

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

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Abstract

The study revealed the phytochemical screening and antioxidant potential of *Rheum webbianum* Royal an important medicinal plant from Naltar Valley, Gilgit-Baltistan, Pakistan. The grinded material of root and aerial part plant 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 the root and aerial parts of plant, while carbohydrates were exclusively detected in roots. Steroids were absent in root extract. The antioxidant potential was investigated using DPPH free radical scavenging assay, with all extracts showing significant activity. 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 percentage inhibition of aerial part revealed moderate potential as compare to root. Among the fractions of aerial parts dichloromethane fraction showed highest percentage of inhibition (85.3%), followed by n-hexane (83%), methane (81.6%) and ethyl acetate showed relatively low inhibition (70.97%). The strong correlation between phenolic, flavonoid content and antioxidant capacity suggests the extract as major contributors to the observed bioactivity. These findings prove and validate the traditional medicinal uses of *R. webbianum* and highlights its potential as a natural source of antioxidants for pharmaceutical applications. The superior activity of root extracts, particularly the ethyl acetate fraction, suggest further investigation into isolation of bioactive compounds and their mood of action. The study contributes to the scientific validation of tradition knowledge while emphasizing the conservation value of medicinal plants in the Karakoram 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 nature 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, a pivotal role in healthcare have played by medicinal plants, 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 basic and important 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 began in ancient times, as early civilizations discovered their uses for food, shelter, and medicine (Tanczuk 2022). Indigenous communities have a deep understanding of local medicinal plants and use them to treat illnesses caused by their environment (Aziz et al. 2018). The therapeutic potential of these plants is largely due to phytochemicals naturally occurring bioactive compounds that show defensive and protective properties against disease causing agents and chronic conditions (Riaz et al. 2023).

A process called Oxidative stress, triggered by reactive molecules, is a major factor in the development of long-term diseases such as asthma, diabetes, brain disorders, cancer, and heart conditions (Jomova et al. 2023). Antioxidants play a key role to decrease oxidative damage by neutralizing free radicals and maintain redox balance (Chaudhary et al. 2023). These compounds are classified into enzymatic antioxidants (e.g. superoxide dismutase, and catalase) and non-enzymatic antioxidants (e.g. vitamins C and E, flavonoids, phenolics, and carotenoids) (Ezema et al. 2024). The compounds like phenolics, alkaloid and flavonoids are particularly known for their potential ability to scavenge peroxides and lipid radicals, thereby inhibiting oxidative degradation (Shi et al. 2022).

Rheum webbianum Royle belongs to Polygonaceae family. It is medicinally important plant native to Asia, distributed across the regions such as China, India, Nepal, Kashmir, and Pakistan (Tabin et al. 2022).

It is known by different vernacular names Himalayan Rhubarb (English), Ravanchini (Hindi), and it is known as Chantal (Shina) in Gilgit-Baltistan, Pakistan. This medicinal plant species is renowned for its anthraquinone derivatives, including aloë-emodin, chrysophanol, emodin, physcion, and rhein, which underpin its pharmacological value (Maqbool et al. 2014).

