



RESEARCH ARTICLE

Microscopical and Preliminary Physicochemical Studies of Two Important Endangered Ayurvedic Medicinal Plants *Kutki* and *Trayamana* to establish their Identity

¹Anupam K Mangal, ²Chinmay Rath, ³Devesh Tewari, ⁴Sreya Dutta, ⁵Narayanam Srikanth, ⁶Kartar S Dhiman

ABSTRACT

Kutki is an important Ayurvedic drug native to the Himalayan region, and its official source is *Picrorhiza kurroa* Royle ex Benth., belonging to the family Scrophulariaceae. The plant is a small, hairy perennial herb with creeping and woody rhizome and used in various Ayurvedic medicines. It is useful as a laxative, liver-stimulant, galactagogue, and appetite-stimulant and is beneficial in bronchial asthma. Due to its high demand, the plant is often adulterated/substituted with the roots of *Trayamana* (*Gentiana kurroa* Royle), as one of the source of the *Kutki*. The present study has focused to establish the identity of *Picrorhiza kurroa* Royle ex Benth through microscopical investigations and comparison of the genuine drug with its substitute *Gentiana kurroa* Royle. Powder of the rhizomes of *Picrorhiza kurroa* Royle ex Benth. and roots of *Gentiana kurroa* Royle were subjected to microscopic characterization and physicochemical analysis, which would not only serve in the identification of both the drugs, but also contribute toward establishing pharmacopoeial standards.

Keywords: Ayurvedic, Endangered, High altitude, *Kutaki*, Microscopy, Rhizomes, *Trayamana*.

How to cite this article: Mangal AK, Rath C, Tewari D, Dutta S, Srikanth N, Dhiman KS. Microscopical and Preliminary Physicochemical Studies of Two Important Endangered Ayurvedic Medicinal Plants *Kutki* and *Trayamana* to establish their Identity. J Drug Res Ayurvedic Sci 2017;2(1):18-22.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Kutki, botanically equated with *Picrorhiza kurroa* Royle ex Benth., is a small perennial herb growing in the hilly areas of the northwestern Himalayan region in India and

Nepal, the flowering and fruiting occurs in the month of June to September. Regionally, *Kutki* is known as *Katuka*, *Tikta*, *Katki*, *Kadu*, *Kutki*, *Karru*, etc.¹⁻³

The rhizome of the plant is used in Ayurvedic system of medicine for treating primarily gastrointestinal problems and is useful as a laxative, liver-stimulant, appetite-stimulant, febrifuge and is beneficial in bronchial asthma.^{4,5} 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, hyperlipidemia, and as a diuretic. The bitter rhizomes of *Picrorhiza kurroa* Royle ex Benth have been used for thousands of years in India to treat people with indigestion and constipation due to insufficient digestive secretion.⁶⁻¹¹

Main chemical constituents of *Kutki* 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 cucurbitacins have been shown to be highly cytotoxic and possess antitumor effects.¹²⁻¹⁴

Different botanical sources are used in the name of *Kutki* in different parts of the country. The roots of *Trayamana* are used as substitute for *Kutki*. *Trayamana*, botanically known as *Gentiana kurroa* Royle, belongs to family Gentianaceae. Other adulterates or substitutes are the roots/rhizomes of *Plectranthus amboinicus* (Lour.) Spreng, *Helleborus niger* L., *Neopicrorhiza scrophulariiflora* (Pennell) D.Y.Hong, *Actaea spicata* L., *Cimicifuga foetida* L., *Coptis teeta* Wall., *Coscinium fenestratum* (Goetgh.) Colebr., *Sorbus cashmiriana* Hedl., *Delphinium zalil* Aitch. & Hemsl., *Delphinium saniculifolium* Boiss., *Ficus heterophylla* L.f., *Thalictrum foliolosum* DC., *Gentianella moorcroftiana* (Wall. ex Griseb.), Airy Shaw *Gentiana paludosa* Munro ex Hook. f., is used as substitute in Amchi system of medicine. The dried stem barks of *Azadirachta indica* A.Juss., and *Nymphoides cristata* (Roxb.) Kuntze are also used as substitute.^{5,15,16}

To evaluate the botanical identity of genuine *Kutki* rhizomes and its comparison with substitute, the powder forms of both the drugs were subjected to microscopic characterization and physicochemical studies. The experiment has revealed a set of diagnostic characters

