

A Comprehensive Review on *Picrorhiza kurroa* Royle ex Benth.

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Review Article

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ABSTRACT

There exists a wide diversity of medicinal plants in Western Himalaya and among them, the drug “Kutki” (*Picrorhiza kurroa* Royle ex Benth.) is one of the highly renowned medicinal plants of this region. More than 50 secondary metabolites have been recorded from the dried rhizomes and roots of this endangered plant and it is harvested for medicinal purpose to treat various types of diseases such as asthma, bronchitis, malaria, chronic dysentery, viral hepatitis, upset stomach, scorpion sting, stimulation of appetite and improvement of digestion, liver protection, skin conditions, peptic ulcer and neuralgia, vitiligo and rheumatic arthritis. Overexploitation and consequent degradation of natural habitat are reported to be a major threat to this plant. Due to its remote location, narrow distribution range and small population size, scientific investigations on this species are sporadic and fragmentary. Much information on this species is scanty and fragmented and need to be compiled. It is important because they provide insight into the scientific information available, even if too little, with a view to determining the gaps that need to be addressed in the future. This article tries to consolidate all the available information into a single compilation. After thorough investigation and literature search, it was observed that less work has been done on this plant especially on its extraction methods and storage conditions and durations.

INTRODUCTION

Picrorhiza kurroa Royle ex Benth., the well known medicinal herb for rhizomes and roots, belongs to the family Scrophulariaceae. It is known variously in different parts of the country like Katki and Karu (Bengali), Kadu (Gujarati), Katki, Karu and Katki (Hindi), Katu, Khurohani (Malayalm), Kutaki(Marathi), Kalikutki, Karru, and Kaur (Punjabi), Katuka Katurohini, Kedarakatuka and Tiktaranica (Sanskrit), Kutki (Unani), Katukurogani (Tamil), Katukurogani and Katukurohini (Telegu), Kutakisafed (Urdu), Kadvi (UP) and Hellebore (English). In Himachal Pradesh it is locally known as Kadu in Chamba, Keylong and Manikaran, Kutki in Pangī and Karu in Rotang. This species is variously known in different countries like Kutakee in Nepal, Koorean in Japan, Huang line in China, Katukarasana in Sri Lanka, Khairbague in Arab, Kharbaq Sijah in Persia and Hon-Len in Tibet. [1-4] Scientific investigations on this species are sporadic and fragmentary. This article tries to consolidate all the available information into a single compilation to provide insight into the scientific information available with a view to determining the gaps that need to be addressed in the future.

METHODOLOGY

A large number of the published documents and literatures were collected using Google Scholar, Educational Resources Information Center, Research Gate, Bielefeld Academic Search Engine and Semantic Scholar. Library sources such as books, journals, and newsletters were also browsed to locate any available information on this species. All those documents were published prior to 2018. Furthermore, several scientists who have worked on temperate medicinal plants were personally contacted to elicit any further information on this species.

CHARACTERISTICS OF PLANT

Picrorhiza kurroa Royle ex Benth. is a perennial herb up to 20 cm tall at flowering stage possessing 15-25 cm long branched stoloniferous root stock which perennates during the most parts of the year.[1-5] Stem is small, weak, creeping or erect, leafy and slightly hairy.[6] Leaves are 5-15 cm long, mostly radical, cauline absent or appearing in the form of bracts at fruiting stage.[7] Flowers are very small, in dense spicate racemes and white or pale blue-purple colored with exerted stamens. [1,4,6-8]

HABITAT AND DISTRIBUTION

It is an important alpine herb of Himalayan region growing at an altitudinal range of 3,000 to 5000 m above mean sea level in meadows.^[9,10] Though this plant grows naturally at altitude above 3000 m asl, it can be cultivated at lower altitudes.^[11,12] It is endemic to Western Himalayas extending up to mountains of Yunnan in China.^[6] It has a long creeping rootstock that grows in rock crevices and moist, sandy soil.^[9] In Himachal Pradesh it is found in the higher reaches of Chamba, Kangra, Mandi, Shimla, Kinnaur, Lahaul and Spiti districts.^[13] In Kashmir Himalayas it grows in high reaches of Gurez valley, Lolab, Keran, Sindh and Lidder valleys. It is commonly seen associated with the herbs like *Aconitum violaceum*, *Lagotis cashmiriana*, *Potentilla kashmirica*, *Sedum ewersii* and *Senecio jacquemontianus*. It is observed to be common in Gurez, occasional in Lolab and Keran valleys whereas, rare in Sindh valley. On the whole from Kashmir Himalayas, this species is considered to be an endangered one.^[4]

