Review Article

Picrorhiza kurroa: An ethnopharmacologically important plant species of Himalayan region

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Abstract

Picrorhiza kurroa Royle ex Benth. commonly known as Kutki, belongs to family Scrophulariaceae. It is found in the Himalayan regions of China, Pakistan, India, Bhutan and Nepal. It is considered as an important medicinal plant which is mostly used in the traditional medicinal system for asthma, jaundice, fever, malaria, snake bite and liver disorders. Different pharmacological activities of *P. kurroa* include anti-microbial, anti-oxidant, anti-bacterial, anti-mutagenic, cardio-protective, hepato-protective, anti-malarial, anti-diabetic, anti-inflammatory, anti-cancer, anti-ulcer and nephro-protective activities were recorded from this plant. So far, Iridoids (Picroside I and II), Cucurbitacins and Phenolic components are the different phytochemicals which are extracted from *P. kurroa*. The authentication of *P. kurroa* raw material for commercially available herbal/botanical products is essential and it is done by the DNA fingerprinting of *P. kurroa*. Because of the over-exploitation of *P. kurroa* for medicinal purposes, the conservational status of *P. kurroa* in different regions became endangered. It is the need of the hour to utilize different conservational strategies and save this medicinal wealth from extinction as it is widely used by the local people for curing different diseases and thus it cause immense pressure on the plant population.

Keyword: Ethnopharmacology; Conservation; Extinction.

Introduction

Geographical distribution

Kutki is a perennial herb found in the Himalayan region (Garhwal to Bhutan), West China, South-East Tibet and North Burma. It grows in wild form in alpine regions on rock crevices and also in organic soils. In Nepal, Kutki is found in abundance in alpine Himalayan region between altitudes of 3500m and 4800m and also in the western

regions of Nepal where it grows on the rock's crevices on the north facing slopes, cliffs and the turf of glacial flats.

P. kurroa Royle ex Benth. is present in wild form in the north-western Himalayan region from Kashmir to Sikkim [1]. It is found in the North-Western Himalayan region from Kashmir to Kumaun and Nepal and Garhwal regions in India [2]. It is found in Nepal, China, India, Pakistan and Bhutan [3].

Taxonomy and Morphology

The complete botanical name is *Picrorhiza kurroa* Royle ex Benth. It has got two synonyms i.e. *Picrorhiza lindleyana* (Wall.) Steud. and *Veronica lindleyana* Wall.

In Greek, “picros” means bitter, while “rhiza” means root. The specific epithet of plant is taken from the Punjabi name of the plant “Karu”, which means bitter [2].

Vernacular names being used for *P. kurroa* include Balakadulen, Hellebore, hohwangryun, honglen, hunglen, honkadu, huhuanglian, kadu, kadukrohini, kadugurohini, kalikutki, karupicrorhiza, karru, katki, katu, katuka, Karukarohini, katukaa, Kaurohini, Kavi, Kauka, katuka, katukarohini, katukarogani, katukarohini, katuki, katuko, katukurohini, katurohini, katvi, kaur, kharbhaswael, kharbaqe-hindi, kotkaanphraao, koouren, kotkaanphraao, kour, kurri, kuru, kutaki, kutki, kutta, rohini, sutiktaka, tikturohini, Tikti, xi zanghuhuanglian [4-8].

Morphological and Taxonomic Features

Picrorhiza kurroa Royle ex Benth. belongs to Scrophulariaceae family. This is a large plant family, with around 200 genera and 3000 species, mainly found in the northern temperate regions of the world. Scrophulariaceae includes the plants such as popular garden plants (including tiny alpines); and also some other plants grown for their aesthetic value (include Penstemon, Mimulus and Calceolaria) [9].

