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Phytochemical analysis of wild plant of *Rheum Webbianum* L species a potential medicinal plant utilized by humans

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ABSTRACT

In modern medicines, the plants are either directly used as medicine or provide bioactive compounds or raw material for semi synthetic drugs or as markers for discovery of new bioactive compounds. *R. webbianum* is used for treatment of indigestion, abdominal disorders, boils, wounds and flatulence. It improves the memory of senile patients. The present study was carried out in the Kupwara Forest divisions in three different altitudes site 1=1800m, site 2= 2700m and site 3= 3500m. The species was identified by consulting the herbarium specimens and published data. Extraction of plant material The powdered roots and rhizomes of each sample (25 g) were charged in a soxhlet apparatus and extracted with 500 ml of HPLC methanol on water bath. All the data were subjected to two-way ANOVA analysis and Bonferroni multiple comparison test. Total carbohydrates were seen decreasing significantly with increasing altitude for both the years with maximum value at Site 1 42.4mg/g) during 1st year and minimum value at study Site 3= 3500m (30.5mg/g) during 2nd year, that is, the carbohydrate content was observed to be highest in low and mid elevations and lowest in high elevation. Total flavonoid content increased in high altitude areas. Moisture was screened in roots, leaves and callus of Rheum species. The moisture was least at Study Site 1= 1800m as 5.8% and highest at Study Site 3= 3500m as 6.7%. Crude fiber and crude fat in our study were observed in higher amounts at high altitude in comparison to the middle and low elevation. Due to high medicinal value, it is being over exploited and has been included in red list of threatened species of IUCN, thus necessitating the need for conservation and cultivation.

Keywords: *R. webbianum*, Carbohydrates, Extraction of plant material, Kupwara.

Introduction

The genus Rheum L. (commonly called Rhubarb), belongs to the family Polygonaceae of group Monochlamydae of Dicots, is world over represented by 60 species[1], of which only 7 species have been reported from the Indian subcontinent.[2] According to Stewart[3], all the seven species (including R. webbianum) reported from the Indian subcontinent, are also present in the Kashmir Himalaya. The genus "Rheum" was first reported by Carl Linnaeus in 1753.[4] Globally, this genus is represented by about sixty perennial species distributed in the mountains of Asia and Europe.[5] In the Indian subcontinent, this genus is represented by seven species,[6] all of them are present in Kashmir Himalaya.[3] Rheum webbianum is an important species of this genus inhabiting alpine regions between 2,400 and 4,300 m.a.s.l .[7]

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In modern medicines, the plants are either directly used as medicine or provide bioactive compounds or raw material for semi synthetic drugs or as markers for discovery of new bioactive compounds. As per WHO (World Health Organization) estimation, the present demand for plant based raw material is approximately USD (United States dollar) 14 billion per year with 15-25% annual increase. India is the second largest supplier (next to China only) of raw material of medicinal plants. In India, the medicinal plant related trade is estimated to be USD 1 billion per year.[8]

In India, most of these medicinal plants (more than 90%) are collected from wild source with unplanned, unscientific and unregulated way leading to shaky, exploitative and unsustainable supply. Further, local people are unaware of morphological features, leading to mixing of different many cases leading to intentional adulteration (Schippmann et al., 2006).[9] Commonly known as "Pambhak" (leaves) or "Pambchalan" (rhizome), it is medicinally important finding wider utilization in the pharmaceutical sector in the preparation of drugs combating cancer (Srinivas et al., 2007)[10] and body cholesterol.[11] It is also highly important in treating indigestion, abdominal disorders, boils, wounds, and gastritis through traditional medicinal practices.[7] Incessant exploitation of the plant along with cattle grazing and trampling has threatened its existence in nature. Unprecedented overexploitation has squeezed its populations and as such, it has been listed as a vulnerable medicinal herb from North–West Himalaya.[12, 13] In Kashmir Himalaya, which constitutes a part of North-West Himalaya, R. webbianum grows at higher elevations between 2,836 and 4,497 m.a.s.l.[14]

