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Elite chemo-types of a critically endangered medicinal plant *Picrorhiza kurroa* Royle ex Benth from Indian Western Himalaya

Mehra TS, Raina R, Rana RC, Chandra P and Pandey PK

Abstract
Picrorhiza kurroa Royle ex. Benth belonging to family Scrophulariaceae is a high value perennial medicinal herb endemic to the alpine Himalayan region of India. The present study is focused towards the evaluation of Picrosides (Picroside-I and Picroside-Il) content in roots and rhizomes of *P. kurroa* populations collected from different parts of Himachal Pradesh and Uttarakhand, which could be utilized by the Indian drug industries for the preparation of quality herbal drug. Maximum Picroside-I content was recorded in rhizomes in Kafnu (HP) populations (1.85 %), while in roots it was recorded maximum in Rampur (UK) populations (0.50%). It is also notable that the concentrations of both Picroside I & Picroside II were found maximum in rhizomes as compared to roots. P- II content in rhizomes was maximum in the Sainj (HP) populations (3.54%), while in roots it was found maximum (3.38 %) in Sairopa (HP) populations.

Keyword: *Picrorhiza kurroa;* Picroside; Medicinal plant; Herbal; HPLC; Endangered

Introduction
Picrorhiza kurroa Royle ex Benth. is a critically endangered high value medicinal plant distributed throughout Himalayan region from Kashmir to Arunachal Pradesh, used in both traditional as well as modern day systems of medicine, the rootstock of which constitutes the drug which is used as hepato-protective, anti-periodic, cholagouge, stomachic, anti-amoebic, anti-oxidant, anthelmintic, anti-inflammatory, cardio-tonic, laxative, carminative, expectorant, choleretic, hypolipidemic and antispasmodic etc. (Tiwari *et al.*, 2012; Bhattacharjee *et al.*, 2013; Sultan *et al.*, 2016; Mahajan *et al.*, 2016) [27, 2, 26, 14]. It is widely used as liver-stimulant, appetite stimulant, as a laxative and febrifuge. It is also used to treat various ailments such as bronchial asthma, epidemic jaundice, viral hepatitis diabetes, jaundice, liver, spleen disorders, skin diseases gastrointestinal problems such as indigestion and constipation (Meena *et al.*, 2010) [17]. It is reported that the underground portions of *P. kurrooa* produced a crystalline product "Kutkin", which constitute two principle iridoid glycosides Picroside and Kutkoside (Patil *et al.*, 2013) [19]. "Kutkin" is composed of picroside-I and picroside-II. Rhizomes of *P. kurroa* show the presence of iridoids, acetophenones and cucurbitacins in different chemical study. It is known to be a good source of biologically active compounds picroside-I and II (Bantawa *et al.*, 2010; Gaikwad *et al.*, 2011; Sah and Varshney, 2013) [1, 8, 20]. Among the several active chemical constituents reported, its main constituents arepicroside I, II and III, picrorhizin, kutkoside, kurrin, kuthinol, kutkiol, kutkisterol, kutkoside, androsin, apocynin, drosin and cucurbitacin (Meena *et al.*, 2010) [17], Aromatic esters Vanillic acid capriate, Myristylpicraldehyde and Lauryl picraldehyde (Kumar and Sharma, 2014) [12]. Biosynthesis of Picrosides is an amalgamation four biosynthetic route involving non-mevalonate (MEP), mevalonate (MVA), phenylpropanoid and iridoid pathways. Picrosides are monoterpenoids consisting iridoid backbone and glycoside moiety. Among Picrosides, P-I consists cinnmate and P-II consists vanillate moiety and thus, both classified accordingly, derived from phenylpropanoid pathway. Their Iridoid backbone is a resultant of geranylpyrophosphate (GPP), which is further synthesized from isopentenyl pyrophosphate (IPP) and its allelic isomer dimethyl allyldiphosphate (DMAPP) by head to tail condensation through cytosolic mevalonate (MVA) and/or plastidic non-mevalonate (MEP) pathway (Singh *et al.*, 2012; Mahmoud and Croteau, 2001; Hampel *et al.*, 2006; Shitiz *et al.*, 2015) [24, 15, 9, 23].
Extensive harvesting and limited cultivation of *P. kurroa* has threatened its natural presence and lead it to listed as "critically endangered" species by International Union for Conservation of Nature and Natural Resources. The plant has been included in the Appendix II of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora- an