Kutki is a perennial herb with an elongated rhizome. The leaves are basal and alternate, approximately 5–10 cm long (Figure 1). Terminal Spikes are present. Calyx divide equally in 5 parts. The corolla has 4 or 5 lobes, 4–5 mm long, bilobate with lobes more or less spreading or nearly actinomorphic. Stamens 4, inserted on corolla tube, slightly didynamous. Stigma capitate. Fruit an acute capsule, tapered at top, dehiscent into 4 valves, 12 mm long. Seeds numerous, ellipsoid: seed coat very thick, transparent.

Pollen grains round, tri-colpate, with incomplete or perforate tectum, the partial tectum micro-reticulate, colpus membrane smooth or occasionally coarse [10, 11].

Rhizome is 2.5–12.0 cm long and 0.3–1.0 cm thick, sub-cylindrical, straight or to some extent curved, externally greyish-brown, external surface is coarse due to longitudinal furrows and spherical scars of roots, tip ends in a growing bud enclosed by a crown of leaves (Figure 2). Root is elongated, tubular, 5–10 cm in length and 0.5–1.0 mm in diameter, straight or marginally curved with a few longitudinal and dotted scars, mostly associated with rhizomes [12].

The anatomy of rhizome shows 20–25 layers of cork consisting of tangentially extended, suberized cells; 1–2-layered cork cambium; cortex single layered or not present, main cortex continues in some cases, 1 or 2 small sized vascular bundles (xylem and phloem) present in the cortex. Vascular bundles are surrounded by fibrous bundle sheath. Secondary phloem is made up of parenchyma cells and a few dispersed fibers. 2–4 layered thick cambium is present. Secondary xylem consists of tracheids, vessels, fibers and parenchyma cells. Vessels vary in size and shape, tracheids long, thick walled, lignified, more or less cylindrical with blunt pointed ends. Starch grains are abundantly present, 25–105 µm in diameter. Anatomy of root shows when root is young, it shows single layered epidermis, some epidermal cells stretch forming unicellular hairs. Hypodermis is single-layered. Cortex 8–14 layered, consisting of ovoid to polygonal, thick-walled parenchymatous cells. Primary stele, tetrarch to heptarch, enclosed by a single-layered pericycle and single layered thick-walled cells of endodermis. Mature roots show 4–15 layers of cork, 1–2 layers of cork cambium. Vessels vary in size and shape, some tubular with tail-like, tapered ends; some barrel shaped with perforation on

end walls or adjacent walls. Tracheids are cylindrical with tapered sharp ends [1].

The flowering period of Kutki is from June to August. It is found far away from the community and takes from hours to days to walk to its growing habitat. It has been reported that plant has been harvested to near extinction.

Pharmacological Activities

The pharmacological properties of *Picrorhiza kurroa* Royle ex Benth. include anti-microbial, hepatoprotective, anti-oxidant, anti-bacterial, anti-mutagenic and anti-cancer activities.

Rhizomes of *Picrorhiza kurroa* Royle ex Benth., when phytochemically screened showed the presence of bioactive components, which have been linked to antimicrobial properties. Numerous chemical experiments and TLC studies revealed the occurrence of sterols, glycosides and phenolic compounds when tested on various extracts of rhizomes of *P. kurroa*. The main chemical components found in *P. kurroa* include iridoid glycosides and cucurbitacins (triterpenoids). The effects of aqueous and methanolic extracts on pathogenic fungal (including *Candida albicans*, *Aspergillus niger*, *Pseudomonas aeruginosa*) and bacterial strains (including *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli*) indicated that the plant part can be used to treat infections caused by these bacteria and fungi. The aqueous and methanolic extracts showed antibacterial activity but the significant antimicrobial activity was shown by methanolic extract only, against *P. aeruginosa* and *S. aureus*. The aqueous extract was not much effective for anti-microbial activity and also it showed no activity against fungal strains. The efficacy of the extracts was less than ciprofloxacin, which is the conventional antibiotic [13].

