Evaluation of Phamacognostic and Physicochemical parameters of *Picrorrhiza kurroa* Royle ex Benth

A K Meena[1]*, M M Rao[1], C M Krishna[3], Komalpreet[1],
K N Sunil Kumar[4], M M Padhi[2], Ramesh Babu[2]

Abstract

Picrorrhiza kurroa Royle ex Benth is commonly known as *Kutki* belonging to family Scrophulariaceae. It is useful as a laxative, liver-stimulant, improving lactation, appetite stimulant, fabrifuge and as beneficial in bronchial asthma. This plant is native to the Himalayan region and is part of Ayurvedic medicine as a treatment for various diseases. The leaf, bark and the underground parts of the plant, mainly rhizomes are widely used in the traditional Indian (Ayurvedic) systems of medicine since ancient times. Rhizomes of *Picrorrhiza kurroa* Royle ex Benth plant material was subjected to microscopic characterization and physico-chemical studies. TLC has also been studied to fix the quality standards of this drug. The experiment has revealed a set of diagnostic characters essential for its standardization. Photomicrography and TLC profile were employed to fix standards. In this article attempt has been made to standardize the *Picrorrhiza kurroa* Royle ex Benth obtained from reliable source for its physico-chemical parameters like loss on drying, total ash value, acid insoluble ash, water soluble extract, alcohol soluble extract, pH etc.

Keywords: *Picrorrhiza kurroa* Royle ex Benth, Rhizomes, Ayurvedic

Introduction

Picrorrhiza kurroa Royle.ex Benth is commonly known as *Kutki* belonging to family Scrophulariaceae. It is useful as a laxative, liver-stimulant, sialogogue, appetite stimulant, fabrifuge and as beneficial in bronchial asthma. The plant and its formulations are widely used in therapy of epidemic jaundice. Clinical studies have been carried out with the root powder of the plant in patients with viral hepatitis with significant improvement in symptoms like anorexia, nausea and vomiting. It is bitter, carminative, digestant and has a cooling effect and is used as a cardiotonic, antipyretic and anthelmintic. It is also used in diabetes, jaundice, liver, spleen disorders and skin diseases. The *Kutki* plant is native to the Himalayan region. It is used in Ayurvedic system of medicine for treating primarily gastrointestinal problems such as indigestion and constipation. Usually the root or rhizome of the plant is used medicinally. *Picrorrhiza kurroa* Royle ex Benth *is* a small perennial herb growing in the hilly areas of the North-Western Himalayan region in India and Nepal. Although it shows anti-oxidant, anti-inflammatory and immunomodulatory activities, it is most valued for its hepatoprotective effect. The bitter rhizomes of *Picrorrhiza kurroa* Royle ex Benth have been used for thousands of years in India to treat people with indigestion (1) and constipation due to insufficient digestive secretion (2). *Picrorrhiza kurroa* Royle ex Benth *is* considered as a trophorestorative herb for the liver as well as a potent immunostimulant (3). Its constituent, picroliv is also reported to possess choleretic effect (4), and prevent hepatic

1* Corresponding Author, National Institute of Ayurvedic Pharmaceutical Research, Patiala-147001, Punjab. Tele Fax. No. +91-175-2212393; +91-9465159789 (M), E-mail: ajaysheera@yahoo.co.in.
1. National Institute of Ayurvedic Pharmaceutical Research, Patiala-147001, Punjab, 2. Central Council for Research in Ayurveda and Siddha, Janakpuri, Delhi, 3. National Institute of Indian Medical Heritage, Hyderabad, India, 4. Captain Srinivasa Murti Research Institute for Ayurveda and Siddha Drug Development, Chennai.

injury caused by ethanol (5) and chemicals.

Picrorrhiza kurroa Royle ex Benth is a small, hairy perennial herb with small white or pale bluish flowers in cylindrical spikes and woody rhizomes. The dried rhizomes of the plant constitute the drug. *Picrorrhiza kurroa* Royle ex Benth is highly beneficial in treating liver cirrhosis among adults. Its root is given in powdered form. It stimulates the liver to produce more bile. *Picrorrhiza kurroa* Royle ex Benth is also used with good results in the treatment of ascites, a disease characterized by the accumulation of fluid in the peritoneal cavity of the abdomen.

