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PHYTOPHARMACOLOGICAL REVIEW ON GENUS *PICORHIZA*

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ABSTRACT

The genus *Picrorhiza* comprises of two species. These are useful in treatment of various ailments. The phytochemical and pharmacological evaluation by various researchers has authenticated their usage in traditional system of medicine. In this paper the authors have compiled a review of their traditional uses, pharmacological activity and phytochemical profile in the present day research.
INTRODUCTION:
The genus *Picrorhiza* (Scrophulariaceae) is widely distributed in China, Nepal and India. It contains two species namely *Picrorhiza scrophulariiflora* Pennell and *Picrorhiza kurroa* Royle. The genus *Picrorhiza* and its species *Picrorhiza kurroa* Royle appeared first time on a drawing published by Royle on August 24, 1835 in his illustration of botany and still known by names *Picrorhiza kurroa* Bentham or alternatively *Picrorhiza kurroa* Royle ex Bentham [1]. In Greek, *Picros* means bitter and *rhiza* means root. The specific name derived from Karu, the Punjabi name of the plant, which means bitter as well [2].

TRADITIONAL USES
*Picrorrhiza kurroa* is an important medicinal plant used in traditional as well as modern medicines. It is used in treatment of liver disorder, fever, asthma, jaundice caused by environmental pollution, industrial toxicants, food adulteration, malnutrition, excessive consumption of alcohol and certain infections. It is also used in gastrointestinal, urinary disorder, leukoderma, snake bite, scorpion sting, antimalarial and inflammatory affections [3,4,5,6,58].

In Ayurveda, *Picrorhiza kurroa* Royle is called Katuka. It has many synonyms like tikta - bitter, mahausadhi - the great medicine [7]. It is also known by another name Dhanvantarigrasta means the plant was self administered by Dhanvantri God of Indian Medicine [8].

The medicinal plants are the back bone of the traditional medicine. The many countries of World, specially the poor and less developed countries, the population still used folklore medicinal plant for the treatment of disorder or ailments. The present paper on genus *Picrorhiza* gives a comprehensive review of the phytochemistry and pharmacological profile.

HABITAT AND GEOGRAPHICAL DISTRIBUTION
*Picrorrhiza* is a small reputed alpine herb, endemic to Himalayan region of Pakistan, India, Nepal and China. Both species is restricted to the Himalayan region and China. While *P. kurrooa* occurs mainly in the Western Himalaya at an altitude of 3000-4300 meters, *P. scrophulariiflora* is found mainly in the Eastern Himalaya at an altitude of 4300-5200 meters [9]. In India *P. kurrooa* Royle is distributed from Kashmir to Sikkim at altitude ranging from 2700 to 5000 m.

The root occurs as pieces, 2-4 cm long and 0.3 -1.0 mm in diameter. The scales are present at distant intervals, frequently small protuberances, which probably represent accessory buds. The buds present on both at rhizomes and stolons. Rhizomes are 2.5 - 8 cm long and 4 - 8 mm thick, sub-cylindrical, straight or slightly curved, externally greyish– brown, surface rough due to longitudinal wrinkles. The tip ends of growing bud are surrounded by tufted crown of leaves. Fracture - short, odour - pleasant and taste - bitter. Leaves are flat, oval and sharply serrated. The flowering occurs in May to June and August to September, but in alpine region (>4000m), the
flowering occurs only once in July - August. The flowers are purple coloured. The fruits are ovoid shaped capsules. The average dimensions of fruits are 9 x 5 mm. Seeds are numerous, ellipsoid and extremely small, about 1-1.3 mm in size [10, 11,12,13].

PRODUCTION, CULTIVATION AND COLLECTION

The production from cultivated source is not fixed, but from the wild source the total annual production is about 2500 metric tons mainly from Nepal and India (mainly from Himachal Pradesh and Assam). The crop is cultivated under experimental conditions; however the cultivated material is yet not being used for commercial purpose. The roots and rhizomes are harvested during the senescence of aerial parts in the month of September at the lower altitudes and in October at higher altitude get the maximum bioactive compounds [14].

TAXONOMY OF THE GENUS PICRORHIZA

_Picrorhiza_ is a more or less hairy herb. The leaves are subradical and serrate; flowers with radical leafy flowering stems, bracteates, abracteolate and dimorphic; stipulate imbricate in bud. Corolla is long and stamened membranous. Capsule is ovoid in shape. Seeds are oblong, curved, nucleus is in the large bladdery loose hyaline reticulated testa[15,16,55].