The antimicrobial potential of acetone, aqueous, ethanol, hexane and methanol

extracts of rhizome of *P. kurroa* was checked against bacterial strains which belonged to gram positive and gram negative bacteria. Ethanolic extract of rhizome of *P. kurroa* exhibited high antibacterial activity against *B. cereus*, *E. coli*, *K. pneumoniae*, *S. aureus*, *S. pyogenes* and *S. typhi*. The methanolic extract of rhizome showed greater antibacterial activity against *S. aureus* and *P. aeruginosa*, while hexane and acetone extract showed intermediate activity against *B. cereus*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *S. typhi*. Aqueous extract of rhizome did not show antibacterial activity against the tested bacterial strains [14].

To evaluate mutagenic potential of *P. kurroa* root extract, the extract was tested in *Salmonella typhimurium* reverse mutation assay with five histidine-requiring strains of *Salmonella typhimurium* (TA1537, TA1535, TA98, TA100 and TA102). The test was done in two autonomous experiments (viz., Dose Selection Study/Cytotoxicity Study and Mutagenicity Study) in the absence and presence (10% S9 v/v) of metabolic activation system in triplicates. In the dose range finding study, the extract was tested at the dose levels of 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/ml in the absence and presence (10% S9 v/v) of metabolic activation system. The results of solubility, precipitation of test item and cytotoxicity study/dose range finding study, root extract of *Picrorhiza kurroa* was verified in the mutagenicity assay at the subsequent dosage levels. Mutagenicity Study with metabolic activation: 312.5, 625, 1250, 2500 and 5000 µg/ml. *P. kurroa* root extract did not induced a significant dose-linked rise in the quantity of revertant (his+) colonies in each of the five tester strains of *Salmonella typhimurium* in the absence and presence (10% S9 v/v) of metabolic activation system. It was concluded that *Picrorhiza kurroa* root extract is non-mutagenic in the *Salmonella*

typhimurium reverse mutation assay (Ames test). Thus it verifies the Anti-mutagenic activity of *P. kurroa* root extract [15].

Oral administration of ethanolic extract of *Picrorhiza kurroa* rhizome at a dose of 20mg/kg of the body weight, for 10 consecutive days, was found to increase the healing speed on Indomethacin-induced abdominal ulcer in mice, compared to the mice having ulcer but without treatment. The level of peroxidised fat, in terms of thiobarbituric acid reactive species (TBARS), in abdominal tissue, was increased in ulcerated mice which was restored to normal on treatment with ethanolic extract. The specific activity of in-vivo antioxidant enzymes i.e. SOD, catalase and total tissue sulfhydryl group, which were distinctly reduced in ulcerated group, were found to be considerably raised ($p < 0.05$), on treatment with the above extract, at the specified dose, compared to the indomethacin-induced ulcerated group without treatment. So it suggests that ethanolic extract of *P. kurroa* rhizome, at the dose of 20mg/kg, enhanced the healing of stomach wall of indomethacin induced gastric ulcerated rats by an in-vivo free radical scavenging mechanism [16].

The ethanolic extract of rhizome of *P. kurroa* was checked for nephroprotective and nephrocurative activity in female Wistar mice against Cisplatin induced nephrotoxicity, by estimating blood urea and serum creatinine levels. One of the Ayurvedic preparations i.e. Arogyawardhini, comprising *P. kurroa* as a major ingredient was also studied for the nephrocurative and nephroprotective effects against Cisplatin induced nephrotoxicity. The preparation was standardized for the existence of total polyphenols. Treatments with the ethanol extract of the rhizome in the dose of 600 mg/kg could considerably decrease the high serum levels of blood urea and creatinine. So the preparation was found to have better effect as compared to the rhizome [17].

Anti-inflammatory effect of *P. kurroa* extract (PK) by β -adrenergic blockade was confirmed, which suggests alteration in cell-surface biology by PK action. Obstruction of protein synthesis by cycloheximide pre-treatment reduced PK effect suggesting protein mediation. Dinitrophenol which is a metabolic inhibitor, repressed inflammatory oedema likewise in control and PK treated animals, and masking of PK effect was concluded. Selective PK effect on membrane related activation procedures in inflammatory effector cells could be the origin of anti-inflammatory and perhaps other biological activities [18].

