J. Indian Chem. Soc.,
Vol. 92, March 2015, pp. 375-378

Variation in picrotin and picrotoxin content of *Picrorhiza kurroa* Royle ex Benth rhizomes from Garhwal Himalaya

Neetu Bohra[a], P. Prasad[c], Geeta Tewari*[b] and Lalit M. Tewari[a]

[a]Department of Botany, [b]Department of Chemistry, D. S. B. Campus, Kumaun University, Nainital-263 002, Uttarakhand

E-mail : geeta_k@rediffmail.com

[c]High Altitude Plant Physiology Research Centre (HAPPRC), H. N. B. Garhwal University, Srinagar Garhwal-246 174, Uttarakhand

Manuscript received online 18 August 2014, revised 04 September 2014, accepted 04 September 2014

Abstract : *Picrorhiza kurroa* Royle ex Benth (Scrophulariaceae), commonly known as Kutki, is an important medicinal herb in the traditional ayurvedic system of medicine and has been used to treat various diseases. It is known to contain iridoid glycosides (picrotin and picrotoxin) which are found responsible for the medicinal effects of Kutki. This study addresses variability at active ingredients level among populations of rhizomes of *Picrorhiza kurroa* Royle ex Benth growing at two different altitudes of Garhwal Himalayas. The plants were collected from two different altitudes, viz. Tungnath (3600 m) and Pothivasa (2200 m) in Rudraprayag district of Garhwal Himalaya, Uttarakhand, India, and analyzed by HPLC (High Performance Liquid Chromatography). A considerable degree of variation in amount of active contents was observed. Picrotin and picrotoxin content was found highest in populations collected from Tungnath compared to Pothivasa suggesting that active content accumulation is directly correlated with altitudinal variation. The present study reveals that there is a remarkable variation in the amount of picrotin and picrotoxin content with increasing altitude.

Keywords : *Picrorhiza kurroa* Royle ex Benth, HPLC, picrotin, picrotoxin, altitude.

Introduction

Picrorhiza genus belonging to family Scrophulariaceae is comprised of two Indian species, *Picrorhiza kurroa* Royle ex Benth and *P. scrophulariiflora* Pennel. The common trade and vernacular name kutki is applied to both. It is distributed in the Himalayas from Kashmir to Sikkim at an elevation of 2700–4500 m. It is found in rocky slopes, grasslands in the alpine region Himalayas and is propagated by rhizomes, stolons and offsets. The plant is a small, hairy perennial herb with a woody elongate creeping root, stock, leaves almost radical spathulate, base narrowed into winged sheathing petiole, flowers white or blue, stamens four, capsule are egg shaped flowering fruiting occurs in June-August[1].

It secretes a large quantity of glycosylated bitter principles named kutkosides and picrosides, which are the main constituent of the drug "Kutkin"[2]. The drug, in the form of dried roots and stolons, is prescribed in the treatment of several ailments of the liver and spleen, and in cases of fever and asthma[3,4]. It is antiperiodic, cholagoque, stomachic, laxative in small doses and cathartic in large doses[5]. Kutkin, a glycoside mixture showed hepatoprotective and bile flow enhancing activities[6]. A drug named picroliv (iridoid glycoside fraction of roots and rhizomes of *Picrorhiza kurroa* Royle ex Benth containing at least 60% of 1 : 1.5 mixture of picroside-I and kutkoside) has been developed for the treatment of acute and chronic hepatitis and healthy carriers[7].

There are several High Performance Liquid Chromatography (HPLC) methods[8,9] in the literature for the estimation of picroside I and kutkoside which is part of the formulation picroliv. A number of HPLC methods[10,11] have been reported for the quantification of picroside-I and picroside-II. Several other methods are proposed by many workers for the quantification of iridoid glycosides from *Picrorhiza* species[12–15]. Variations among the populations of some high altitude species have been reported[16]. Increasing demand for the kutki drug has prompted many

researchers to search for sources of genotypes of *Picrorhiza kurroa* Royle ex Benth rich in picrotin content. Effect of altitude on picroside content of *Picrorhiza kurroa* Royle ex Benth from Kashmir[17] and Shimla[18] has been investigated. To, the best of our knowledge, no work has been reported on the effect of altitude on picrotin and picrotoxin content of *Picrorhiza kurroa* Royle ex Benth from Garhwal Himalayas. Therefore, the objective of this study was to assess the active content in *Picrorhiza kurroa* Royle ex Benth populations collected from high altitudes of Garhwal Himalayas to identify superior genotypes for further cultivation, exploitation, and utilization in Pharmaceutical Industry and determine if picrotin and picrotoxin content correlates with different elevation zones.

Results and discussion

Picrotin and picrotoxin content were estimated in samples collected from different altitudes in the Garhwal Himalayas. HPLC results revealed variations in the distribution and relative content of picrotin and picrotoxin extracted from two different altitudinal populations. This variation was found associated with the altitude. It appears that, *Picrorhiza kurroa* Royle ex Benth from Tungnath population was characterized by the maximum picrotin and picrotoxin contents (Figs. 1 and 2).