international agreement between governments) list with the aim of sustainable use and conservation without any compromise (Shitiz *et al.*, 2013) [22]. *Picrorhiza kurroa* is among one of the topmost traded 15 plant species in India in relation to their economic value and more than 10,000 kg of their marketing takes place only in the Delhi market. The prices of *Picrorhiza kurroa* varied in range of Rs. 220- 340 /kg during 2007- 10 which is hiked up to Rs. 475 /kg in 2016 in Indian market (Shitiz *et al.*, 2013; Anonymous, 2016) [22]. India contributed only about 70 tonnes of *Picrorhiza kurroa* supply in context to global supply of 375 tonnes (excluding China and Pakistan) in their actual demand of 5000 tonnes annually which is mainly collected from Himachal Pradesh, Jammu and Kashmir, Uttarakhand and Sikkim to satisfy the demand of drug industries (Shitiz *et al.*, 2013) [22]. Hence, to fulfill the actual demands of 5000 tonnes and this is also going up year by year the study and conservation of *Picrorhiza kurroa* has become essential.

A perusal of literature revealed that although *P. kurroa* is very well known for its medicinal importance, but informations regarding variability in active content (chemo-types) are scanty. Such information is essential in isolating better strains in terms of active content, understanding and further refinement of propagation methodology for cost-effective large scale cultivation. The major aim of the present study is to evaluate the Picroside (Picroside-I and Picroside-II) content of *P. kurroa* found in the different location of Himachal Pradesh and Uttarakhand utilized by the Indian drug industries for the preparation of quality herbal drug with respect to standard Picroside compounds.

Materials and methods

The studies of active content of picroside I and picroside II from *P. kurroa* was undertaken in the laboratory and experimental farms of Department of Forest Products, Dr. Y.S. Parmar University of Horticulture and Forestry at Nauni (1250 m amsl, Distt. Solan) and Rahla (2800 m amsl, Distt. Kullu) respectively.

Evaluation of different populations for bitter principle content

P. kurroa populations, collected from different places located in Himachal Pradesh and Uttarakhand, were maintained at Rahla farm of the university under uniform environmental conditions for more than two years. 15 treatments of different populations in triplicate were analyzed for picroside I peroxide II content in rhizomes and roots, separately for each population through HPLC method. Data were analyzed by RBD.

Preparation of standard curve

Five different known concentrations (50, 75, 100, 125 and 150 µg/ml) of standard compounds were injected to HPLC (Shimadzu) on shim-pack C-18 Reversed column (4.6 x 250 mm) with a reversed phase guard column, LC-10 AT pump, spherical silica (5 µm particle and 100 Aº pore diameter). Mobile phase was methanol: water (35: 65) and flow rate was 1.5 ml/minute. The chromatogram was scanned up to 35 minutes and detection was done at 270 nm on SPD-M10 photodiode array detector with the help of SPD-MXA software.

The calibration graph was plotted between the concentration and area of respective standards. A linear regression equation was obtained to estimate concentrations of Picroside I and Picroside II in test samples. The equation for Picroside I and Piroside II was Y = 12594 X and Y = 12592 X respectively with 96 % correlation coefficient.Where,Y = corresponding peak area of compound in test sample and X = concentration of compound analysed in µg.

Sample preparation

Oven dried (50ºC) finely ground material (1 gm) of each rhizome and roots of different populations were refluxed (30 minutes) with methanol (2x15 ml) on a boiling water bath. Each time the contents were filtered and residue was washed with methanol. The filtrates and washings were combined and final volume was made to 50 ml with methanol. 1 ml of the extract was further diluted to 10 ml and subjected to HPLC analysis. All the rhizome and root samples were analyzed by the same procedure (used for preparation of standard curve) in triplicate and accordingly percentages of Picroside I and Picroside II were worked out in all the populations with the help of regression equation.