The antioxidant properties of *P. kurroa* were evaluated in-vitro using different radical scavenging methods. Moreover, the antioxidant activity of extract was tested by using liver slice culture system and ethanol was used as a hepatotoxin to generate oxidative stress. Discharge of intracellular marker enzymes such as glutamate oxaloacetate transaminase, lactate dehydrogenase and glutamate pyruvate transaminase indicated hepatotoxicity. Ethanol induced oxidative stress and its modulation in the presence of *P. kurroa* extract was verified by measuring the quantities of antioxidant enzymes such as catalase, glutathione reductase, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase and of antioxidant molecules i.e. uric acid and reduced glutathione that were quantified along with lipid peroxidation. The results clearly prove that aqueous extract of *P. kurroa* with high antioxidant activity, as confirmed using different radical scavenging methods, was active in reducing the harmful effects of ethanol. Addition of *P. kurroa* aqueous extract along with ethanol reestablished the activities of antioxidant enzymes and significantly suppressed lipid peroxidation [19].

Phytochemistry of *P. kurroa*

Picrorhiza kurroa Royle ex Benth. comprises of iridoid glycosides i.e. picroside I and II [Figure 3(a), 3(b)] as key bioactive components. The high pressure liquid chromatography (HPLC) method was used to determine the picroside-I and picroside-II content in rhizome of seven accessions [Rohtang-3978 m (PKR-1); Marhi-3300 m (PKM-2); Manali-2050 m (PKM-3); Keylong-3350 m (PKK-4); Khoksar-3160 m (PKK-5); Manikaran-1737 m (PKM-6) and Chamba-996 m (PKC-7)]. Both components i.e. picroside I and II have been detected in the rhizome of all seven accessions of *P. kurroa*. After thorough evaluation, it was confirmed that the fraction of both analytes were found to be maximum, that is, picroside-I (3.5%) and picroside-II (2.0%) in rhizome of *P. kurroa* collected from Rohtang area (3978 m). The chemical studies on the rhizomes of *P. kurroa* indicated the presence of iridoids, acetophenones and cucurbitacins. It is known to contain picroside I and II, as major bioactive compounds. Both species of genus *Picrorhiza*, that is, *P. scrophulariiflora* Pennell and *P. kurroa* Royle ex Benth are rich source of picroside I and II. *P. kurroa* also contains phytochemicals including pikuroside, veronicoside, phenol glycosides, a number of cucurbitacin glycosides and 4-hydroxy-3-methoxy acetophenone [Figure 3(n)] [20].

A method to determine picroside and kutkoside [Figure 3(e)] in *Picrorhiza kurroa* and its herbal preparation was high performance thin layer chromatography (HPTLC). The amount of picroside content in *P. kurroa* and its formulations were found to contain 7.27%, 6.83% and 7.03% respectively. The amount of kutkoside in the same plant extract and its formulation was found to be 3.22%, 3.17% and 2.97% respectively. The plant phytochemicals include iridoid glycosides i.e. picroside I and kutkoside as major bioactive components.

Other minor constituents include picroside-III [Figure 3(c)], veronicoside [Figure 3(i)], minecoside, phenol glycoside i.e. Picein [Figure 3(o)] and androsin [Figure 3(j)], cucurbitacin glycosides and 4-hydroxy-3-methoxy acetophenone [21].

Kutkin, which is one of the active constituent of *Picrorhiza kurroa* is composed of kutkoside and the iridoid glycoside (picrosides I, II, and III). Other active constituents identified from plant extract are apocyanin [Figure 3(h)], drosin and nine cucurbitacin glycosides. Apocynin is a catechol that can prevent neutrophil oxidative burst, also it is a powerful anti-inflammatory agent, while the cucurbitacins are highly cytotoxic and antitumor in action [22-24].

