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Phytochemical studies on Indian market samples of drug "Kutki" (*Picrorhiza kurroa* Royle *ex* Benth)

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Abstract

The present investigation entitled "Phytochemical studies on Indian market samples of drug Kutki (Picrorhiza kurroa Royle ex Benth.)" was carried out in the department of forest products under the University of Horticulture and Forestry, Nauni, Solan (India) during 2012-2014 with an aim of evaluating the adulteration of the raw material of drug "Kutki" in the Indian markets and assessing their actual status of major chemical constituents (prcroside-I and picroside-II) to provide information about the level of authenticity of plant materials for the preparation of different drug formulations. The market samples of drug "Kutki" were procured from 35 different retail shops from different market places of India. The samples were evaluated for chemical profile through HPLC analysis. Out of 35 samples, 34 samples were genuine having all the characteristics resembling to drug "Kutki" although sufficient degree of variation was observed and one sample was spurious. There is adulteration as well as lots of variation in the phytochemical constituents of Kutki all over the India. Therefore, this study suggest pharmaceutical industries to purchase the quality of raw material by taking into consideration the presence of phytochemical constituents for the production of desired pharmacological efficacy.

Keywords: Kutki, picroside-I, picroside-II, extraction, quantification, adulteration.

Introduction

The important medicinal herb Kutki (*Picrorhiza kurroa* Royle *ex* Benth.) belongs to the family of Scrophulariaceae and the dried underground part (rhizomes and roots) of this plant has found to possess hepatoprotectice, antioxidant, antiallergic, antiasthamatic, anticancerous and immunomodulatory properties[1]. This is one of the renowned plants in India, China, Tibet, Nepal and Sri Lanka from the prehistoric period having been used for the treatment of numerous immune-related diseases in Ayurvedic as well as other different traditional system of medicine[2]. It is traditionally applied in the treatment of disorders like liver and upper respiratory tract, fevers, dyspepsia, chronic diarrhea, and scorpion sting[3].

There are more than 50 secondary metabolites reported from the plant including iridoid glycosides, cucurbitacins and phenolic compounds[4]. However, the pharmacological importance of this species has been revealed owing to prcroside-I and picroside-II[5].

Picrorhiza kurroa is an important alpine herb of Himalayan region growing at an altitudinal range of 3,000 to 5000 m above mean sea level especially in crevices and moist, sandy soil[6-8]. It is endemic to Western Himalayas extending up to mountains of Yunnan in China[9].

Chamba, Kangra, Mandi, Shimla, Kinnaur, Lahaul and Spiti districts are the important habitat in Himanchal Pradesh for this plant[10]. In Kashmir Himalayas it grows in high reaches of Gurez valley, Lolab, Keran, Sindh and Liidder valleys. It is commonly seen associated with the herbs like *Aconitum violaceum*, *Lagotiscashmiriana*, *Potentillakashmirica*, *Sedum ewersii* and *Seneciojacquemontianus*. It is observed to be common in Gurez, occasional in Lolab and Keran valleys whereas, rare in Sindh valley[6].

FAO report shows that the demand of Kutki is increasing each year. For example, its demand was recorded 220 tonnes in 2001-2002 and 317 tonnes during 2004-2005 which means demand increased by 12.9% between that time periods[11]. In India, it comes under the 15 top traded plant raw materials in the sense economic value[12,13]. For example, only Delhi market deals with more than 10,000kg annually[14]. Globally (excluding China and Pakistan) its' supply is around 375 tonnes where India itself contributes about 70 tonnes.

This plant is uprooted from its natural habitat for obtaining mature roots and rhizomes. More than 90% of its annual demand is fulfilled from the wild sources. Total 300 to 400 individual plants should be uprooted to get 1 kg dry weight of underground parts from this plant[14]. Now, the size of the wild populations has been declining and being critically endangered as a result of overexploitation, habitat fragmentation, lack of organized cultivation practice, which may become extinct, if reckless exploitation continues, therefore, this species has been listed as endangered species by international Union for Conservation of Nature and Natural Resources[15]. It has also been categorized among the 37 identified as top priority species

for conservation and cultivation in Western Himalaya because of narrow distribution range, small population size and high use value.