Results

A total of 15 populations (11 from Himachal Pradesh and 4 from Uttarakhand) were evaluated for picroside I and picroside II content in rhizomes and roots separately and the data is presented in Table 1 (Figure1 – 3).The Picroside II content ranged from 1.30 to 3.54 per cent in rhizomes and 1.17 to 3.36 per cent in roots, whereas Picroside I content ranged from 0.13 to 1.85 per cent in rhizomes and 0.05 to 0.50 per cent in roots. Maximum Picroside II content of 3.54 and 3.36 % was obtained in rhizomes of Sainj (HP) and roots of Sairopa (HP) population, respectively, whereas the minimum 1.30 and 1.17 per cent was observed in rhizomes as well as roots of Rampur (UK) population respectively. Maximum Picroside I content of 1.85 & 0.50 per cent was observed in rhizomes of Kafnu (HP) and roots of Rampur (UK) population while as rhizomes and roots of Pulga (HP) contained the minimum percentage of 0.13 & 0.05 percent respectively.

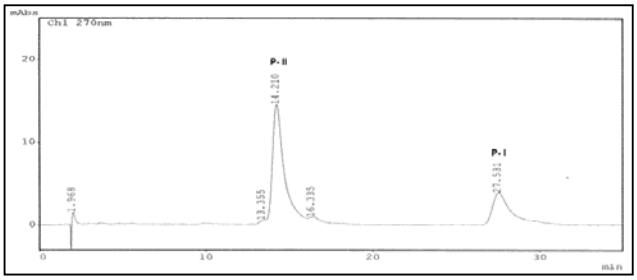

Fig 1: Reference compound Chromatograms (HPLC) of Picroside I and II.

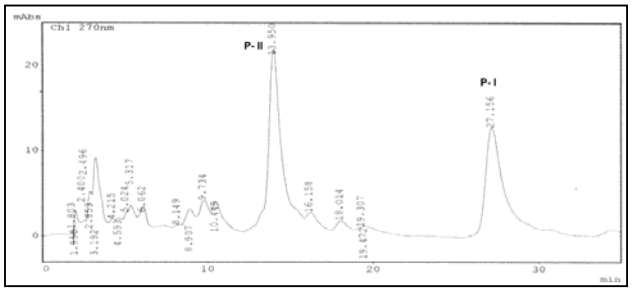

Fig 2: Rhizome extracts Chromatograms (HPLC) of Picroside I and II.

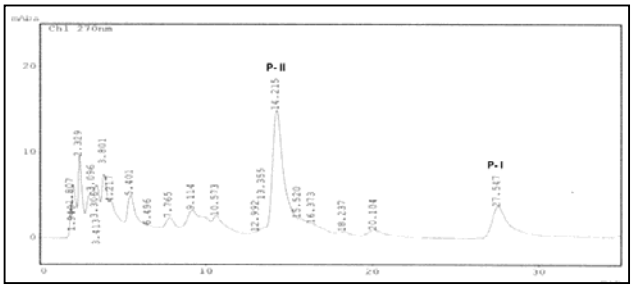

Fig 3: Root extracts Chromatograms (HPLC) of Picroside I and II.

Discussion

The medicinal properties of plants are primarily due to the presence of secondary metabolites with specific therapeutic value. Often the nature of the germplasm resource of a particular medicinal plant is determined on the basis of relative percentage of such secondary metabolites. Picroside I and Pricroside II are the two medicinally important secondary metabolites occurring in rhizome and roots of *P. kurroa*.

As evident from the Table- 1, it has been observed that, rhizome contains more Picroside-II and Picroside-I than the roots, which is also supported by earlier studies (Kaul and Kaul, 1996) [10]. Again amongst these two, concentration of Picroside-II was found to be higher as compared to Picroside-I in rhizomes as well as roots and this observation confirms the earlier studies (Dutt *et al.*, 2004) [4].