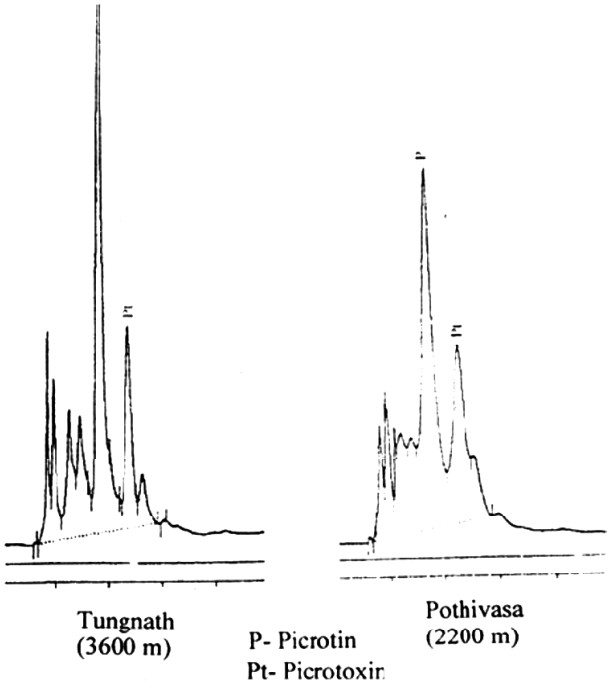

Fig. 1. Quantitative determination of picrotin and picrotoxin in *Picrorhiza kurroa* Royle ex Benth at two altitudes by HPLC.

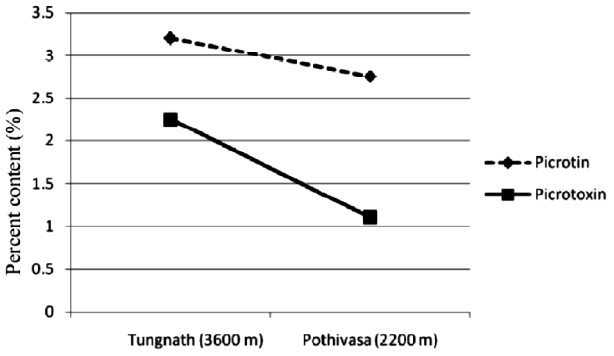

Fig. 2. Variation in active constituents in *Picrorhiza kurroa* Royle ex Benth at two diffe-rent altitudes.

Picrotin and picrotoxin content was found to be 3.20% and 2.25% for Tungnath while it was 2.7% and 1.10% for Pothivasa populations.

The data showed that as the altitude increases, the concentration of picrotin and picrotoxin also increases, which suggested that the concentration of active contents is affected with the change in altitude. The alpine climatic conditions are characterized by high ultra-violet radiation, low atmospheric pressures and oxygen concentration, with the minimum air temperature dropping near freezing point every night. The sharp change in day and night temperature and the permafrost conditions which are often found a few inches below the soil surface, even during the active growth season, forms the stress conditions for the plant growth. Alpine plants however still manage to grow under these conditions. Plants growing there are morphologically, genetically and physiologically well adapted. They appear as different growth forms, secrete secondary metabolites to tolerate stress conditions. Therefore, it can be concluded that the higher concentration of active contents at higher altitude may help the plants better adapt to their climatic conditions. Earlier studies reported that altitude affects the quantity and quality of secondary metabolites[18–21].

Zidron *et al.* (2005)[19] suggested that there was positive correlation between the altitude of collection site and contents of flavonoids and phenolic acid in *Crepis capillaris*, *Hieracium pilosella* and *Hypochaeris radicata* from New Zealand. In case of *Arnica montana*, sesquiterpene lactones and flavonoids were not positively correlated with the altitude of the growing side[20]. In a study from Kumaun Himalaya, it was observed that the content

of germacrene D in *Craniotome furcata* was positively correlated to the altitude of the collection region[21]. Results of Pandit *et al.*[18] revealed that picrosides content of *Picrorhiza kurroa* Royle ex Benth was highest at high altitude as compared to lower altitude collections. In our study, similar results for picrotin and picrotoxin were observed as their content was higher in *Picrorhiza kurroa* Royle ex Benth collected from high altitude. Study revealed that plants growing in Tungnath had better glycoside contents than that of Pothivasa. So it is concluded that Tungnath population had superior genotype than that of Pothivasa and this elite germplasm can be used for exploitation and multiplication of genotype for commercial cultivation and its utilization in Pharmaceutical industries.

Experimental

Solvents and chemicals :

All the solvents and chemicals used were HPLC grade purchased from Ranbaxy Chemicals (Mohali, Punjab, India). Standard picrotin and picrotoxin were purchased from Sigma-Aldrich, New Delhi, India. HPLC grade water was purchased from Merck, India.