¹Assistant Director (Pharmacognosy), ²Research Officer (Botany), ³Senior Research Fellow (Pharmacognosy), ⁴Assistant Research Officer (Botany), ⁵Deputy Director General, ⁶Director General

^{1-3,5,6}Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Government of India, New Delhi, India

⁴Central Ayurveda Research Institute for Drug Development Kolkata, India

Corresponding Author: Anupam K Mangal, Assistant Director (Pharmacognosy), Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Government of India, New Delhi, India Phone: +918826210975, e-mail: anupam.mangal68@gmail.com



essential for its standardization. To the best of our knowledge, the study is the first study so far to ascertaining the comparable cytological diagnostic characters of *Picrorhiza kurroa* Royle ex Benth and *Gentiana kurroo* Royle.

MATERIALS AND METHODS

Plant Material

The authenticated samples of rhizomes of *Picrorhiza kurroa* Royle ex Benth. and roots of *Gentiana kurroo* Royle were supplied by Regional Ayurveda Research Institute, Itanagar, India, and authentication of the rhizomes and roots of both the drugs was done by the first author (AKM).

Macroscopic Evaluation

The macroscopic evaluation of both the plant samples was carried out according to standard procedures.^{17,18}

Microscopic Evaluation

Free hand sections were taken, and dried roots and rhizome material was grinded in the electric grinder separately. About 2 gm (each) of rhizome powder of *Picrorhiza kurroa* Royle ex Benth. and root powder *Gentiana kurroo* Royle were warmed separately with chloral hydrate, washed and mounted in 50% glycerine, powder treated with iodine solution and mounted in 50% glycerine, and observed under trinocular microscope (Olympus). The microscopical characters of both the drug powder were studied to establish its identity. Line drawing of the diagnostic cell structures was obtained by observing the powder drug with the help of Camera Lucida, and cell measurements were also carried out by using ocular micrometer.

Physicochemical Evaluation

The physicochemical parameters like ash value, acid-insoluble ash value, water-soluble extractive value, alcohol-soluble extractive value, and foreign matter were determined according to the procedures of the Ayurvedic Pharmacopoeia of India (API).¹⁹

RESULTS AND DISCUSSION

Macroscopical Characters of *Kutki*

Picrorhiza kurroa Royle ex Benth. is a small, hairy perennial herb with creeping and woody rhizome, rooting at nodes. Rhizome is 2.5 to 6.0 cm long and 0.5 to 1.0 cm thick, subcylindrical, straight, externally grayish brown, rough surface, circular scars of roots, and bud scales attached. Odor is pleasant and tastes bitter.

Microscopy of Rhizome of *Kutki*

Diagrammatic sections of the rhizome consist of cork, cortex, endodermis, and pith in the center. Transverse

section of the rhizomes showed 3 to 4 layers, thick-walled oval to polygonal cells of brown color cork; 20 to 24 layered cortex cells, thick and loosely arranged in definite radial rows; 5 to 7 ovate vascular bundles were present in between cortex and the pith. In the center, there was parenchymatous pith with pitted walls. Simple starch grains were found in the parenchyma of the cortex and the pith.

Powder Microscopy of Rhizome of *Kutki*

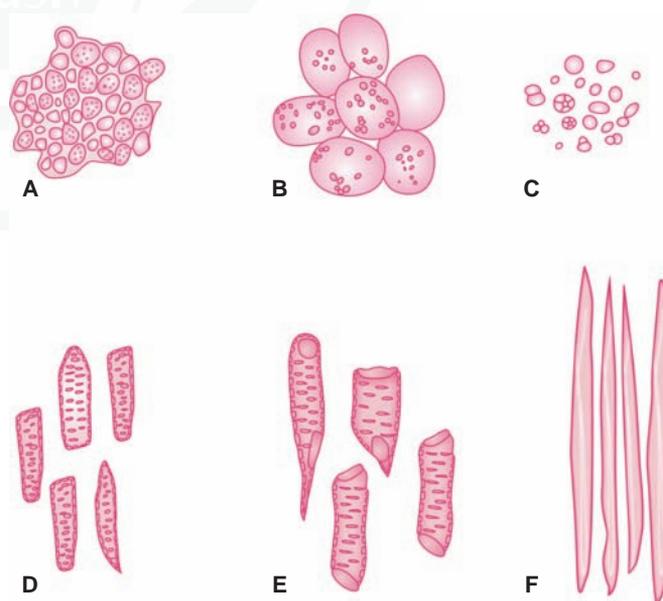
Microscopy of the powder showed cork cells in transversely cut mode and plenty of thick-walled parenchymatous cells with starch grains. Cortex cells were found circular to oval in shape and transverse reticulate vessels with oblique articulation, and tracheids were observed in the powder. Thick-walled aseptate lignified fibers were also observed. Starch grains and oil globules were present in large amount as shown in Figure 1.