The cucurbitacins include triterpenes, known for their bitterness and toxicity. Cucurbitacins isolated from *Picrorhiza kurroa* include cucurbitacin B, D [Figure 3(f), 3(g)] and R. The basic nucleus of cucurbitacins is 9-methyl-19-norlanosta-5-ene and it is also known as 10-cucurbit-5-ene. Some of the iridoids in *P. kurroa* are picroside I, II, III and V [Figure 3(d)], pikuroside, 6-feruloylcatalpol, minecoside and kutkosides. The basic nucleus of the iridoids is cyclopentano-[c]-pyran. *P. kurroa* also contains kutkin and picroliv. Kutkin consists of the picroside I. The kutkoside is present in a ratio of 1:2 along with glycosides. *P. kurroa* also has monocyclic phenolic compounds like vanillic acid and apocyanin. Picein and androsin are phenolic glycosides which are isolated from *P. kurroa*. The plant extract also contains some important chemical constituents like carbohydrates (D-mannitol), aromatic acids like cinnamic acid [Figure 3(m)], vanillic acid [Figure 3(k)] and ferulic acid [Figure 3(l)] [25].

Ethnobotanical Features of *P. kurroa*

Picrorhiza kurroa Royle ex Benth. is widely used by locals in curing diseases like stomachache and high fever. Ten gram

slightly boiled root decoction along with honey is given to patient to cure stomachache. To cure fever, 10 g root powder mixed with 1 g black pepper and honey is given to the adult patient. 0.25g powder of kutki with mother's milk is advised for newborns to cure stomachache [26]. In Kashmir it is also used for veterinary purposes.

In Bhutan, kutki is used as a medicine for coughs, colds and fever. The National Institute of Traditional Medicines and other local hospitals in Bhutan use the rhizome as an ingredient in developing medicines [27].

In China it is used to cure fever, malnutrition due to digestive disorders, jaundice, diarrhoea and dysentery [28]. It is also used in traditional Tibetan medicine. *P. kurroa* rhizome extract can significantly prevent the hepatic injury as it has choleric effect. The plant is bitter in taste and has cold properties. The plant species is used in combination with Chinese herbs including Corium Erinacei and navel gland secretions of musk deer for treating damp-fire accumulation in the large intestine, hemorrhoids, and anal fistula. The dosage of plant used must be in the range of 6-12 gm [55].

Kutki is widely used in Ayurvedic and Unani traditional medicine systems in India with the rhizomes valued for their effectiveness as an antibiotic. It is regarded as one of the major components of Arogyavardhini, an effective Ayurvedic preparation used to treat liver illnesses. Kutki is also used as a substitute for, *Gentiana kurroo* [29, 30]. Pharmaceutical uses of Kutki are also being explored in India.

In Nepal, the rhizome of *P. kurroa* is widely used to treat coughs, skin disease, fever, indigestion, liver disease, jaundice, hepatitis and metabolic disorders. Manufacturers in Kathmandu described that rhizome of plant used as purgative and to treat scorpion bites [31]. Rhizome of the plant is also used in treatment of high blood pressure, intestinal

pain, eye disease, gastritis, bile disease, sore throats, blood, and lung fever [32]. It is considered a bitter tonic, used as a cholagogue (promoting the flow of bile from the gall bladder), stomachic (stimulating gastric activity) and cathartic (purgative) [33]. In Nepal, Kutki is used both by *amchi* (specialists trained in the Tibetan medical system or Sowa Rigpa) and by non-specialists, in the latter case largely for treating coughs and colds [34].

In Pakistan, Kutki is also used in the Ayurvedic and Greek-Arab systems of medicine, most commonly as a stimulant, anaromatic, carminative agent, as a remedy for coughs, bronchial asthma and diseases of blood, liver, kidney and skin. Under the name of Qusttalakh, *P. kurroa* is used in two herbal preparations (Roghane-qust-talakh, an essential oil and *majoon-e-murravehul-azwah*), which are also used for treatment of hypothermia, debility, tremors, tetanus and gout [35].