Picrorrhiza kurroa Royle ex Benth is one of the major drugs useful in jaundice. It is beneficial in the treatment of dyspepsia. It strengthens the stomach and promotes its action, while improving appetite and stimulating the secretion of gastric juices. It cures periodic attacks like hysteria, epilepsy and convulsions and promotes the secretion of bile. It has antibiotic properties and is a mild purgative. Its main chemical constituents are glycosides picroside I, II and III, picrorhizin, kutkoside, kurrin, kuthinol, kutkiol, kutkisterol, kutkoside, androsin, apocynin, drosin and cucurbitacin. Apocynin is a catechol that has been shown to inhibit neutrophil oxidative burst in addition to being a powerful anti-inflammatory agent while the curcubitacins have been shown to be highly cytotoxic and possess antitumor effects.

Here, we attempted to study the physico-chemical standardization parameters as well as analyze the crude drug for its authenticity by microscopic characterization.

MATERIALS AND METHODS

Plant material

The sample of *Picrorrhiza kurroa* Royle ex Benth was collected from local market of Patiala, Punjab. Specimen was identified and authenticated at the National Institute of Ayurvedic Pharmaceutical Research (NIAPR), Patiala (Punjab). Samples were studied for physiochemical evaluation.

Methods for Physicochemical Parameter

Moisture content

4 g of the sample was taken and heated in an oven at 105°C for 5 hour in a previously weighed 100 ml beaker. It was cooled in desiccators and weighed. The procedure was repeated till constant weight is obtained. The percentage of loss in weight of the sample was calculated.

Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to fungal attack. The loss on drying at 105°C of *Picrorrhiza kurroa* Royle ex Benth rhizome was found to be 7.83 %.

Determination of Total ash value

2 g of the sample was taken accurately in a previously ignited and tarred Silica dish. The material was spread evenly and ignited in a muffle furnace by gradually increasing the temperature to 600^{o}C until it is white, indicating the absence of carbon. The crucible was cooled in desiccators and allowed to stand for 30 minutes and weighed.

Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value of *Picrorrhiza kurroa* Royle ex Benth rhizome was 5.92 %.

Determination of Acid insoluble ash value

To the dish containing the total ash, 25 ml of 20 % Hydrochloric acid was added covered with a watch glass and boiled gently for 5 minutes. The watch was rinsed with a hot water and added to the crucible. The residue was washed with the hot water till the washings were neutral

to the litmus. The insoluble material was collected and again placed in a same crucible and again ignited for 6 hr. to constant weight. The residue was cooled a desiccators for 30 minutes and weighed.

Percentage of acid insoluble as was calculated. The amount of acid-insoluble siliceous matter present in the *Picrorrhiza kurroa* Royle ex Benth rhizome was 2.14%.

Determination of Water soluble extractive value

4 g of the sample was taken in a glass stoppered flask.100 ml of distilled water was added. The flasks were shaken occasionally for 6 hours and then allowed to stand for 18 hours. The extract was filtered and 25 ml of the filtrate was pipette out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath.It was kept in a hot air oven for 5 hr at l05°C, cooled in a desiccators for 30 minutes and weighed. The procedure was repeated till constant weight.

The water-soluble extractive value indicated the presence of sugar, acids and inorganic compounds. The water soluble extractive value in the *Picrorrhiza kurroa* Royle ex Benth rhizome sample was found to be 33.10%.

Determination of Alcohol soluble extractive Value

Same procedure as for the water soluble extractive value was followed. Instead of water, rectified spirit was taken as a solvent.

The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and secondary metabolites present in the plant sample. The alcohol soluble extractive value was found to be 25.40% in the *Picrorrhiza kurroa* Royle ex Benth rhizome.