Macroscopical Characters of *Trayamana*

Gentiana kurroo Royle roots are dusky white or brownish, subcylindrical to quadrangular, entire or longitudinal split pieces of roots. The roots and rhizomes are longitudinally wrinkled and somewhat twisted. In dried specimen of rhizome portions, the wrinkles are more or less transverse. Fracture is short and brittle, and taste was sweet followed by bitter with aromatic odor.

Microscopy of Root of *Trayamana*

Diagrammatic section of the roots is subdivided into epidermal cells which are mainly for the absorption of



Figs 1A to F: Powder microscopy of *Picrorhiza kurroa* Royle ex Benth: (A) Cortex cells; (B) parenchyma cells with starch grains; (C) starch grains; (D) tracheids; (E) vessels; and (F) fibers

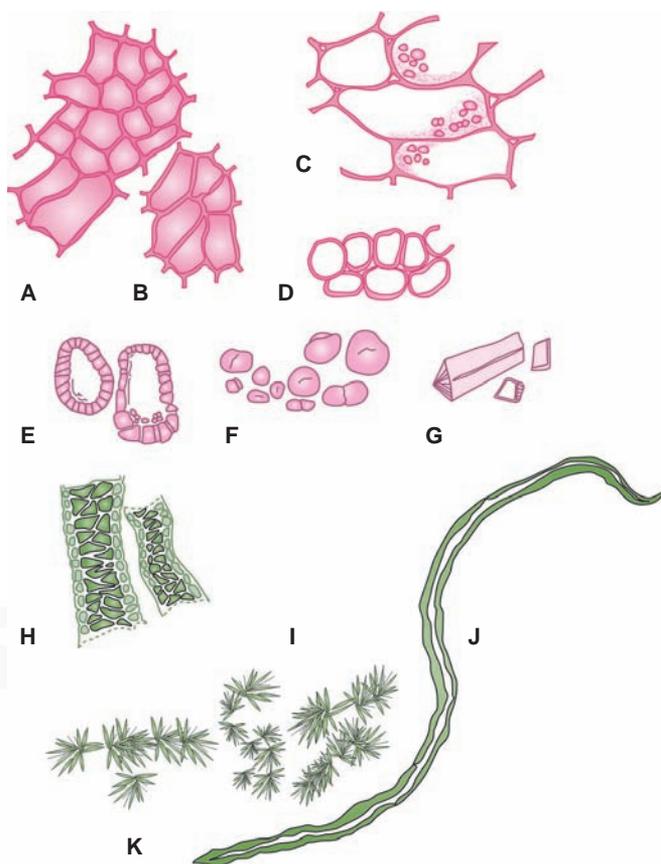
liquids, hypodermis, cortex, and vascular cylinder. Transverse section of root showed brown bark separated by a darker cambium line from the porous and indistinctly radiate wood. The outermost periderm consists of about four to six layers of thin-walled cork cells, presence of tangentially elongated, polygonal to oval parenchyma cells. The phellogen is not distinguishable. The secondary cortex is composed of parenchyma cells containing calcium oxalate crystals and small oil globules. The phloem is composed of tangentially elongated parenchyma and numerous strands of sieve tubes. Cambium ring is distinct; xylem is broad and consists of long annular or scalariform tracheids arranged singly or in groups of about 40 μ in diameter; and reticulate and spiral xylem vessels in group were observed.

Powder Microscopy of Root of *Trayamana*

The powder is yellowish orange in color and shows fragments of elongated, polygonal to oval parenchyma cells. Vessels with annular and scalariform thickenings; cork cells in surface view; prismatic crystals of calcium oxalates, raphides, and oil globules were also observed. Presence of lignified reticulate to oval sclereids and aseptate lignified fibers and few simple to compound starch grains were found in the powder treated with chloral hydrate. Figure 2 illustrates the powder microscopy of *Gentiana kurroo*. The details of the comparative microscopical studies are presented in Table 1.