ADULTERATION

In spite of herbal medicines over many centuries as important mean for medication for treatment of illness or as dietary supplements and health products, little attention has been given towards the standardization of medicinal plants and other natural products. From the time of collection of plant or plant parts to its storage and up to the production of medicines/formulations, chances of deterioration in quality are quite frequent; resulting in decline of efficiency of drugs.^[14] 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. To protect the public health, a number of quality standards have been developed to safeguard against hazardous contamination and other abnormalities of these medicinal materials. These include determination of heavy metals, pesticide residues, mycotoxins, foreign matter, ash, extractives, water content etc. in the products. Of various quality related tests, the level of ash content and extractive values remain one of the most facile means to ascertain the quality of medicinal plant materials. Certain pharmacopoeias and some publications from World Health Organization (WHO) related to quality control aspects of medicinal plants have strongly emphasized these parameters as a requirement for quality standards.^[15] However, intentional as well as unintentional adulteration in the drug is regular phenomenon. 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 1) replacement by exhausted drugs, 2) substitution by superficially similar, but inferior parts 3) substitution by artificially manufactured substitutes, 4) substitution by substandard commercial varieties and 5) substitution by organic matter (non official parts) obtained from the same plant. Thani observed adulteration on *Picrorhiza kurroa* while evaluating the chemical profile of its raw material collected from the from 35 different retail shops from different market places of India.^[16] This species is generally known to be adulterated by roots of *Gentiana kurroa*, *Holoptelea integrifolia* and *Lagotis cashmiriana* Royle ex Benth.^[4] *Lagotis cashmiriana* grows with *Picrorhiza kurroa* at similar elevations between 3,200 – 4,500 m and sometimes sold in the name of 'Kutki'. Adulteration of raw material is one of the greatest drawbacks in the global distribution of herbal products.^[17,18] To maintain the safety, efficacy and quality of such products, a proper process for the authentication of crude drugs is needed. The general methods of authentication to check for adulteration of herbal drugs includes morphological /anatomical characterization, organoleptic markers (odor, color, texture) and chemical testing.^[19]

MORPHOLOGY-CHEMICAL CHARACTERISTICS OF UNDERGROUND PART

Organoleptic and histological characteristics analysis

a) Rhizome

Rhizome 2.5- 6.0 cm long and 0.5 -1.0 cm thick, sub-cylindrical, straight, externally grayish-brown, surface rough due to longitudinal wrinkles, circular scars of roots and bud scales attached; tip ends in a growing bud surrounded by tufted crown of leaves.^[20] It has pleasant odor and bitter taste.^[21,22] Rhizomes are jointed and zigzag, cylindrical, irregularly curved with branching and rooting at the jointed nodes.^[23]

The rhizome consists of 20-25 layers of cork consisting of tangentially elongated, suberised cells; cork cambium is generally 1-2 layered; cortex single layered or absent, primary cortex persists in some cases, one or two small vascular bundles present in cortex; vascular bundles surrounded by single layered endodermis of thick-walled cells; secondary phloem composed of phloem parenchyma and a few scattered fibers; cambium 2-4 layered; secondary xylem consists of vessels, tracheids, xylem fibers and xylem parenchyma, vessels vary in shape and size having transverse oblique articulation; tracheids long, thick-walled, lignified, more or less cylindrical with blunt tapering ends; xylem parenchyma thin-walled and polygonal in shape; centre occupied by a small pith consisting of thin-walled cells; simple round to oval, starch grains, measuring 25-104 µm in dia., abundantly found in all cells.^[20-24,25]

b) Root

Roots are thin, cylindrical, 5-10 cm long 0.05-0.1 mm in diameter, mostly attached with rhizome; inner surface black with whitish xylem. Odor is pleasant and taste is bitter.^[20] They are grayish to brown in appearance, while the fracture is tough.^[26] Root stacks are irregularly curved as thick as the little finger.^[27,28]

Young root generally have single layered epidermis, some epidermal cells elongate forming unicellular hairs; hypodermis single layered; cortex is 8-14 layered consisting of oval to polygonal, thick-walled, parenchymatous cells; primary stele tetrarch to

heptarch, enclosed by single layered pericycle and single layered, thick-walled cells of endodermis; mature root shows 4-15 layers of cork, 1-2 layers of cork cambium; secondary phloem poorly developed; secondary xylem consisting of vessels, tracheids, parenchyma and fibers; vessels have varying shape and size, some cylindrical with tail like, tapering ends, some drum shaped with perforation on end walls or lateral walls; tracheids cylindrical with tapering pointed ends; fibers aseptate, thick-walled, lignified with tapering blunt chisel-like pointed ends. [20-24,25]