R. webbianum is used for treatment of indigestion, abdominal disorders, boils, wounds and flatulence.[15] It improves the memory of senile patients,[16] helpful in managing cancers.[10] Cultivation of plant tissue in synthetic media offers an alternative way of producing metabolites of interest to the traditional cultivation in the fields or greenhouses.[17] In successful cases, cell suspension cultures can offer a repeatable method to produce secondary metabolites from elite mother plants with easily controlled conditions and with a continuous supply of material. Genetic transformation may provide increased and efficient system for in vitro production of secondary metabolites. Tissue culture protocols have been developed for several medicinal plants, but there are many other species, which are over exploited in pharmaceutical industries and need conservation.

Materials and Methods

***Study Area and Plant Identification*:** The present study was carried out in the Kupwara Forest divisions with Latitude: 34° 01' 60.00" N and Longitude: 74° 15' 60.00" E in Kashmir Valley of Jammu and Kashmir, Union Territory of India. The present study was conducted from May 2023 to September 2024.

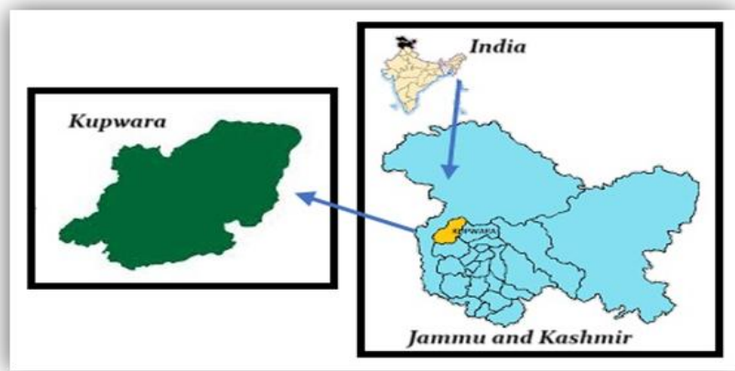

Figure-1: Study Area Kupwara district.

Fieldwork: Fieldwork was carried out in three different altitudes site 1=1800m, site 2= 2700m and site 3= 3500m of Kupwara district forest (**Figure.1)**. Kupwara district is hilly and mountainous in the north, west and east regions comprising of Pir-Panjal ranges of Lesser Himalayas with broad intermountain valley. The altitudes of the hill ranges ranging from 1800 m to 3600m.At all the sites, the species was studied throughout its different phenol phases, and necessary data were recorded and subjected to further analysis.

***Species identification and preservation*:** The species was identified by consulting the herbarium specimens and published data. The specimens collected during the present study were compared with the specimens' submitted at Herbarium of Department of Botany, Glocal University. Earlier published records related to morphology and distribution of this species were also referred for identification.[16, 7, 6] The roots and rhizomes of *R. webbianum* were kept in brown paper bags dried under room temperature. The in vitro explants i.e., leaves, roots and callus of these species were also dried in room temperature in paper bags. The dried roots and rhizomes of all these species were grinded in pestle and mortar to powder form for making methanol (HPLC grade) extracts.

***Extraction and phytochemical screening*:** The glassware and methanol (HPLC grade) was procured from commercial suppliers. Triple distilled water was used in the laboratory for different steps. Extraction of plant material The powdered roots and rhizomes of each sample (25 g) were charged in a soxhlet apparatus and extracted with 500 ml of HPLC methanol on water bath. The extraction was continued for one week. The extract was concentrated and dried on rotary evaporator under reduced pressure. The resultant semisolid, sticky extract of each sample was stored at 4ºC till further analysis. Each extract was subjected to phytochemical screening to detect the different types of constituents present in it. We used Dragendorff's test for alkaloids, Fehling solutions test for carbohydrates, Millions test for proteins, Shinoda test for Flavonoids, FeCl3 test for tannins and Sudan Reagent test for Fats and oils.

