

## Altitude acclimatization and concentration of active constituents and calorific value of two medicinal plant species *Rheum emodi* and *R. nobile* (Rhubarb) in Sikkim Himalaya

*Rheum emodi* Wall. ex. Meissen and *R. nobile* HK.f & Thoms (locally known as khokim, Sikkim cabbage) are herbaceous and rhizomatous species of great medicinal importance. *R. emodi* is included in the list of important species that are banned for export or need special export permit, while *R. nobile* is already a threatened plant species of India<sup>1</sup>.

Rhizomes of two *Rhubarb* species were analysed for the presence and concentration of anthraquinones (emodin and chrysophanol) and glycosides (rutin) from wild and conserved resources by high performance liquid chromatography (HPLC). The active contents recorded in *R. emodi* were greater than those in *R. nobile* in all respects however, conservation by ethnic groups of these high-altitude species at low altitudes showed only minor differences in the active contents.

Energy content for its calorific value was higher in the case of *R. nobile* than *R. emodi*. These two species studied were considered to estimate the superior varieties among the same genus and as a test case for their successful conservation.

*Rheum* (Polygonaceae) is a perennial herbaceous genus, which is commonly known as *Rhubarb*, distributed in the temperate and subtropical alpine regions of the Himalayas<sup>2</sup>. Ten species of *Rhubarb* are recorded from India; two species *R. emodi* and *R. nobile* have been reported from Sikkim Himalayas. *R. emodi* is a stout herb, distributed in the Himalayas from Kashmir to Sikkim at altitudes of 3300–5200 m asl. *R. nobile*, a pretty herb, is distributed in the Himalayas from Nepal to Bhutan, and Sikkim at altitudes of 3900–4800 m asl. *Rhubarb* is recognized by its broad leaf blades, subtended by elongated and often reddish celery-like petioles (leaf stalks).

Indiscriminate and non-systematic collection of these valuable medicinal plants by pharmaceutical companies for their active constituents and by ethnic

people for domestic and traditional herbal mixtures has put severe pressure on the availability of these plants. *Rheum* preparations are well known for their medicinal properties. Pharmacologically, besides the purgative effect which is widely known<sup>3</sup>, this genus has antimicrobial, antitumour and anti-inflammatory activities. Emodin and rutin have been isolated for antitumour activity<sup>4</sup>. The presence of the substituted tricyclic anthrones of emodin and its derived anthrones<sup>5</sup> in a diverse range of natural products, namely purpurquinone, olivine and resistomycin suggests that emodin might serve as a starting material for the synthesis of these compounds.

The investigations were carried out on plants of both the species harvested from and conserved at lower elevation by ethnic people and from their natural habitat in Sikkim Himalayas. The study was aimed to evaluate the effect of the altitude acclimatization of these medicinal plants with respect to their active constituents. The calorific value of root and shoot parts of both the species was estimated. The present work describes the qualitative and quantitative variations in anthraquinones and their glycosides in the rhizomes of two *Rhubarb* (wild and low-elevation conserved plants) species, viz. *R. emodi* and *R. nobile*. This would help to develop a future strategy for conservation of these threatened and valuable species.

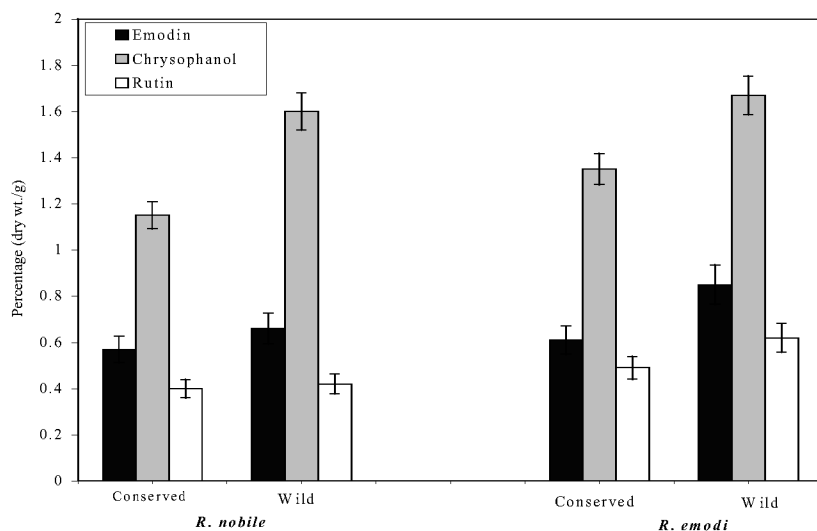
Survey work was conducted through a questionnaire with local users of these species among porters and glaziers. Observations were made in the field as well as among the folklore herbalists. Plant materials of both the species were collected from two altitudes; one from the gardens of local people at low altitudes (conserved) and other from its natural population (wild). *R. emodi* was harvested between 2500 and 2800 m altitude in conserved garden and between 3850 and 4000 m altitude in the wild, while *R. nobile* was harvested between 3000 and 3400 m altitude in