Transverse section of root shows that periderm consist of about 8-10 layers of thin walled cork cells having average dimensions of $35\mu \times 20\mu$. Phellogen cells appear rectangular and radially flattened in transverse section and lack in intercellular spaces. The cells of phellogen are more or less arranged definitely in radial rows. The cortex consists of thin parenchymatous cells with average dimension of $50\mu \times 15\mu$. The secondary phloem consists of layers of cells and they originate from the cambium. The secondary phloem is composed of sieve tubes, companion cells and parenchyma. The phloem ray cells are indistinguishable. Xylem consists of groups of thick walled fibers and few porous tracheae, embedded in between the pith and the cortex. Tracheae with porous wall thickening have an average diameter of 24μ . Cambium cells have average dimensions of $11\mu \times 4\mu$. The pith is characterized by the presence of parenchymatous cells with porous wall thickening having diameter of $50\mu \times 40\mu$. [1,29]

Cortex consists of collenchymatous and parenchymatous cells, cortical bundles, radially arranged xylem which is endarch, and the pith. A large number of root and leaf traces, leaf gaps and branch connection are visible. The vascular ring is not continuous and consists of 3-6 vascular area of different sizes. [30] The cells of cortex and pith are spongy and filled with starch grains. [31] The roots show only thick parenchymatous cells and four to seven arcs of exarch xylem. Cortical bundles and pith are absent. [3]

Chemical characteristics analysis

In Indian Herbal Pharmacopoeia, the quality control parameter for dry "Kutki" (*Picrorhiza kurroa*) powder are mentioned as foreign matter should not be more than 2%, total ash should not be more than 7% and acid-insoluble ash should not be more than 1%. Also alcohol soluble extractives should not be less than 20% and water soluble extractives should not be less than 30%. [20] Besides this, different chemical/ biochemical techniques such as high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GC), gas chromatography mass spectroscopy (GCMS) and liquid chromatography mass spectroscopy (LCMS) have also proven useful in component identification and for the detection of adulterants in traded commodities of plant origin along with immunoassays and DNA based molecular tools. [32-39] For example, TLC of alcoholic extract of the drug on Silica gel G plate using chloroform: methanol (95:5) shows under U.V. light (366 nm) three fluorescent zones at Rf. 0.05 (blue), 0.30 (blue) and 0.35 (green). On exposure to Iodine vapor nine spots appear at Rf. values 0.10, 0.17, 0.21, 0.30, 0.37, 0.41, 0.62, 0.72 and 0.84 (all yellow). On spraying with 5% methanolic sulphuric acid reagent and heating on plate for about ten minutes at 105°C, all brownish grey seven spots appear at Rf. 0.05, 0.10, 0.17, 0.21, 0.30, 0.41 and 0.84. [25] Furthermore, for the quantification of two iridoid glycosides, kutkoside and picroside-I, which have active hepatoprotective principles, a precise and rapid thin layer chromatography (TLC) method was developed. The analysis was performed on a TLC precoated silica gel 60 F254 plate with ethyl acetate: methanol: glacial acetic acid: formic acid (25:5:1:1) as mobile phase.

Densitometric evaluation of kutkoside and picroside-I was carried out at 265 nm and the mobile phase showed good resolution with Rf. values 0.42 ± 0.03 and 0.61 ± 0.03 for kutkoside and picroside-I, respectively. The content of kutkoside and picroside-I was found to be 2.18% and 1.90%, respectively, and was comparable with those obtained by HPLC. The linearity was found to be in the range of 80-480 ng/spot for both kutkoside and picroside-I. The average recovery values were found to be 96.5% and 96.0% for kutkoside and picroside-I, respectively. [40]

CHEMICAL CONSTITUENTS

More than 50 secondary metabolites have been reported from the plant *Picrorhiza kurroa* which includes iridoid glycosides, cucurbitacins and phenolic compounds. The dried rhizome of *Picrorhiza kurroa*, contains at least 60% of a 1:1.5 mixture of Picroside-I and Kutkoside and the remainder 40% is a mixture of iridoid as well as cucurbitacin glycosides. [41]