Physicochemical Parameter

Comparative physicochemical values of root of *Picrorhiza kurroa* Royle ex Benth and rhizome of *Gentiana kurroo* Royle are mentioned and expressed as % w/w (n = 5), and the values for *Picrorhiza kurroa* Royle ex Benth are as within the prescribed limit as mentioned in the API. However, the official standards of *Trayamana* are not available in the official compendium to the best of our



Figs 2A to K: Powder microscopy of *Gentiana kurroo* Royle: (A and B) Group of closely packed rectangular flattened cells with wavy thick cutinized wall and yellowish brown cell contents; (C) group of thin-walled large parenchymatous cells with intercellular spaces and cell contents; (D) group of round to oval smaller closely packed cells; (E) lignified rectangular to oval sclereids; (F) simple to compound (two cells) starch grains; (G) prismatic crystals of calcium oxalate; (H) reticulate xylem vessels; (I) spiral xylem vessels in group; (J) aseptate lignified fiber; and (K) large thick acicular raphides present in groups of peculiar branched manner

knowledge; therefore, the comparison with standards was not possible. Moreover, the study could serve to establish the pharmacopoeial standards of *Trayamana*. The details of the physicochemical parameters are presented in Table 2.

Table 1: Comparative of powder drug analysis of rhizome of *Picrorhiza kurroa* Royle ex Benth and root of *Gentiana kurroo* Royle

Cell structure	<i>Picrorhiza kurroa</i> Royle ex Benth.	<i>Gentiana kurroo</i> Royle
Cork cells	4–5 layers	Single layer
Cortex	20–25 layers of cork, consisting of tangentially elongated suberised cells	4–6 layers, thin walled
Parenchyma cells	Thick-walled, oval to polygonal	Tangentially elongated, polygonal to oval
Vessels	Having transverse, oblique articulation	Reticulate xylem vessels and spiral xylem vessels in groups
Tracheids	Long, thick-walled, lignified, cylindrical with blunt tapering ends	Absent
Sclereids	Absent	Lignified reticulate to oval
Fiber	Thick-walled aseptate lignified	Aseptate lignified
Starch grains	Simple and abundant	Simple to compound and lesser in number
Calcium oxalate crystals	Absent	Few prismatic crystals of calcium oxalate
Raphides	Absent	Large thick, acicular, present in groups of peculiar branched manner
Oil globules	In large amount	Present

Table 2: Comparative physicochemical values of root of *Picrorhiza kurroa* Royle ex Benth and root of *Gentiana kurroo* Royle (expressed as % of dry weight and average of five readings)

Values	<i>Picrorhiza kurroa</i> Royle ex Benth (% w/w)		<i>Gentiana kurroo</i> Royle (% w/w)
Ash values			
Total ash	5.92%		6.1%
Acid-insoluble ash	0.8%		1.8%
Extractive values			
Water-soluble extractive	33.10%		19.1%
Alcohol-soluble extractive	15.40%		31.1%
Foreign matter	1.5%		2.1%

CONCLUSION

Microscopy as well as various phamacognostic aspects of the rhizome sample were studied and described along with physicochemical parameters. These parameters will be useful in authentication and identifying the adulterants and quality control of raw drugs because *Kutki* is considered as one of the important drugs of Ayurveda, and different botanical sources are used in various parts of the country as *Kutki*. *Trayaman* is distinguished from *Kutki* by various diagnostic characters, such as absence of raphides and calcium oxalate crystals in *Kutki*. 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 Ayurvedic compound formulation.