conserved garden and between 4364 and 4500 m altitude in the wild. Samples were dried in hot air at 40°C for 72 h until constant weight and powdered for analysis. Powder of both the species separately was extracted using 70% ethanol in a Soxhlet apparatus for 1 h on a water bath maintained at a temperature of about 60°C. The extract thus obtained was filtered and dried in vacuum.

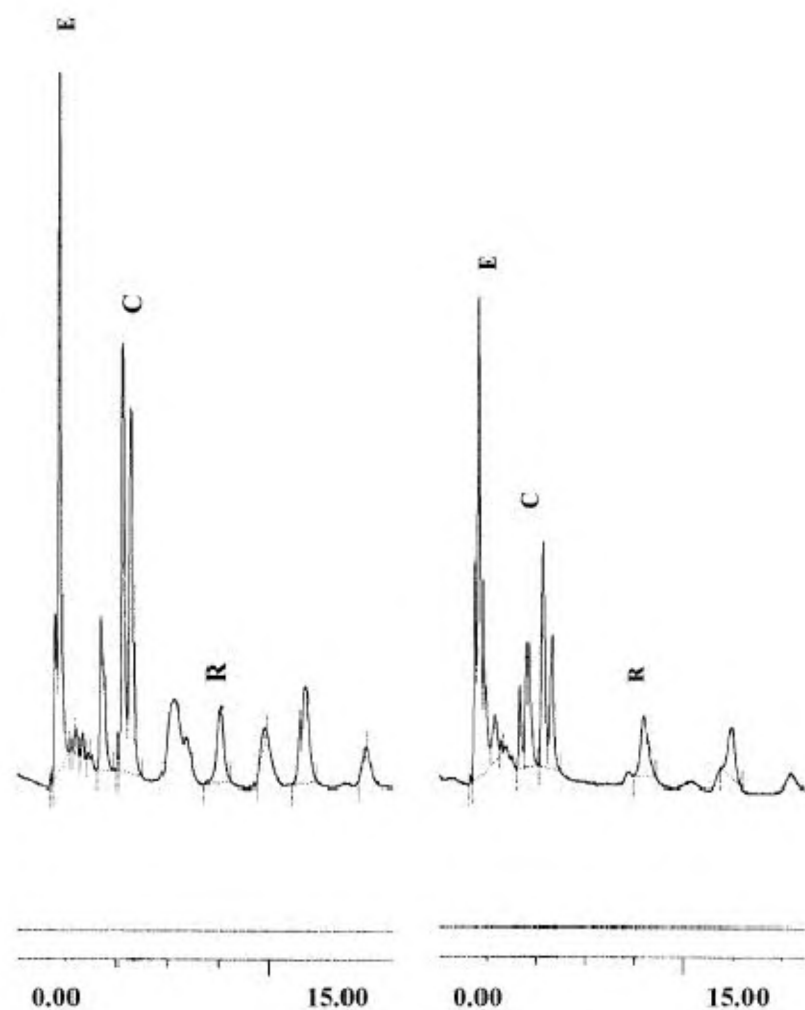
Crude extract (0.1 mg) was dissolved in 10 ml of acetonitrile : water : acetic acid in the ratio of 75 : 20 : 5 and from this stock solutions of 1, 4, 8, 10 and 20 ppm were prepared. Emodin, chrysophanol and rutin (purchased from Sigma Chemicals) were used for establishing the calibration curve. Solutions were filtered before running through a 0.45 µm millipore filter. Beckman system Gold HPLC consisting of two pumps, a 20 µl loop injector and reverse phase DS ultra-sphere column packed with silica (C-18; 4.5 × 250 mm) was used for analysis. A mixture of acetonitrile : water : acetic acid (75 : 20 : 5) as a solvent for the elution at a flow rate of (1 ml/min) was used.