The various populations of this species analysed for active content Picroside I &Picroside II showed large variability (although all the germplasms were maintained under uniform environmental conditions) thereby indicating the existence of several chemo-types. It appears that such chemo-types have arisen as a result of genetic recombinations.

Picroside II conent was found to be minimum in rhizomes (1.30 %) and roots (1.17 %) of Rampur (UK) population, whereas it was observed to be the maximum in rhizomes (3.54 %) of Sainj (HP) population and roots (3.36 %) of Sairopa (HP) population. Pulga (HP) population was observed to have the minimum Picroside I content of 0.13 per cent (rhizomes) and 0.05 per cent (roots), whereas the maximum Picroside I content in rhizomes (1.85 %) was observed in Kafnu (HP) population and in roots (0.50 %) was observed in Rampur (UK) populations. Variation in active content in rhizome and roots of *P. kurroa* has also been reported earlier (Kaul and Kaul, 1996 and Chauhan *et al.*, 1999) [10, 5].

A similar study by Shitiz *et al.*, (2013) [22] also supports our finding of good nature of *P. kurroa* samples from the Himachal Pradesh qualitatively and quantitatively. *P. kurroa* samples; Manali (from Manali Market), Himachal Pradesh (from Amritsar Market), Nepal (from Delhi Market), Uttarakhand (from Delhi Market) and China (from Delhi Market) were assessed for the quality of chemical constituents of Picrosides (Picroside-I and Picroside-II) through Reverse Phase HPLC. It was found that the samples of Himachal Pradesh sold in the Amritsar Market having highest content of Picrosides (10.9%) in comparisons to the other places of samples sold in the market with the samples from China sold in the Delhi market having lowest concentration of Picrosides (2.8%) (Shitiz *et al.*, 2013) [22].

The effectiveness and quality of herbal drug formulated from *P. kurroa* was realized on the accurate concentration and ratio of Picroside (P-I and P-II) available in particular plant genotypes because biosynthesis and accumulation of P-I and P-II varied in different plant's part or tissues. It is also found that the biosynthesis of P-I and P-II takes place in shoots and in roots or stolons respectively but following synthesis their accumulations takes place in rhizomes (Sood and Chauhan, 2010; Pandit *et al.*, 2012; Shitiz *et al.*, 2015) [25, 18, 23].

P. kurroa contains various secondary metabolites and their derivatives utilized to treat different ailments and diseases. Metabolic profiling of leaves and rhizome tissues of *P. kurroa* using NMR, HPTLC and LC-MS/MS confirms several primary and secondary metabolites. Significant qualitative differences were noted in the secondary metabolites profiling of leaves and rhizomes, higher concentration of cucurbitacins and flavonoids were confirmed in leaves while iridoids were found to be more in rhizomes of *P. kurroa*. These types of studies provide an insight of chemical nature and quality in different tissues of *P. kurroa* for their perfect use in different applications and ailments (Kumar *et al.*, 2016) [11].

On the basis of results obtained during the present investigation and also reported earlier and considering the fact the germplasm of *P. kurroa* used in present study was maintained under uniform environmental conditions, it appears that there exist several chemical races of *P. kurroa* in nature. Chemo-types with higher concentrations of Picroside I & II need to be multiplied for their economical production.

Table 1: Evaluation of different populations of *P. kurroa* for Picroside I & II.