Determination of pH Value

10% aqueous solution of sample was prepared and used for determining the pH value by pH meter. The pH value of *Picrorrhiza kurroa* Royle ex Benth *rhizome* was found to be 4.84

RESULT AND DISCUSSION

Microscopy

Rhizome:

Transverse section of the rhizomes showed outermost thin brown colored cork, lacunose black colored bark, a circle of vasculature formed by 5 to 7 ovate vascular bundles.

TS of the rhizome (Figure 1) showed 8 to 12 layers of rectangular cork cells without any intercellular spaces. Cells of the cortex were found thick and loosely arranged in definite radial rows. It showed ovate vascular bundles in between the cortex and pith. In the centre there was parenchymatous pith with pitted walls. Simple starch grains were found in the parenchyma of the cortex and the pith.

Detailed TS of the root was similar to that of rhizome in histology but it showed a central small circular vascular bundle formed by xylem in the centre surrounded by phloem.

Powder:

Microscopy of the powder (Figure 2) showed plenty of thick-walled parenchymatous cells with starch grains. Cortex cells were found circular to oval. Cork cells in transversely cut mode and in surface view were found to contain fragments of pitted vessels and tracheids and groups of cells with orange colored content.

Physico-chemical parameters:

Different physicochemical parameters like Total ash, acid insoluble ash, water soluble extractive value, ethanol soluble extractive value, loss on drying at 105ºC and pH etc. were studied. (Table 1)

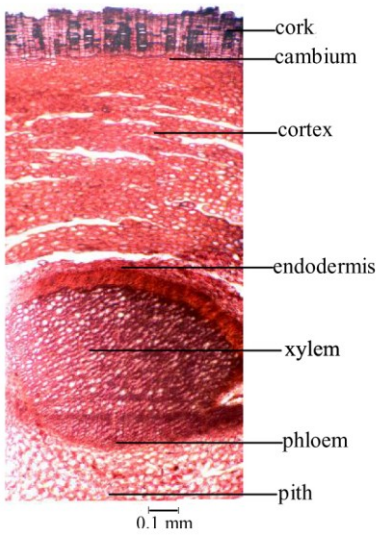


Figure 1. TS of rhizome of *Picrorrhiza kurroa* Royle ex Benth

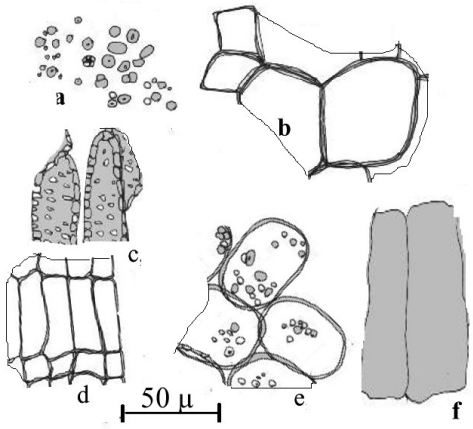


Figure 2. Powder microscopy of *Picrorrhiza kurroa* Royle ex Benth

(a. starch grains; b. cork in surface view; c. fragments of pitted vessels; d. transversely cut cork; e. cortical parenchyma; f. pigment cells.)

Table 1. Physicochemical parameters of *Picrorrhiza kurroa* Royle ex Benth

S. No.	Name of Parameters	Results
1.	pH (10% aqueous solution (v/w)	4.84
2.	Loss on drying at 105°C (% w/w)	8.96
3.	Total Ash (% w/w)	5.92
4.	Acid-insoluble ash (% w/w)	2.14
5.	Water-soluble extractive (% w/w)	33.10
6.	Alcohol-soluble extractive (% w/w)	25.40

Thin Layer Chromatography (Figure 3)

The thin layer chromatography of the *Kutki* was performed and observed under 366 and 254 nm. The plates were also exposed to the iodine vapours and to the vanillin-sulphuric acid solution for Derivatisation (Fig: 3). Under 366 nm the plate showed no spots indicating that the *Kutki* contains no components absorbing at this wavelength. At 254 nm, the samples showed 2-3 spots towards the baseline indicating the highly polar nature of the compounds. After exposing to the iodine vapours, the plate showed few spots in between the base line and solvent front indicating the presence of compounds which can be fixed by the iodine. After dipping the plate in a vanillin-sulphuric acid solution, the plate showed different colored compounds indicating the presence of compounds with strong chromophoric groups and multiple bonds.