Sample solution of 20 µl was injected and components were detected at  $\lambda_{\max} = 254$  nm using variable wavelength UV detector. Components were identified by simultaneous run-off standard compared with their retention time and quantified by standard peak area method.

For the estimation of the energy content, the oven-dried plant material was powdered. One gram powdered dry matter of both the species was taken for estimation of energy with the help of oxygen bomb calorimeter. Platinum wire of 10 cm length was inserted into each 1 g sample before compaction so that it could be tied up with the electrodes. The material was ignited electrically inside the oxygen-filled steel bomb of the oxygen bomb calorimeter and the rise in the temperature was recorded by its thermometer. The energy content was calculated using the formula by Lieth<sup>6</sup>.



**Figure 1.** Emodin, chrysophanol and rutin contents in the rhizomes of two *Rhubarb* species, *R. nobile* and *R. emodi*, collected from conserved low-altitude region and wild alpine region plants.

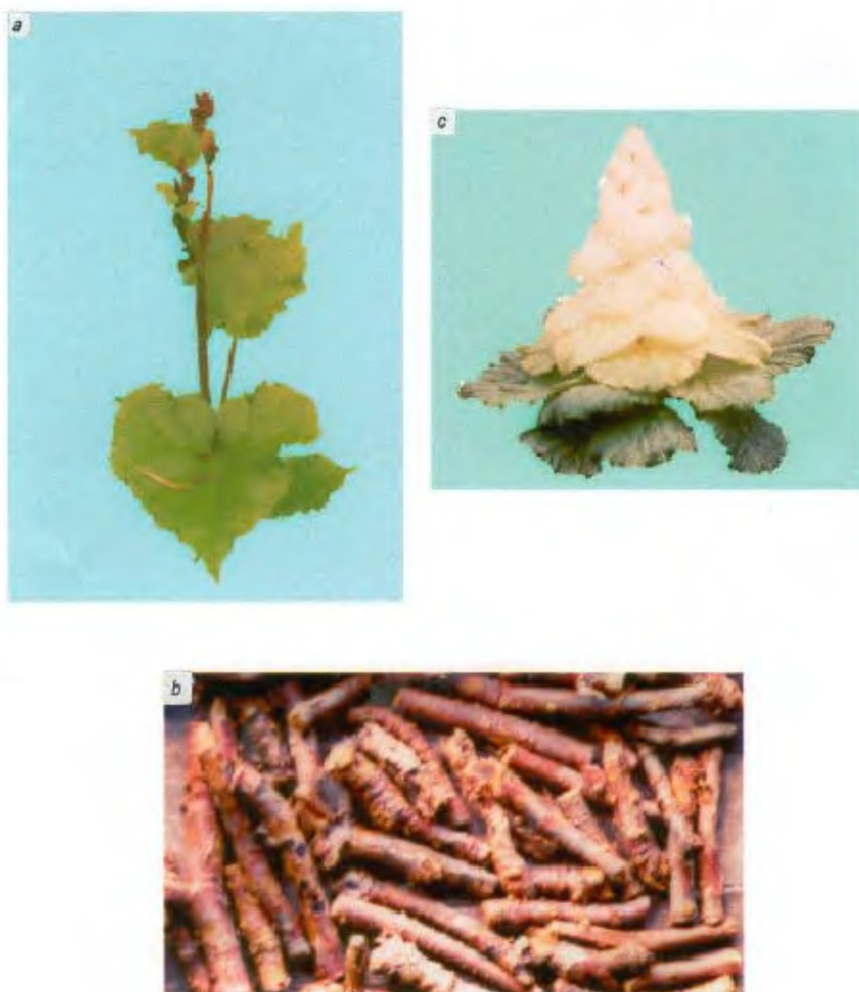


**Figure 2.** HPLC chromatogram of *a*, wild plant extract; *b*, conserved plant extract. E, Emodin; C, Chrysophanol; R, Rutin.