Molecular Studies on *P. kurroa*

Different techniques such as randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) marker were used for authentication of *Picrorhiza kurroa* Royle ex Benth. and its adulterant *Lagotis cashmiriana*. The unique bands obtained in RAPD amplification clearly differentiated between the two groups of *P. kurroa* that had similar morphology. On the basis of marker characteristics of species specific SSR primers, *P. kurroa* is considered genuine only when the motif (PKssrD2F, PKssrD2R) targeting locus (EU883611) is amplifiable in a PCR reaction. Thus RAPD and SSR markers can serve as corresponding tools for quality control. So the authentication of raw material for commercially available herbal products is easy by using this method [36].

Another study was performed to detect proteins linked with picroside biosynthesis in *Picrorhiza kurroa* Royle ex Benth. Differential protein expression was studied

under picroside accumulating versus picroside non-accumulating conditions using SDS-PAGE. A total of 19 differentially expressed proteins were identified. Proteins involved in miscellaneous jobs were identified, the most important proteins being 1-aminocyclopropane-1-carboxylate oxidase, glyceraldehyde-3-phosphate dehydrogenase, photosystem I reaction center subunit V, putative cytochrome P450 super-family protein and 2-oxoglutarate ferrous-dependent oxygenase because of their role in picroside biosynthesis. These identified proteins provide a thorough understanding of biosynthesis of secondary metabolites and various other physiological progressions of *P. kurroa* [37].

Threats and Conservation

The plant is at the verge of being endangered due to its overexploitation and collection from the wild. Samples were taken from different biodiversity zones of India including northeast Himalaya, western Himalaya, western ghats, gangetic plain, semiarid zone and central highlands. Aseptic cultures were grown at morphogenic level of callus, suspension, axillary shoot, multiple shoot and rooted plants. It was effectively hardened under glasshouse conditions and successful in-vitro conservation was done [38].

Classification

Table 1. Hierarchical Classification of *Picrorhiza kurroa* Royle ex Benth.

Rank/Taxa	Classification
Kingdom	Plantae
Division	Dicotyledonae
Class	Asteride
Order	Scrophulariales
Family	Scrophulariaceae
Genus	Picrorhiza
Species	<i>Picrorhiza kurroa</i>

Picrorhiza kurroa Royle ex Benth. is listed as endangered species due to extensive usage of plant from the wild and lack of organized cultivation. Underground parts (roots and rhizomes) are used for extraction of picrosides, which are the medicinally important constituents of *P. kurroa* in herbal medicinal system [39].

Another study was aimed at developing propagation methods and ex-situ conservation for *P. kurroa*, an endangered medicinal plant of western Himalaya. Regeneration using leaves from mature plant is beneficial because the source plant is not deteriorated. A regeneration practice was regulated by using leaves from aseptic shoot cultures, raised from ex-vitro leaves. Micro-shoots with well-developed root system were obtained in MS basal medium after 4 weeks. Incubation of cultures at low temperature (15°C) for ten days enhanced the survival percent under green house conditions and it was correlated with the development of thick cuticle and well differentiated leaf tissues (palisade and spongy parenchyma). Flow cytometric analysis was performed to check the genetic stability of in-vitro plantlets. In a parallel study, seed progenies of these germplasm were raised under ex-situ conditions. Its reproductive cycle was also studied for successful domestication [40].

Fig. 1. Leaves of *P. kurroa* [41]

Fig. 2. Rhizome of *P. kurroa* [42]

Fig. 3. Phytochemicals of *P. kurroa* [43-54]

Conclusion

From the above discussion it is clear that *P. kurroa* is an important plant species with respect to its ethnobotanical importance. In the traditional health care system it is widely used. So this importance builds a pressure on the plant regarding its use. So there is a need to conserve this plant species which is under threat according to the listing of IUCN (International Union for Conservation of Nature). Practical steps are needed for its conservation which include ex-situ and in-situ conservation. Much more work should be done on its molecular studies and phytochemistry. The structures and composition of different chemical components present in it should be determined for recognizing its further activities. So this information is precious for drug production from this plant for treating various diseases.

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