Name of Population	Picroside-II		Picroside-I	
	Rhizomes	Roots	Rhizomes	Roots
Grahan	2.04 (1.74)	1.85 (1.69)	0.68 (1.30)	0.05 (1.02)
Holi	3.44 (2.11)	2.19 (1.79)	0.75 (1.32)	0.12 (1.06)
Kafnu	2.44 (1.85)	1.36 (1.54)	1.85 (1.69)	0.22 (1.10)
Manimahesh	1.88 (1.70)	1.56 (1.60)	0.85 (1.36)	0.06 (1.01)
Pasruthach	2.78 (1.94)	1.51 (1.58)	0.36 (1.17)	0.25 (1.12)
Peokar	3.34 (2.08)	1.50 (1.58)	1.54 (1.57)	0.05 (1.02)
Pulga	2.26 (1.81)	1.66 (1.63)	0.13 (0.34)	0.05 (1.02)
Rohtang	3.19 (2.05)	1.77 (1.66)	0.99 (1.41)	0.09 (1.04)
Sainj	3.54 (2.13)	3.24 (2.06)	0.69 (1.30)	0.06 (1.03)
Sairopa	3.38 (2.09)	3.36 (2.09)	1.79 (1.67)	0.19 (1.09)
SolangNala	1.55 (1.60)	1.41 (1.55)	0.27 (1.13)	0.10 (1.05)
Gursutop	3.20 (2.05)	1.73 (1.65)	0.92 (1.39)	0.21 (1.10)
Kedarnath	1.61 (1.62)	1.39 (1.55)	0.58 (1.37)	0.42 (1.19)
Rampur	1.30 (1.52)	1.17 (1.47)	0.64 (1.28)	0.50 (1.22)
Tungnath	2.01 (1.73)	1.72 (1.65)	0.58 (1.26)	0.24 (1.11)
C D @ 5%	(0.05)	(0.05)	(0.10)	(0.01)

Conclusion

On the basis of present study, it is concluded that the concentrations of both Picroside I &Picroside II were found maximum in rhizomes as compared to roots.Picroside II content in rhizomes was maximum (3.54 %) in the populationss collected from Sainj (HP) and minimum (1.30 %) in the populationss collected from Rampur (UK) populations, while in roots it was found maximum (3.38 %) in Sairopa and minimum (1.17 %) in Rampur (UK) populations.Maximum Picroside I content in rhizomes was observed in Kafnu (HP) populationss (1.85 %) and Pulga(HP) populationss had the least (0.13 %), whereas in roots it was observed maximum in Rampur (UK) populationss (0.50%) and Pulga (HP) populations had minimum (0.05 %).