***Statistical Analysis*:** All the data were subjected to two-way ANOVA analysis and Bonferroni multiple comparison test.

Result and Discussion

Total carbohydrates were seen decreasing significantly with increasing altitude for both the years with maximum value at Site (142.4mg/g) during 1st year and minimum value at study Site 3= 3500m (30.5mg/g) during 2nd year, that is, the carbohydrate content was observed to be highest in low and mid elevations and lowest in high elevation. The roots showed the max number of, protein, fibre and flavonoid in all the observed samples. The maximum content of carbohydrates, fat and tannin was found in leaf sample, whereas least numbers content of fats are observed in roots (Table 1 and Figure 2).

Crude fiber and crude fat in our study were observed in higher amounts at high altitude in comparison to the middle and low elevation which was in agreement with the study done on red clover cultivars, where crude fat content was higher in mountain region in comparison to the low land region (Wang ETAL., 2007).[17] Total flavonoid content increased in high altitude areas. Total flavonoid content of the plant also significantly increased with increasing altitude, maximum amount of flavonoids was observed at study site 3= 3500m, that is, 5.6 mg/g and least present at study site 1=1800m, namely, 4.2 mg/g .Protein was high at Study Site 1=1800m as 30.5 mg/g and lowest as middle altitude as 27.8 mg/g.

The Rheum contains a number of anthraquinone derivatives. The free anthraquinones viz. rhein, emodin, aloe-emodin, physcion and chrysophamol are present in nearly all species (Peigen et al., 1984 and Li et al., 2000).[18, 5] Stilbene glycosides, including rhaponticin and the metabolite rhapontigenin, have been identified in the root (Ganie et al., 2017).[6]

Tannins, sennosides, catechins, gallic acid and cinnaminic acid have been identified (Zhu et al., 2005).[19] Lindleyin, a phytochemical with estrogenic activity, has also been described .[20] Oxalic acid, as well as 2-methylbutanol and 4-methylhexanol are present in the leaf blades. In the present study, the species of Rheum i.e., *R. webbianum* were screened for the presence of different phyto constituents. These were collected from Kashmir (India) at different altitudes. Rashid et al., has also reported the similar phytochemical behavior for *R. webbianum*. [21] In present studies, the moisture were screened in roots, leaves and callus of Rheum species. The moisture was least at Study Site 1= 1800m as 5.8% and highest at Study Site 3= 3500m as 6.7%. Apoptosis, as well as anti-tumor action, has been demonstrated and Rhubarb extract has been suggested as an adjunct to chemotherapy. Antiangiogenic action of Rhubarb has also been shown.[10, 17] It has been used in cases of GI bleeds to eliminate extra vasated blood.[10] Plants at high altitudes possess UV protective systems to cope up with the increasing harmful UV radiations with altitude. Phenols play a vital part in defence and other mechanisms of the plant for instance, hydrogen scavenging which could function less effectively when the temperature remains less and therefore, more phenolic compounds are required to avoid the loss happening to plants at low temperatures.[22] Total flavonoid content increased in high altitude areas. Total flavonoid content of the plant also significantly increased with increasing altitude, maximum amount of flavonoids was observed at site 3=3500m, that is, 5.6mg/g and least present at site 1= 1800m as 4.2mg/g [Table 1].

Table-2: Variation in phytochemical elements of R. webbianum L. growing in different altitudes.