In spite of herbal medicines over many countries as important mean for medication for treatment of illness or other dietary supplements and health products, little attention has given towards the standardization of medicinal plants and or other natural products[16]. The herbal drugs can be used as a therapeutic agent only if they are genuine and their standard and quality are up to the mark. A disease cannot be managed comprehensively until the delivery of genuine sample of drug is ensured[17].

Large scale collection from unmanaged wild sources not only depletes the natural availability of these sources, but also leads to intentional as well as unintentional adulteration in the drug which reduces its potency. The reasons of intentional adulterations are the high price of the drug in the market and extreme scarcity.

Herbal drugs are adulterated by a variety of ways such as: i. replacement by exhausted drugs such as cloves, saffron, tea, fennel, ginger etc., ii. substitution by superficially similar, but inferior parts as in case of saffron where *Calendula officinalis*, *Carthamus tinctorious* and *Arnica montana* are used for adulteration, iii. substitution by artificially manufactured substitutes, iv. substitution by substandard commercial varieties and v. substitution by organic matter (non-official parts) obtained from the same plant. *Picrorhiza kurroa* is known to be adulterated by roots of *Gentiana kurroa*, *Holoptelea integrifolia* and *Logotis cashmiriana* Royle *ex.* Benth[6]. *Logotis cashmiriana* is found growing at same altitude with *Picrorhiza kurroa* which is sometimes traded by traders as an herb'Kutki'. Adulteration of raw material has been playing vital disadvantage for the maintenance of safety, efficacy as well as quality of herbal products in the global distribution for a long time[18,19]. Therefore, appropriate method of authentication for each and every crude drug like morphological / anatomical, organoleptic and chemical study should be carried out before its final usage[20].

The present research work was carried out with an objective of evaluating adulteration of the raw material of drug "Kutki" in the different markets of India and assessing their actual status of major chemical constituents (prcroside-I and picroside-II) to provide information about the level of authenticity of plant materials for the preparation of different drug formulations.

Materials and methods

Sources of drug "kutki" market samples: The market samples of drug "Kutki" were procured from 35 different retail shops from different market places of India. These samples were procured through scientists working under AICRP on Medicinal and Aromatic Plants and Betel-vine in different institutes/agricultural universities of India. The detail of samples procured from different markets is given in Table-1.

Table-1: Source of market samples of drug "Kutki".

Sample No.	Market Name	State
1	Akola	Maharashtra
2	Akola	Maharashtra
3	West Godavari	Andhra Pradesh
4	Vizag district	Andhra Pradesh
33	Srikakumam	Andhra Pradesh
5	Udaipur	Rajasthan
6	Udaipur	Rajasthan
7	Udaipur	Rajasthan
8	Udaipur	Rajasthan
9	Udaipur	Rajasthan
10	Palakkad	Kerala
11	Thrissur	Kerala
12	Changaramkulam	Kerala
13	Pollachi (Coimbatore)	Tamilnadu
14	Attur (Coimbatore)	Tamilnadu
35	Tripur	Tamilnadu
15	Hissar	Haryana
16	Hissar	Haryana
17	Hissar	Haryana
18	Hissar	Haryana
31	Hissar	Haryana
32	Hissar	Haryana
34	Hissar	Haryana
23	Kalka	Haryana
29	Solan	Himachal Pradesh
30	Solan	Himachal Pradesh
24	Shimla	Himachal Pradesh
21	Mandi	Himachal Pradesh
22	Mandi	Himachal Pradesh
19	Indore	Madhya Pradesh
20	Mandsaur	Madhya Pradesh
27	Bharsar	UttraKhand
28	Bharsar	UttraKhand
25	Bhubaneshwar	Odissa
26	Bhubaneshwar	Odissa

Extract preparation: For conducting the experiment, samples (underground parts) of drug "Kutki" which were collected from the 35 markets of different parts of India were dried at room temperature after removing unwanted materials and cutting into small pieces. The dried samples were then grinded to form uniform powder. Accurately weighted (2g) air dried powdered material of each market sample was extracted using soxhlet apparatus for 5 hours with methanol. The methanolic extract was dried over water bath to remove methanol and each dried extract was used for quantitative estimation of important phytochemicals (picroside-I and picroside-II) through High Performance Liquid Chromatography (HPLC) analysis.