Study area and collection of plant material :

Subalpine-alpine region of Garhwal Himalaya (77° 33′5″ to 80° 6′E longitudes and 29° 31′9″ to 31° 26′5″ N latitudes) known for the occurrence of the species was surveyed and two locations namely; Tungnath (3600 m) and Pothivasa (2200 m) in Rudraprayag district of Garhwal, Uttrakhand, India were selected for collection. The rhizomes of each collection, was air-dried separately and ground to a fine powder for preparation of plant extract.

Preparation of Picrorhiza kurroa Royle ex Benth extract :

The dried powered rhizomes (100 mg) were extracted using 20 ml of 70% ethanol in a Soxhlet apparatus for 4–5 h maintained at temperature of about 60 °C. The extract thus obtained was filtered through Whatman No. 1 filter paper and dried in vacuum pressure. The dry extract (0.1 mg) was dissolved in 10 ml of HPLC grade mobile phase, water : methanol : isopropanol : acetonitrile in the ratio 60 : 30 : 5 : 5. Picrotin and picrotoxin were used for preparation of calibration curves. Stock solutions of 1000 mg/ml were prepared and from these stock solutions, working solutions (10, 20, 40, 80 and 100 ppm) for each reference compound were prepared by using HPLC grade water. Ten micro-liter of working solutions were taken for establishing calibration curves. Solution was filtered through Millipore filter paper (0.45 μm) before using them.

HPLC analysis :

The standard HPLC system consisted of 1.25 Beckman System Gold with two liquid pressure pumps, a high value equipped with a 20 μL loop as a control on retention behaviour of standard and a normal phase injector, reverse phase ODS Bondapak Column (4.5 × 250 mm). Mixture of water : methanol : isopropanol : acetonitrile (60 : 30 : 5 : 5) being the mobile phase at a flow rate 1 ml/min 20 μL of sample solution was injected and glycosides were detected at emox 220 nm using 166 Beckman System Gold variable wavelength UV detector. Components were identified by simultaneous run of standard with its retention time.

References

1. N. Chettri, E. Sharma and S. D. Lama, *Lyonia*, 2005, **8**, 89.
2. B. K. Kapahi, T. N. Srivastava and Y. K. Sarin, *Int. J. Pharmacogn.*, 1993, **31**, 217.
3. G. N. Chaturvedi and R. H. Singh, *J. Res. Ind. Med.*, 1966, **1**, 1.
4. W. Dorsch, H. Stuppher, H. Wagner, M. Gropp, S. Demolin and J. Ring, *Int. Arch. All. Appl. Immunol.*, 1991, **95**, 128.
5. V. J. Ram, *Drug News Perspect.*, 2001, **14**, 353.
6. S. S. Handa, A. Sharma and K. K. Chakravarty, *Fitoterapia*, 1986, **58**, 307.
7. B. N. Dhawan, "Plant Based Pharmaceuticals in Developing Countries", Current R & D High Lights, Central Drug Research Institute, Lucknow, 1993.
8. H. Stuppner and H. Wagner, *Sci. Pharm.*, 1992, **60**, 73.
9. S. Cheng, J. Huang and J. He, *Pharmazie*, 2007, **62**, 577.
10. S. Sturm and H. Stuppner, *Chromatogr.*, 2001, **53**, 612.
11. B. Singh, P. Bhandari, N. Kumar and V. K. Kaul, *J. Chromatogr. (A)*, 2008, **1194**, 257.
12. R. C. Gupta, K. Vipul and M. Nitin, *J. Chromatogr. (B)*, 2005, **820**, 221.

13. P. Bhandari, N. Kumar, B. Singh and V. K. Kaul, *J. Chromatogr. (A)*, 2008, **1194**, 257.

14. P. Bhandari, N. Kumar, B. Singh, A. P. Gupta, V. K. Kaul and P. S. Ahuja, *Chromatogr.*, 2009, **69**, 221.

15. H. Junqi, M. Yanmei, C. Shan, L. L. Michael and L. Dali, *Chromatogr.*, 2008, **68**, 1027.

16. H. Purohit, B. P. Nautiyal and M. C. Nautiyal, *J. Plant Physiol.*, 2008, **3**, 154.

17. M. Katoch, I. S. Fazil, K. A. Suri, A. Ahuja and G. N. Qazi, *J. Nat. Med.*, 2011, **65**, 578.

18. S. Pandit, K. Shitiz, H. Sood and R. S. Chauhan, *J. Plant Biochem. Biotechnol.*, 2012, **22**, 335.

19. C. Zidorn, B. Schubert and H. Stuppner, *Biochem. Syst. Ecol.*, 2005, **33**, 855.

20. R. Spitaler, P. D. Schlorhaufer, E. P. Ellmerer, I. Merfort, S. Bortenschlager, H. Stuppner and C. Zidorn, *Phytochemistry*, 2006, **67**, 409.

21. S. Singh, G. Tewari, C. Pande and C. Singh, *Rec. Nat. Prod.,* 2013, **7**, 332.