REFERENCES

- Nadkarni, KM. Indian Meteria medica. Mumbai: Popular Prakashan; 1999. p. 573.
- Chopra, RN.; Chopra, IC.; Handa, KL.; Kapur, LD. Indigenous drugs of India. Kolkata: UN Dhur and Sons; 1958. p. 181, 509.
- Sharma, PV. Dravya guna vijnana. Vol. II. Varanasi: Chowkhambha Orientalia; 1978. p. 696.
- The wealth of India. Raw materials. Vol. IV. New Delhi: Publication and Information Directorate, Council of Scientific and Industrial Research (India); 1956. p. 125.
- Chauhan, NS. Medicinal and aromatic plants of Himachal Pradesh. New Delhi: Indus Publishing Company; 1999. p. 207-210.
- Grieve, A. Modern herbal. Middleburg (VA): Penguin; 1984. ISBN 0-14-046-440-9.
- Bown, D. Encyclopaedia of herbs and their uses. London: Dorling Kindersley; 1995.
- Pushpangadan, P.; Mehrotra, SR.; Rao, CV.; Ojha, SK.; Raghavan, G.; Rao, GMM.; Padmavathi, S., inventors. Synergistic composition for treating hyperlipidemia. US patent. 6,989,165. 2006.
- Lust, J. The herb book. New York: Bantam Books; 1983.
- Launert, E. Edible and medicinal plants. Middlesex (UK): Hamlyn; 1981.
- Chiej, R. Encyclopaedia of medicinal plants. Edinburgh: MacDonald; 1984.
- Rastogi, RP.; Mehrotra, BN. Compendium of Indian medicinal plants. Vol. V. New Delhi: Publication and Information Directorate, CSIR; 1990-1994. p. 387.
- Kumar V, Chand R, Auzi A, Ikeshiro Y, Sarker SD. 2'-(2,3-Dihydroxybenzoyloxy)-7-ketologanin: a novel iridoid glucoside from the leaves of *Gentiana kurroo*. Pharmazie 2003 Sep;58(9):668-670.
- Garg, S. Substitute and adulterant plants. New Delhi: Periodical Experts Book Agency; 1991. p. 43-44, 128.
- Guhabakshi, DN.; Sensharma, P.; Pal, DC. A lexicon of medicinal plants in India. Vol. II. Kolkata: Naya Prakashan; 1999. p. 247-248.
- Kirtikar, KR.; Basu, BD. Indian medicinal plants. Vol. III. New Delhi: Indian Book Centre; 1993. p. 1661.
- Evans, WC.; Trease, D. Pharmacognosy. Edinburgh: Saunders Company; 2002. p. 519-520.
- Quality control methods for medicinal plant materials. Geneva: World Health Organization; 1998.
- Government of India, Ministry of Health and Family Welfare, Department of AYUSH. The Ayurvedic Pharmacopoeia of India. Part 1. Vol. I. 2004. p. 84-86.

हिन्दी सारांश

दो महत्वपूर्ण लुप्तप्राय आयुर्वेदीय औषधीय पादपों कुटकी तथा त्रायमान की पहचान स्थापित करने हेतु सूक्ष्मदर्शी अध्ययन

अनुपम के. मंगल,¹ चिन्मय रथ², देवेश तिवारी,³ श्रेया दत्ता,⁴ नारायणम श्रीकांत,⁵
करतार एस. धीमान⁶

कुटकी एक महत्वपूर्ण आयुर्वेदीय औषध है, जो हिमालयी क्षेत्र में पाई जाती है एवं इसका वानस्पतिक नाम पिकरोराइजा कुर्रो रोयल बेंथ है, जो कि स्क्रोफुलेरियेसी कुल से संबंधित है। यह पादप लघु, बालदार बहुऋतुजीवी औषध है एवं विभिन्न आयुर्वेदीय औषधियों में उपयोग किया जाता है। इसकी अधिक मांग के कारण, इस पादप को अक्सर त्रामायन की जड़ों के साथ अपमिश्रित किया जाता है जो कुटकी के स्थान पर प्रायः उपयोग में लाया जाता है। यह रेचक, यकृत-उत्तेजक, स्तन्यवर्धक एवं क्षुधा उत्तेजक के रूप में भी उपयोग किया जाता है तथा ब्रॉन्किअल अस्थमा में लाभदायक है। वर्तमान अध्ययन पिकरोराइजा कुर्रो की परिशुद्ध पहचान के लिए सूक्ष्मदर्शी अन्वेषण के माध्यम से मूल्यांकन पर केन्द्रित है तथा वास्तविक औषध का उसके स्थान पर प्रयोग होने वाले पादप जेन्शियाना कुर्रो रोयल के साथ तुलना पर भी प्रकाश डालता है। पिकरोराइजा कुर्रो के कंद एवं जेन्शियाना कुर्रो की जड़ों का सूक्ष्मदर्शी अध्ययन न केवल औषध की पहचान का विवरण देगा वरन् भेषज मानकीकरण स्थापित करने में भी सहायक होगा।