Quantitative estimation of picrosides through HPLC: Quantitative analysis of picrosides was performed by HPLC equipment consisted of Water's binary HPLC unit with Waters HPLC pump 515 and dual λ absorbance detector 2487. Sunfire C-18 (4.6 × 250 mm, 5µm) column was used.

Mobile phase was prepared as methanol: water (40: 60). The vacuum dried extract was 1000 times diluted with mobile phase, 20µl of each diluted sample was injected into the column and the column oven temperature was set to $24^0 \pm 1^0$C. The flow rate was optimized and set to 0.9 ml/min.

Area under Curve for peaks of picroside-I and picroside-II was recorded at 270 nm wavelength and then percentage of these two phytochemicals in each sample was calculated separately by using the formula as given below where percent purity of standard compound was taken as 95%:

$$\text{Picrosides content (\%)} = \frac{\text{Test Area}}{\text{Standard Area}} \times \frac{\text{Wt. of Standard}}{\text{Standard Dilution}} \times \frac{\text{Test Dilution}}{\text{Test Weight}} 100 \times \text{percent purity}$$

Results and discussion

The major two active constituents (picroside-I and picroside-II) of market samples of drug "Kutki" were qualitatively and quantitatively estimated through HPLC. Among the HPLC profile of 35 market samples, only34 samples showed the existence of these two chemical compounds and one sample which was procured from Solan market (sample no. 29) didn't show any existence. The picroside-I and picroside-II content in other remaining different market samples ranged between 0.399 per cent to 7.383 per cent and 0.517 per cent to 7.66 percent respectively.

Maximum picroside-I content (7.383 %) in sample number 22 which was procured from Mandi, Himachal Pradesh and minimum (0.399%) in sample number 32 which was procured from Hissar, Haryana. The maximum picroside-II content (7.666 %) was recorded in sample number 13 which was procured from Pollachi, Tamilnadu and minimum (0.517%) in sample number 32 which was procured from Hissar, Haryana.

Since these are the major phytochemicals of *Picrorhiza kurroa*, it is essential to be present on the sample. However under the study of 35 different market samples, one sample i.e. sample number 29 procured from Solan market has lack of their content which raises doubt for its genuineness.

Discussion: Structural evaluation of herbal drugs is useful for both authentication and checking for adulterants but it requires expertise in analyzing the macroscopic and microscopic features of plant parts, especially those that are ground to very fine powders, mixed with other plants, or degraded due to poor storage or processing. Likewise, chemical profiling is very useful for detecting adulterants such as synthetic drugs and phyto-chemicals from unwanted plant material[21]. During the present study, the market samples of Kutki were evaluated by HPLC analysis for qualitative and quantitative estimation of important phyto constituents (picroside-I and picroside-II).

On the basis of HPLC analysis, out of 35 market samples procured from different markets of India, one sample was spurious whose identity couldn't be established, and only 34 samples were genuine i.e. of drug "Kutki" however, sufficient degree of variation was observed in different market samples. Same result was found while experimenting through organoleptic and histological characteristics of those samples[22].

Such a wide variation of those two chemical compounds content in the 34 market samples may be due to various factors like storage conditions, adulteration etc. The phytochemical content in *Picrorhiza kurroa* has also been reported to vary with altitudinal zone, among populations, plant age, plant part, collection time and place of collection[5,6,23,24].

Proper concentration of desired phytochemicals in different systems of medicine is in fact utmost essential for effective treatment of disease, therefore, it becomes necessary to evaluate and apply only suitable plant material to get herbal drug formulations with required pharmacological efficacy.