The pharmacological importance of *Picrorhiza kurroa* has been demonstrated due to picroside (picroside-I and picroside-II) and other metabolites like picroside-III, picroside-IV, kutkoside, etc. The bioactive compounds picroside and kutkoside belong to the category of iridoids. Besides these iridoid glycosides, other minor iridoids such as vernicoside, minecoside and 6-feruloyl catalpol and phenol glycoside picein and androsin have also been identified in this plant. [42,43] Jia et al. also isolated a neval iridoid glucoside pikroside (0.20%), besides already known iridoid glycosides i.e. picroside-I (0.78%), picroside-II (0.69%) and 6-feruloyl catalpol (0.50%) and has been characterized as 6-vanniloyl catalpol. [44] Similarly, Mandal and Mukhopadhyay isolated a new iridoid glucoside picroside-V. [45] Besides iridoid glycosides, a total of 23 different cucurbitacin glycosides and a single aglucone. phenolic compounds including apocynin/I androsin4 and vanillic acid14 and flavonoids have also been identified in *Picrorhiza kurroa*. [46]

The content of these chemical compounds on raw material depend upon different factors. For example, picroside-I and picroside-II are the effective ingredients responsible for its medicinal effect and these chemical constituents vary according to different plants at different altitudes. The plants collected from the lower altitude contains less picroside content as compared to plants collected from higher altitude. [47] Similarly, the bitter glycoside content has been shown to vary with plant age, plant part, collection time and place of collection. Kaul reported that the two years old rhizome and rootlets yield about 1.5 times more kutkin as compared to one year old rhizomes and rootlets of *Picrorhiza kurroa*. [4] The content in rootstock collected from Himachal Pradesh was observed

to be 0.70- 0.90 percent as compared to 1.00 percent in rootstock collected from Garhwal (Uttaranchal) region and the kutkin content was almost double in rhizomes compared to rootlets. Dutt et al. also reported the differential accumulation of picroside-I, and picroside-II in leaves and rootstock of 30-240 days old tissue culture raised plants of *Picrorhiza kurroa*. The picroside-II content was observed higher (1.01%-3.03%) as compared to picroside-I (0.08%-0.29% in roots, whereas picroside-I was more (0.31%-1.62%) as compared to picroside-II in leaves. [48]

EXTRACTION METHOD

Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plant. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/ inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. The purpose of standardized extraction procedures for crude drugs is to attain the therapeutically desired portion and to eliminate unwanted material. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction(Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave- assisted extraction, ultrasound extraction (Sonication), supercritical fluid extraction and phytonic extraction. [49]

In addition to this, the basic parameters influencing the quality of an extract are plant parts used as starting material, solvent used for extraction and extraction procedure. [50] The selection of method to isolate active components with best yield and higher purity from natural sources is mainly dependent on the nature of compounds and raw material which is going to be processed. [51] A typical extraction process includes, collection and authentication of plant material and drying, size reduction, extraction, filtration, concentration and drying and reconstitution. [49]

Since, very little research has been done to find efficient extraction methods in order to get high efficiency and efficacy from the *Picrorhiza kurroa* and therefore, it is very difficult to make comparison on them. However, Thani et al.has reported sonication method best in terms of time consumption for extraction and yield while comparing four extraction methods, soxhlet extraction, extraction by refluxing, microwave assisted extraction and sonication assisted extraction. [52]

MEDICINAL USE

Picrorhiza kurroa is an important herb in the Indian medical system of Ayurveda. It has been found to possess' hepatoprotective, antioxidant, anti-inflammatory, immunomodulatory properties along with antiallergic, antiasthmatic, anticancerous, cardiovascular, choleric, hypoglycemic, hypolipidemic, antiviral, purgative, anti-phosphodiesterase, neuritogenic, molluscicidal, and leishmanicidal activities. [53-59] Its' underground part has been used traditionally for Asthma, bronchitis, Malaria, chronic dysentery, viral hepatitis, upset stomach, Scorpion sting, as a bitter tonic(stimulating the appetite and improving digestion), and as a liver protectant. [60,61] Besides, it has been used in the treatment of skin conditions, peptic ulcer and neuralgia, vitiligo and rheumatic arthritis. [42,43] Recently its' root and rhizome extracts of have also shown to possess anti malarial activity. [62]

There are several commercially available drug formulations of *Picrorhiza kurroa* in India which contain P-I and P-II, in different concentrations. [63] **Table 1.**

EFFECT OF STORAGE CONDITIONS ON PHYTO-CHEMICALS

Medicinal properties of plants are manifested due to the presence of bioactive compounds present in them. The stability of bioactive compound largely depends upon their chemical structure. Some of these compounds undergo degradation in the presence of heat, moisture, light, solvents and enzymes. The concentrations of these compounds in plants are greatly influenced by genotype, plant growth and development stages, and environmental factors. Immediately after harvesting the medicinal plants, it becomes important to minimize the loss of active constituents during drying conditions and storage of dried material till it is finally converted into formulation to be used by the consumers. Not much work pertaining to effect of different storage conditions and storage durations on stability of bioactive constituents of plant *Picrorhiza kurroa* has been done. However, Thani and Sharma

Table 1. Commercially available drug formulations of *Picrorhiza kurroa*.