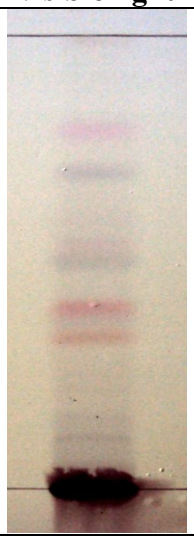
The R_f of all the spots was calculated and mentioned in the table 2.

Table 2. TLC profile of Picrorrhiza *kurroa* Royle ex Benth

S.No	254 nm		After Derivatisation with Iodine in visible light		After Derivatisation with Vanillin sulphuric acid in visible light	
	Colour	R_f.	Colour	R_f.	Colour	R_f.
1.	Purple	0.11	Yellow	0.09	Purple	0.11

2.	Purple	0.16	-	-	Purple	0.16
3.	Purple	0.36	Yellow	0.36	Pink	0.36
4.	-	-	-	-	Pink	0.41
5.	-	-	-	-	Violet	0.51
6.	-	-	-	-	Light Pink	0.55
7.	Purple	0.72			Violet	0.72
8.	-	-	Yellow	0.82	Pink	0.82

Figure 3: TLC profile of *Picrorrhiza kurroa* Royle ex Benth rhizome (Toluene: Ethyl acetate 7.5:2.5)

λ 254 nm	After Derivatisation with Iodine in visible light	After Derivatisation with Vanillin Sulphuric acid in visible light
		

Conclusion

Morphology as well as various phamacognostic aspects of the rhizome sample was studied and described along with physio-chemical parameters and TLC. These parameters will be useful in authentification and identifying the adulterants and quality control of raw drugs. Whole plant of *Picrorrhiza kurroa* Royle ex Benth exhibited a set of diagnostic characters, which will help to identify the drug in dried condition. It has been concluded from this study that estimation of these parameters is highly essential for raw drugs or plant parts used for the preparation of compound formulation. The periodic assessment is essential for quality assurance and safer use of herbal drugs.

Acknowledgement

Authors acknowledge the secretarial assistance rendered by Ms. Rekha in the preparation of this paper.

References

1. Chevallier A: The Encyclopedia of Medicinal Plants. New York: DK Publishing; 1996.
2. Shukla B, Visen PK, Patnaik GK: Choleretic effect of Picroliv, the hepatoprotective principle of Picrorhiza kurroa.
3. Saraswat B, Visen PK, Patnaik GK: Ex vivo and in vivo investigations of Picroliv from Picrorrhiza kurroa Royle ex Benthin an alcohol intoxication model in rats.

4. Visen PK, Saraswat B, Dhawan BN: Curative effect of picroliv on primary cultured rat hepatocytes against different hepatotoxins: An in vitro study.
5. M, Tiwari V, Jain A, Ghoshal S: Protective activity of picroliv on hepatic amoebiasis associated with carbon tetrachloride toxicity.
6. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi. 1956; 94.
7. Anonymous. The Wealth of India—Raw materials vol. IX: Rh–So. CSIR, New Delhi, 1989; 393–394.
8. Yelne MB, Sharma PC, Dennis TJ. Database on Medicinal Plants used in Ayurveda, Central Council for Research in Ayurveda & Siddha, New-Delhi, 2000, Vol.2: 69 - 73.
9. The Ayurvedic Pharmacopoeia of India, part- 1,volume – I, Government of India, Ministry of Health and Family Welfare, Department of AYUSH.
10. AOAC Official methods of analysis of AOAC International, 16th edition. AOAC International, Suite 400, 2200 Wilson Boulevard, Arlington, Virginia, USA, 1995.
11. World Health Organization: Quality control methods for medicinal plant materials. Published by WHO, Geneva, 1998.

******