Conclusion

From obtained result, it is concluded that, there is adulteration as well as lots of variation in the phytochemical constituents of *Picrorhiza kurroa* all over the India. Therefore, this study suggests concerned bodies, who are engaged with pharmaceutical products, to procure quality of Kutki from the market by taking into consideration the presence of phytochemical constituents for the production of desired pharmacological efficacy.

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Table-2: Picrosides content of samples from different markets of India.

Sample No.	Name of market (state)	Picroside -I (%)	Picroside -II (%)
1	Akola (Maharashtra)	4.528	2.602
2	Akola (Maharashtra)	3.496	1.167
3	West Godavari (AP)	1.235	1.584
4	Vizag district (AP)	3.792	1.633
5	Udaipur (Rajasthan)	6.507	1.791
6	Udaipur (Rajasthan)	3.088	2.541
7	Udaipur (Rajasthan)	4.320	1.572
8	Udaipur (Rajasthan)	3.149	1.152
9	Udaipur (Rajasthan)	3.666	3.568
10	Palakkad (Kerala)	2.978	3.332
11	Thrissur (Kerala)	1.930	2.337
12	Changaramkulam (Kerala)	2.060	2.199
13	Pollachi (Tamilnadu)	7.249	7.666
14	Attur (Tamilnadu)	2.779	3.926
15	Hissar (Haryana)	2.474	4.712
16	Hissar (Haryana)	5.839	5.740
17	Hissar (Haryana)	5.989	6.491
18	Hissar (Haryana)	3.888	5.443
19	Indore (MP)	2.035	2.798
20	Mandsaur (MP)	1.870	2.612
21	Mandi (HP)	4.572	3.578
22	Mandi (HP)	7.383	4.932
23	Kalka (Haryana)	6.445	4.279
24	Shimla (HP)	3.273	5.128
25	Bhubaneshwar (Odissa)	1.775	1.027
26	Bhubaneshwar (Odissa)	2.622	3.435
27	Bharsar (UttraKhand)	2.505	3.246
28	Bharsar (UttraKhand)	0.740	5.622
29	Solan (HP)	0	0
30	Solan (HP)	0.481	0.740
31	Hissar (Haryana)	3.216	6.621
32	Hissar (Haryana)	0.399	0.517
33	Srikakumam (AP)	0.478	0.738
34	Hissar (Haryana)	3.534	0.531
35	Tirupur (Tamilnadu)	0.539	0.835

References

1. Rastogi R., Saksena S., Garg N.K., Kapoor N.K., Agarwal D.P. and Dhawan B.N. (1996). Picroliv protects against alcohol induced chronic hepatotoxicity in rats. *PlantaMedica,* 62(3), 283-285.

2. Bhandari P., Kumar N., Singh B. and Kaul V.K. (2008). Simultaneous determination of sugars and picrosides in Picrorhiza species using ultrasonic extraction and high-performance liquid chromatography with evaporative light scattering detection. *Journal of Chromatography A*, 1194(2), 257-261.

3. Sood H. and Chauhan R.S. (2010). Biosynthesis and accumulation of a medicinal compound, Picroside-I, in cultures of Picrorhiza kurroa Royle ex Benth. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 100, 113.

4. Stuppner H. and Wagner H. (1989). New cucurbitacin glycosides from *Picrorhizakurroa*. *PlantaMedica*., 55(6), 559-563.

5. Katoch M., Fazli I.S., Suri K.A., Ahuja A. and Qazi G.N. (2011). Effect of altitude on picroside content in core collections of Picrorhiza kurrooa from the north western Himalayas. *Journal of natural medicines*, 65(3-4), 578-582.

6. Kaul M.K. and Kaul K. (1996). Studies on medicoethnobotany, diversity, domestication and utilization of Picrorhiza kurroa. *Supplement to cultivation and utilization of medicinal plants*, Jammu, edited by Handa SS, Kaul MK, CSIR, 333-348.