	Manufacturer	Picroside-I (%)	Picroside-II (%)	Total Picrosides (%)
Katuki	Zandu Pharma Works Ltd. (Mumbai)	1.29	1.16	2.45
Arogya	Zandu Pharma Works Ltd. (Vapi, Gujrat)	1.01	0.55	1.56
Kutaki	Tansukh Herbal Pvt. Ltd. (Lucknow, UP)	4.17	3.25	7.42
Livocare	Dindayal Aushdi Pvt. Ltd. (Gwalior, MP)	0.06	0.14	0.20
Livocare	Maharishi Ayurveda Pvt. Ltd. (New Delhi)	0.12	0.06	0.18
Livomyn	Charka Pharma Pvt. Ltd. (Mumbai)	0.07	0.01	0.08
Livplus	BACFO Pharmaceuticals Ltd. (Noida, UP)	0.22	0.49	0.71
Pravekliv	Pravek Kalp Herbal Products Pvt. Ltd. (Noida, UP)	0.15	0.12	0.27
Vimliv	OM Pharma Ltd. (Bangalore)	0.001	Traces	0.001

have reported the decrease of bioactive compound, picroside-I and picroside-II content, with the increase in storage duration irrespective of different storage conditions. Furthermore, on their comparative study they have claimed that the loss in picroside-I and picroside-II with storage was maximum when “Kutki” samples were stored under humid condition (85% humidity, 25 °C) and minimum loss was observed when samples were stored at low temperature (4-6 °C). So, they suggested that to minimize the loss of picroside-I and picroside-II content in the drug Kutki during storage, Kutki must be stored at low temperature.^[64]

TRADE AND THREAT PERCEPTIONS

Natural products are becoming increasingly important as sources of pharmacotherapeutics, either as herbal drugs for treatment of chronic diseases or as raw materials from which compounds with particular biological activities are isolated. More than 60% of newly approved anticancer and anti-infection drugs are derived from natural sources including picrorhiza.^[65,66] Therefore, the demand for Picrorhiza kurroa is continuously increasing, e.g. an annual growth rate of 12.9% was recorded for its demand of 220 tonnes in 2001-2002 and 317 tonnes during 2004-2005.^[67] It is one of the top 15 plant species traded in India in terms of the economic value of traded materials.^[68,69] Annual transaction of more than 10,000kg of Picrorhiza kurroa takes place only in Delhi market.^[70] Global supply (excluding China and Pakistan) of Picrorhiza kurroa is around 375 tonnes, with India contributing around 70 tonnes. In India annual demand for Picrorhiza kurroa is more than 5000 tonnes; however, its supply is less than 100 tonnes.^[71] Increasing demand for the Kutki drug has prompted many researchers to search for source of genotypes of Picrorhiza kurroa rich in picroside content.^[47]

Overexploitation and consequent degradation of natural habitat are reported to be a major threat to this plant. Over 90% of the market demand for this species is met from the wild. Uniyal et al., reported that to get 1kg dry weight of Picrorhiza kurroa plant, as many as 300 to 400 individual plants are uprooted.^[70] Due to narrow distribution range, small population size and high use value, the species figure among the 37 identified as top priority species for conservation and cultivation in Western Himalaya. Indiscriminate, unscientific harvesting and lack of organized cultivation of the plant has threatened its status in wild and listed as ‘endangered’ species by International Union for Conservation of Nature and Natural Resources.^[72]

CONCLUSION

The present work was carried out on medicinal herb Picrorhiza kurroa which comes under the family of Scrophulariaceae. Here the review was carried out from the different aspects of plant such as habitat, morphology, anatomy, chemical composition, adulteration, pharmacology and medicinal usage, storage characteristics, extraction method and threat. After thorough investigation and literature search, it was observed that less work has been done on this plant especially on its extraction methods and storage conditions and durations

Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plant. The purpose of standardized extraction procedures for crude drugs is to attain the quantitative and qualitative therapeutically desired portion and to eliminate unwanted material. Similarly, medicinal properties of plants are manifested due to the presence of bioactive compounds present in them which undergo degradation in the presence of heat, moisture, light, solvents and enzymes. Immediately after harvesting the medicinal plants, it becomes important to minimize the loss of active constituents during drying conditions and storage of dried material till it is finally converted into formulation to be used by the consumers. Therefore more detail study is needed in extraction methods, storage conditions and durations.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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