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Bantawa P, Ghosh SK, Bhandari P, Singh B, Ghosh PB, Ahuja PS *et al*. Micropropagation of an elite line of *Picrorhiza scrophulariiflora*, Pennell, an endangered high valued medicinal plant of the Indo China Himalayan region. Med Aro Plant Science Biotechnology. 2010; 4(1):1-7.
2. Bhattacharjee S, Bhattacharya S, Jana S, Baghel DS. A review on medicinally important species of *Picrorhiza*. IJPRBS. 2013; 2(4): 1-16.
3. Bhattacharjee SK. Handbook of medicinal plants. Pointer Publisher, Jaipur, 2004, 261.
4. Chandra B, Palni LMS, Nandi SK. Propagation and conservation of *Picrorhiza kurrooa* Royle ex Benth: an endangered Himalayan medicinal herb of high commercial value. Biodiversity and Conservation. 2006; 15:2325-2338.
5. Chauhan SK, Singh BP, Agrawal S. Simultaneous determination of Picroside-I and Kutkoside from *Picrorhiza kurroa* by HPLC. Indian Drugs. 1999; 36(9):604-605.
6. Coventry BO. Wild flowers of Kashmir. Reprint, Bishen Singh Mahendra Pal Singh, Dehra Dun. 1927-1984; 2:89-90, XLV.
7. Dutt S, Kiddle G, Singh B, Khambay B, Foyer CH. Differential accumulation of picrosides in *Picrorhiza kurroa* Royle ex Benth. plants. http/www.rothamsted-international.org/HTML/ Publications/ News-letters DonorDec04.pdf. 2004.
8. Gaikwad PS, Bhope SG, Kuber VV, Patil MJ. Validated TLC method for simultaneous quantitation of kutkoside and picroside-I from kutki extract. Phytochemical Anal. 2011; 22(1):36-41.
9. Hampel D, Mosandl A, Wust M. Biosynthesis of mono and sesquiterpenes in straw berry fruits and foliage: 2H labelling studies. J Agr Food Chem. 2006; 54:1473-1478.
10. Kaul MK, Kaul K. Studies on medico-ethnobotany, diversity, domestication and utilization of *Picrorhiza kurroa*. In: Supplement to cultivation and utilization of medicinal plants, S S Handa and M K Kaul (ed), Jammu RRL, CSIR, 1996, 333-348.
11. Kumar D, Kumar R, Singh B, Ahuja PS. Comprehensive Chemical Profiling of *Picrorhiza kurroa* Royle ex Benth Using NMR, HPTLC and LC-MS/MS Techniques. Comb Chem High Throughput Screen. 2016; 19(3):200-15.
12. Kumar N, Sharma SK. A new aromatic ester from *Picrorhiza kurroa* Royle ex Benth. Journal of Pharmacognosy and Phytochemistry. 2014; 3(1):96-98.
13. Kutki- *Picrorhiza kurroa* – Home, 2016. http://jagdishrajiv.com/index1.php?c=55&p=90
14. Mahajan R, Kapoor N, Singh I. Somatic embryogenesis and callus proliferation in *Picrorhiza kurroa* Royle ex. Benth. Journal of Experimental Biology and Agricultural Sciences. 2016; 4(2):201-209.
15. Mahmoud SS, Croteau RB. Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and mentho furan synthase. P NatlAcadSci USA. 2001; 98:8915-8920.
16. Masood M, Arshad M, Qureshi R, Sabir S, Amjad MS, Qureshi H *et al*. *Picrorhiza kurroa*: An ethnopharmacologically important plant species of Himalayan region. Pure Appl Biol. 2015; 4(3):407-417.
17. Meena AK, Rao MM, Krishna CM, Komalpreet, Sunil Kumar KN, Padhi MM *et al*. Evaluation of Phamacognostic and Physicochemical parameters of *Picrorrhiza kurroa* Royle ex Benth. International Journal of Ayurvedic Medicine. 2010; 1(1):41-47.
18. Pandit S, Shitiz K, Sood H, Chauhan RS. Differential biosynthesis and accumulation of picrosides in an endangered medicinal herb *Picrorhiza kurroa*. J Plant Biochem Biot. 2012; doi:10.1007/s13562-0120136-z
19. Patil AA, Sachin BS, Shinde DB, Wakte PS. Supercritical CO_2 assisted extraction and LC-MS identification of picroside I and picroside II from *Picrorhiza kurroa*. Phytochemical Analysis. 2013; 24:97-104.
20. Sah JN, Varshney VK. Chemical constituents of *Picrorhiza* genus: a review. American Journal of Essential Oils and Natural Products. 2013; 1(2):22-37.
21. Shah NC. Rhizomes of *Picrorhiza kurroa*. Science and Culture.1969; 35:687-688.
22. Shitiz K, Pandit S, Chauhan RS, Sood H. Picrosides content in the rhizomes of *Picrorhiza kurroa* Royle ex Benth. traded for herbal drugs in the markets of North India. Int J Med Arom Plants. 2013; 3(2):226-233.
23. Shitiz K, Sharma N, Pal T, Sood H, Chauhan RS, NGS. Transcriptomes and Enzyme Inhibitors Unravel Complexity of Picrosides Biosynthesis in *Picrorhiza kurroa* Royle ex. Benth. PLoSONE. 2015; 10(12):e0144546.
24. Singh H, Gahlan P, Kumar S. Cloning and expression analysis of ten genes associated with picrosides biosynthesis in *Picrorhiza kurroa*. Gene. 2012, 320-328.
25. Sood H, Chauhan RS. Biosynthesis and accumulation of a medicinal compound, Picroside-I, in cultures of *Picrorhiza kurroa* Royle ex Benth. Plant Cell Tiss Org. 2010; 100:113-117.
26. Sultan P, Jan A, Pervaiz Q. Phytochemical studies for quantitative estimation of iridoid glycosides in *Picrorhiza kurroa* Royle. Botanical Studies An International Journal. 2016; 57:7.
27. Tiwari SS, Pandey MM, Srivastava S, Rawat A. KTLC densitometric quantification of picrosides (picroside-I and picroside-II) in *Picrorhiza kurroa* and its substitute *Picrorhiza scrophulariiflora* and their antioxidant studies. Biomedical Chromatography. 2012; 26:61-68.