Biochemical Analysis	Year	Study Site 1 1800m	Study Site 2 2700m	Study Site 3 3500m
Carbohydrate (mg/g)	1st Year	42.4 ± 4.5	31.9±4.07	**32±5.29**
	2nd Year	41.6 ± 2.68	35.8±4.09	**30.5±1.99**
Protein (mg/g)	1st Year	30.5 ± 0.97	27.8±3.65	**28.2±2.84**
	2nd Year	28.9 ± 0.68	28.4±1.34	**28.3±1.42**
Crude Fibre (%)	1st Year	4.4 ± 0.62	6.5±1.16	**6.6±0.78**
	2nd Year	6.7 ± 0.9	6.2±1.35	**6.7±0.58**
Ash Content (%)	1st Year	4.8 ± 0.46	5.4±1.26	**5.9±0.24**
	2nd Year	5.7 ± 0.77	5.8±0.46	**5.94±0.56**
Moisture content (%)	1st Year	5.8 ± 0.46	6.8±0.9	**6±0.2**
	2nd Year	6.0 ± 1.42	6±0.67	**6.7±0.73**
Crude fat (%)	1st Year	6.4 ± 0.62	6.5±1.16	**6.6±0.76**
	2nd Year	6.7 ± 0.9	6.2±1.35	**6.7±0.58**
Alkaloid (%)	1st Year	47.9 ± 0.9	48±3.96	**46.9±4**
	2nd Year	42.5 ± 5.07	47.3±2.69	**47.4±6.47**
Flavonoid (mg/g)	1st Year	4.2 ± 0.49	4.4±1.45	**5.6±0.17**
	2nd Year	4.3 ± 0.74	5.2±0.19	**5.5±0.86**
Tannin (mg/g)	1st Year	4.8 ± 0.08	4.8±0.07	**4.9±0.23**
	2nd Year	5.2 ± 0.38	4.9±0.08	**4.9±0.12**

Data are represented as Mean ± Standard Deviation.

Some studies have revealed that due to the various environmental factors happening at high altitudes, flavonoid accumulation was induced as a consequence of UV radiations. Flavonoid is considered as a foremost. Plant trait variations

are mostly environmentally based but sometimes, these can have a genetic background. In a study, variations were found in the morphological attributes and still retained when the plants were grown under similar environmental conditions which suggested a possible genetic background for those traits.

Conclusion

Plants are not randomly distributed in a particular elevational site, their distribution depends on the environment in which they grow and are able to functionally adapt themselves. Plant's ability to adapt to various ecological conditions relies on the morphological and physiological properties exhibited by its organs. Phytochemical variability helps plants to cope up with unsuitable environment for instance, in this study, In *R. webbianum* spiciforme, all constituents i.e., alkaloids, proteins, flavonoids, carbohydrates, tannins and, fats and oils were also present in all the samples. Total carbohydrates were seen decreasing significantly with increasing altitude for both the years with maximum value at Site 142.4 mg/g) during 1st year and minimum value at study Site 3=3500m (30.5mg/g) during 2nd year, that is, the carbohydrate content was observed to be highest in low and mid elevations and lowest in high elevation. Total flavonoid content increased in high altitude areas. Moisture was screened in roots, leaves and callus of Rheum species. The moisture was least at Study Site 1= 1800m as 5.8% and highest at Study Site 3= 3500m as 6.7%. Crude fiber and crude fat in our study were observed in higher amounts at high altitude in comparison to the middle and low elevation. Phenols and flavonoids which are also powerful antioxidants increase their levels in *R. webbianum* L. plants growing at higher elevations to combat stress. *R. webbianum* chemically adapted itself along the altitudinal range by regulation of its phyto-chemicals. Plants at high altitudes are equipped with defense system which activates biosynthesis of secondary metabolites, and the results demonstrate that plants can adapt to varying environmental circumstances happening along altitudinal gradient. All the phytochemicals are present in all the three sites with consistent variation. Hence, the findings of the present study illustrated the general phenomenon that the plants possess the medicinal ability in all the altitudes and to deal with the environmental stress by alternate mechanisms be it morphological, physicochemical, or phyto-chemical. Due to high medicinal value, it is being over exploited and has been included in red list of threatened species of IUCN, thus necessitating the need for conservation and cultivation.

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