The average changes of active compounds are shown in Figure 1. On comparing the contents of both *Rhubarb* species, the highest values were obtained for emodin, chrysophanol and rutin in the wild and conserved harvested tubers of *R. emodi* (Figure 1). As for the total amount, the highest values for emodin (0.85% dry wt/g), chrysophanol (1.67% dry wt/g) and rutin (0.62% dry wt/g) and the lowest value for emodin (0.61% dry wt/g), chrysophanol (1.35% dry wt/g) and rutin (0.49% dry wt/g) were observed in the tubers harvested from wild and conserved plants of *R. emodi* (Figure 1). A fair amount of emodin (0.66% dry wt/g), chrysophanol (1.60% dry wt/g) and rutin (0.42% dry wt/g) and the lowest values for emodin (0.57% dry wt/g), chrysophanol (1.15% dry wt/g) and rutin (0.40% dry wt/g) were estimated in the tubers harvested from wild and conserved plants of *R. nobile* (Figure 1).

High calorific values have been recorded in *R. nobile* in comparison to *R. emodi*. Regarding total amount of energy, the highest values in root ( $4078 \text{ cal g}^{-1}$ ) and shoot ( $3645 \text{ cal g}^{-1}$ ) were obtained in the *R. nobile* plant parts and lower values in root ( $3625 \text{ cal g}^{-1}$ ) and shoot ( $3466 \text{ cal g}^{-1}$ ) were obtained in *R. emodi* plant parts.

The major active constituents are qualitatively comparable between both the *Rhubarb* species; however, in the presence and absence of glycosides and high variations in the quantity of anthraquinones, *R. nobile* seems to differ from *R. emodi* (Figure 1). Active constituents of both the plant species were found to decrease in low-altitudes conserved plants compared to the wild plants, however, differences in each active constituent, viz. chrysophanol, emodin and rutin were small (Figure 1). These differences which were consistent for all active constituents could have resulted either because of shifting the plants from their alpine to semi-natural conditions or from wild to cultivation conditions or synergistic both. The results suggest that among these *Rhubarb* species, *R. emodi* is superior in terms of high-yielding active constituents. It has been reported that qualitative and quantitative changes in alkaloids of *Aconitum* depend on the plant species as well as on seasonal and ecological factors<sup>7</sup>. It has been noticed



**Figure 3.** *Rheum emodi*, **a**, Flowering branch; **b**, Harvested rhizomes/roots; **c**, *Rheum nobile*, a young-grown herb of Sikkim.

that *R. emodi* occurs at lower elevation, from 3300 m, while *R. nobile* occurs at higher elevation from 3900 m. Data indicated that high percentage of active constituents was obtained in the tubers harvested from wild as well as conserved plants of *R. emodi* (Figures 1 and 2). The results suggested that *R. emodi* could be cultivated in further moderate elevations; semi-natural conditions in Sikkim and Himalayan regions can meet the demand for the use of these plants by pharmaceutical companies and in ethnic medicines.

To conclude, at lower elevation both the *Rhubarb* species numbers may increase in cultivation for higher yield of their constituents. These results suggest that *R. emodi* can be cultivated in further low altitudes, while *R. nobile* in moderate elevation. Therefore, cultivation of these plant species in semi-natural conditions *ex situ* can meet the demand for these plants, while *in situ* conservation in nature may be planned for the future of these species. Protection of these plant species in the natural environment will be the best conserva-

tion technique, because of its high altitude and low climatic adaptability.

1. Jain, S. K. and Sastry, A. R. K., *Threatened Plants of India*, 1980.
2. Anon, *The Wealth of India*, 1972. vol. 2, pp. 3–6.
3. Peigeon, X., Liyi, H. and Liwei, K. S. J., *Ethnaopharmacology*, 1984, **10**, 273.
4. Chiunghua, C., Yougchuan, C. and Diendong, I., *Acta Pharmacol. Sin.*, 1966, **13**, 362.
5. Jacobson, R. and Adams, R. J., *Ann. Chem. Soc.* 1924, **46**, 1321.
6. Lieth, H. F. E., Proceedings of the Copenhagen Symposium, 1968.
7. Okamoto, T., *Kagaku to Kogyo*, 1972, vol. 14 (in Japanese).

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