Material and Methods

Plant Collection and Identification

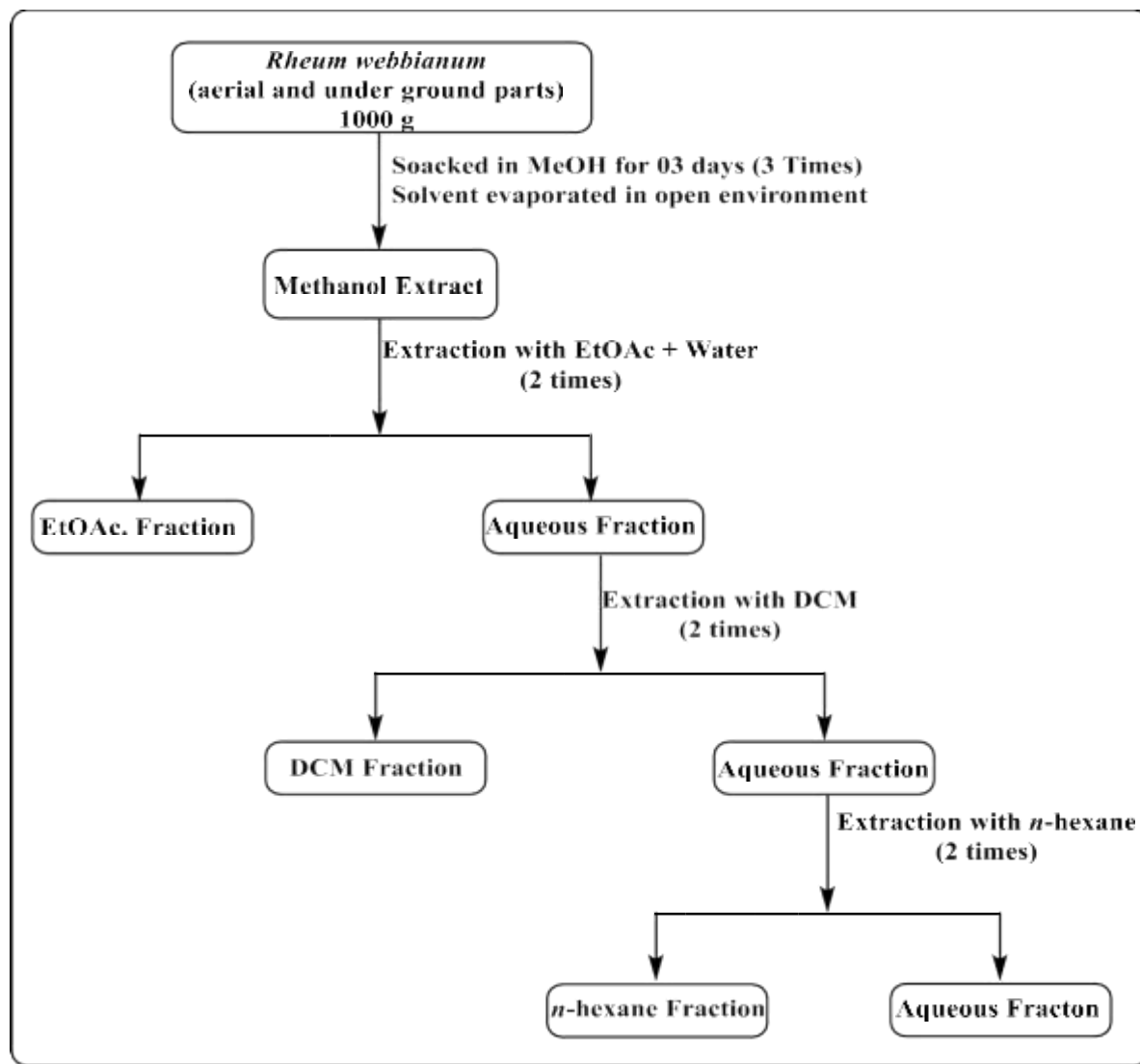
The plant specimen as well as fresh amount of roots and aerial material of *R. webbianum* were collected at the elevation of 3800 meters, in September 2023 from Naltar Valley Gilgit-Baltistan, Pakistan. The collected plant specimen was identified by Professor Dr. Sher Wali Khan Karakoram International University and the identified herbarium specimen (KIU-407) was deposited in the KIU herbarium (Kumar et al. 2018). The plant material was carefully shade-dried at room temperature and ground to a fine powder using standard protocols (Singh et al. 2024).



Figure 1 *Rheum webbianum* Royle

Extraction and Fractionation

500g of aerial and root dried and grinded material soaked for 72 hours and repeat the process three times to ensure the complete extraction. Following the filtration process and obtained crude extract by using a rotary evaporator at 40°C. The methanolic 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 and ethyl acetate (Ezebo et al. 2021). All fractions were stored at 4°C for 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). Reliability of each test's result was ensured by performing it in triplicate.

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). 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:

$$\text{Percentage Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

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	+	+	+	+	-
Aerial	+	+	+	-	ND

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

Antioxidant Potential

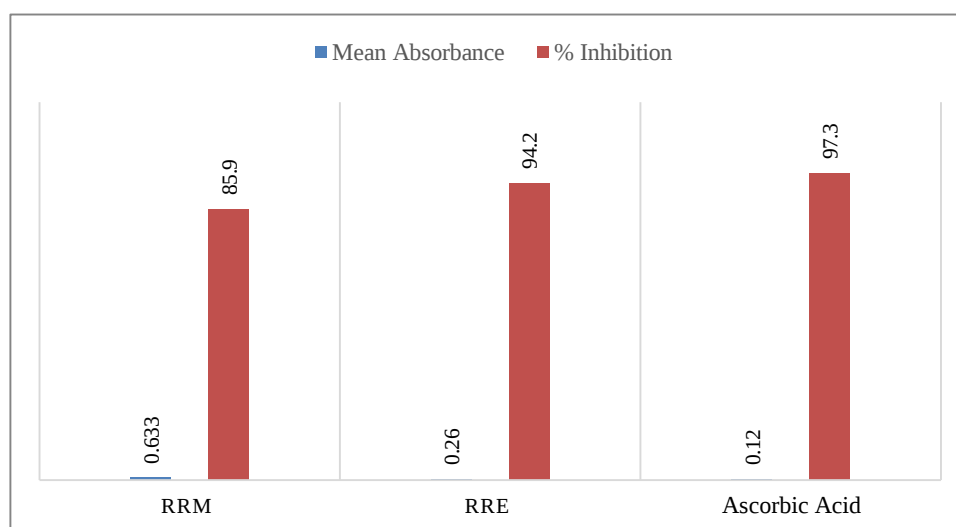
The DPPH assay revealed remarkable free radical scavenging activity across all tested fractions (Table 2). The ethyl acetate root fraction exhibited the highest activity (94.2% inhibition at 100 µg/mL), followed by dichloromethane (91.3%) and n-hexane (90.4%) fractions of roots. Aerial parts showed relatively 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 Extracts	Reading Sample 1	Reading Sample 2	Reading 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	RAM	0.821	0.844	0.821	0.828	81.6
6	RAN	0.72	0.78	0.782	0.763	83
7	RAD	0.66	0.658	0.665	0.661	85.3
8	RAE	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, RAM= Rheum Aerial methanolic Extract, RAN= Rheum Aerial n-Hexane Extract, RAD= Rheum Aerial DCM Extract, RAE= Rheum Aerial 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 drug 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 Karakoram region.

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