7. Anonymous (2001). Alternative Medicine Review. *American Botanical Council*., 6(3), 319-321. *cms.herbalgram.org/herbclip/pdfs/100612-209.pdf*

8. Kumar P.V., Sivaraj A., Madhumitha G., Saral A.M. and Kumar B.S. (2010). In vitro antibacterial activities of Picrorhiza kurroa rhizome extract using agar well diffusion method. *Int J Curr Pharm Res*, 2(1), 30-33.

9. Anonymous (1969). The wealth of India: a dictionary of Indian raw materials and industrial products. VII: Ph-Re. New Delhi: Publication and Information Directorate, CSSIR, 49-50.

10. Uniyal S.K., Singh K.N., Jamwal P. and Lal B. (2006). Traditional use of medicinal plants among the tribal communities of ChhotaBhangal, Western Himalaya. *Journal of Ethnobol. Ethnomedicine*., 2, 14.

11. FAO. (2005). Trade in medicinal plant. p.8; ftp://ftp.fao.org/docrep/fao/008/af285e00.pdf

12. Malaisamy A. and Ravindran C. (2003). Medicinal plants: Where do we stand globally?. *Science Tech Entrepreneur Magazine*., 2(5), 42-49.

13. Ved D.K. and Goraya G.S. (2008). Demand and Supply of Medicinal Plants in India. Bishen Singh, Mahendra Pal Singh,Dehra Dun & FRLHT, Bangalore, India.

14. Uniyal A., Uniyal S.K. and Rawat G.S. (2011). Commercial Extraction of Picrorhiza kurrooa Royle ex Benth. in the Western Himalaya: Patterns of Collection. *Processing, and Conservation Threats. Mountain Research and Development*, 31(3), 201-208.

15. Nayar M.P. and Sastri A.R.K. (1990). Red data plants of India. CSIR Publication, New Delhi, 271.

16. Jahan N., Afaque S.H., Khan N.K., Ahmad G. and Ansari A.A. (2008). Physico-chemical studies of the Gum Arabica. *Natural Products Radiance.,* 7**,** 335-337.

17. World Health Organization (1999). WHO monographs on selected medicinal plants. Geneva, Switzerland, 1.

18. Khatoon S., Mehrotra S., Shome U. and Mehrotra B.N. (1993). Analysis of commercial Ratanjot by TLC fluorescence fingerprinting. *International Journal Pharmacology.,* 31(4), 269-277.

19. Dubey N.R., Kumar R. and Tripathi P. (2004). Global promotion of herbal medicine: India's opportunity. *Current Science.,* 86(1), 37-41.

20. Shaw P.C., Ngan F.N., But P.P.H. and Wang J. (2002). Molecular markers in Chinese medicinal materials. Shaw, P C, Wang J and But PPH. **eds.** Authentication of Chinese medicinal materials by DNA technology. *World Scientific Publishing Co Hong Kong,* 1-23.

21. Joshi V.C., Srinivas P.V. and Khan I.A. (2005). Rapid and easy identification of Illicium verum Hook. f. and its adulterant Illicium anisatum Linn. by fluorescent microscopy and gas chromatography. *Journal of AOAC International*, 88(3), 703-706.

22. Thani P.R. (2015). Morpho-chemical studies of market samples of drug Kutki (*Picrorhizakurroa*Royle*ex*Benth.)–A study. Master Thesis. Dr YS Parmar University of Hurticulture and Forestry, Nauni, Solan, India. *http://krishikosh.egranth.ac.in/bitstream/1/5810010774/1/PDF%20M.Sc.%20Thesis%20Parbat%20Raj%20Thani%20%282015%29.pdf*

23. Singh N., Gupta A.P., Singh B. and Kaul V.K. (2005). Quantification of picroside-I and picroside-II in *Picrorhizakurroa* by HPLC. *Journal Liqid Chromatography Related Technology*., 28(11), 1679-1691.

24. Purohit H., Nautiyal B.P. and Nautiyal M.C. (2010). Interpopulation variation in Picrorhiza kurrooa Royle ex Benth-step towards identifying genetic variability and elite strains for crop improvement study. *American Journal of Plant Physiology*, 5(4), 193-203