plant material was authenticated by Professor Dr. Sher Wali Khan Karakoram International University, while specimen (KIU-407) was deposited in the KIU herbarium (Kumar et al. 2018). The plant parts were carefully shade-dried at room temperature and ground to a fine powder using standard protocols (Singh et al. 2024).

Extraction and Fractionation Procedures

The extraction process involved soaking 500g each of aerial parts and roots in methanol for 72 hours with three repetitions to ensure complete extraction. Following filtration, the combined methanolic extracts were concentrated using a rotary evaporator at 40°C. The concentrated extract was then subjected to sequential solvent partitioning, first with ethyl acetate and water (1:1 ratio), followed by further fractionation of the aqueous layer with dichloromethane and n-hexane (Ezebo et al. 2021). All fractions were concentrated under reduced pressure and stored at 4°C until further analysis.



Scheme-1: Preparation of plant samples

Phytochemical Screening

Comprehensive phytochemical analysis was conducted using standard qualitative tests. Flavonoids were detected through the aluminum chloride test, while phenolics were identified using ferric chloride reagent. Carbohydrates were tested via Fehling's test, alkaloids with Wagner's reagent, and steroids through the Liebermann-Burchard test (Shah and Yadav 2015). Each test was performed in triplicate to ensure reliability of results.

Antioxidant Activity Assessment

The free radical scavenging activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to the method described by (Baliyan et al. 2022). Briefly, various concentrations of plant extracts (10-100 µg/mL) were mixed with DPPH solution (0.1 mM in methanol) and

incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer, with ascorbic acid as the positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula: Percentage Inhibition = $(A_{control} - A_{sample})/(A_{control})\times100$.

Results and Discussion

Phytochemical Screening

The phytochemical screening revealed significant differences in secondary metabolite content between root and aerial parts (Table 1). Both plant parts contained substantial amounts of flavonoids, phenolics, and alkaloids, while carbohydrates were exclusively detected in roots. Steroids were absent in root samples, consistent with previous reports on Rheum species (S et al. 2016). These findings support the traditional use of different plant parts for specific therapeutic purposes in local medicine.

Table-1: Secondary metabolites *Rheum webbianum* Root

Plant Part	Flavonoids	Alkaloids	Phenolics	Carbohydrates	Steroids
Root	+	+	+	+	-
Arieal	+	+	+	-	ND

(+ = Present; – = Absent; ND = Not Determined)

Antioxidant Potential

The DPPH assay demonstrated remarkable free radical scavenging activity across all tested fractions (Table 2). The ethyl acetate root fraction exhibited the highest activity (94.2% inhibition at 100 µg/mL), followed by dichloromethane (91.3%) and n-hexane (90.4%) fractions of roots. Aerial parts showed relatively lower but still significant activity, with inhibition ranging from 70.9% to 85.3%. These results correlate well with the total phenolic and flavonoid content, suggesting these compounds as major contributors to the observed antioxidant effects (Park and Lee 2021). The superior activity of root extracts may be attributed to higher concentrations of anthraquinone derivatives, known potent antioxidants in Rheum species (Öztürk et al. 2007).

Table 2. DPPH radical scavenging activity of *Rheum webbianum* extracts

S.No	Plants Extract samples	Reading of Sample 1	Reading of Sample 2	Reading of Sample 3	Mean	Percentage of Inhibition
1	RRM	0.626	0.641	0.633	0.633	85.9
2	RRN	0.433	0.425	0.433	0.43	90.4
3	RRD	0.385	0.38	0.407	0.39	91.3
4	RRE	0.261	0.262	0.261	0.26	94.2
5	RLM	0.821	0.844	0.821	0.828	81.6
6	RLN	0.72	0.78	0.782	0.763	83
7	RLD	0.66	0.658	0.665	0.661	85.3
8	RLE	1.252	1.332	1.336	1.306	70.97
9	Standard	0.12	0.12	0.121	0.12	97.3
10	Blank	4.5	4.5	4.5	4.5	0

RRM= Rheum Root Methanolic Extract, RRN= Rheum Root n-Hexane Extract, RRD= Rheum Root DCM Extract, RRE= Rheum Root Ethyl Acetate Extract, RLM= Rheum leaf methanolic Extract, RLN= Rheum leaf n-Hexane Extract, RLD= Rheum leaf DCM Extract, RLE= Rheum leaf Ethyl Acetate Extract, Std.= Standard (Ascorbic acid), Blank (Solution DPPH).

Figure 1: Percentage of Inhibition of Plant Extract

Conclusion

This comprehensive study demonstrates that R. webbianum from Naltar Valley possesses significant medicinal potential, particularly in its root extracts which showed remarkable antioxidant activity. The presence of diverse bioactive compounds supports its traditional use in folk medicine and suggests potential for pharmaceutical development. Future research should focus on isolation and characterization of active principles, toxicological evaluation, and clinical studies to fully exploit its therapeutic potential. The findings also highlight the importance of conserving such valuable medicinal plants and associated indigenous knowledge in the Himalayan region.

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Conflicts of Interest: The authors declare no conflicts of interest

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