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CHEMICAL CONSTITUENTS OF GENUS PICRORHIZA

Generally there is no distinction made between samples from Nepal (_Picrorhiza scrophulariiflora_) and India (_Picrorhiza kurroa_). The main chemical constituents are;

Cucurbitacins

The cucurbitacins constitute a group of triterpenic substances, which are specially known by their bitterness and toxicity. Cucurbitacins are isolated from _Picrorhiza kurroa_. The cucurbitacins B, cucurbitacins D and cucurbitacins R also derived from _P. kurroa_. The basic nucleus of cucurbitacins is 9 β-methyl-19-norlanosta-5-ene and it is also known as 10α-cucurbit-5-ene [17,18,19].

Iridoids

Iridoids represent large groups of mono-terpenes and accumulate in a large number of plant families. _P. kurroa has_ large number of iridoids. Some of the iridoids are picroside - I,
picroside - II, picroside - III, picroside - V, pikuroside, 6-feruloylcatalpol, minicoside, kutosides. The basic nucleus of the iridoids is cyclopentano-[c]-pyran [19,20,21,22,23,24]. The standardized iridoid fraction of *P. kurroa* also contains kutkin and picroliv. Kutkin consists of the glucoside picroside-I. The kutoside is present in a ratio of 1:2 along with glycosides [18,5,25,26].

**Phenolic compounds**

*Picrorhiza* has a monocyclic phenolic compound like vanillic acid, apocyanin and their respective glucosides are also present. Picein and androsin are phenolic glycosides which are isolated from *Picrorhiza kurroa* [18,19,27].

**Other chemical constituents**

*Picrorrhiza kurroa* also contains some important chemical constituents like carbohydrates (D-mannitol), aromatic acids like cinnamic acid, vanillic acid and ferulic acid [28,29].

**PHARMACOLOGICAL ACTIVITIES**

**Antimicrobial Activity**

The antimicrobial potential of acetone, ethanol, methanol, aqueous and hexane extracts of rhizomes of *Picrorhiza kurroa* against selected bacterial strains belonging to two different genera of gram positive and gram negative bacteria were studied by agar well diffusion technique. Ethanol extract of *Picrorhiza kurroa* showed high antibacterial activity against *S. aureus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *S. typhi* and *S. pyogens*. Methanol extract showed high antibacterial activity against *S. aureus* and *P. aeruginosa*, whereas acetone and hexene extract showed intermediate activity against *S. aureus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *S. typhi* and *S. pyogens*. Aqueous extract of rhizomes of *Picrorhiza kurroa* do not show antibacterial activity against the *S. aureus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *S. typhi* and *S. pyogens* strains [30,56,57].

**Hepatoprotective activity**

The effect of picroliv has been studied on ethanol induced toxicity in isolated rats. The level of all the marker enzymes (GOT, GPT, alkaline phosphatase and aldehyde dehydrogenase) reduced significantly in rats’ hepatocytes with 40 µg/ml of 40% ethanol solution at 37°C for 24 hrs. The results were compared with other putative hepatoprotective agents such as andrographolide, silymarin and catapol. Picroliv showed a significant dose dependent preventive effect on alcohol-induced hepatocyte toxicity [31].

Picroliv showed a hepatoprotective action against ethanol- induced hepatic injury in rats. The picroliv treatment (3-12 mg/kg p.o. x 45days) restored the altered parameters in a dose dependent manner [32]. The hepatoprotective action of picroliv was evaluated against carbon tetra chloride (*CCl₄*) and *E. histolytica* infection by using three animal models. The studies showed a significant recovery in serum enzyme levels in all animal models and against amoebic liver abscess in gerbils.
on treatment with picroliv. Hence these studies showed that picroliv possesses hepatoprotective action against *E. histolytica*, which damages hepatic function. It is also reported that kutkins are more potent than silymarin when hepatoprotective activity was concerned [33]. The ethanol extract of *P. kurroa* rhizomes and roots showed hepatoprotective effect on liver mitochondria, although antioxidant defence system was also observed with isoniazid and rifampicin induced hepatitis in rats. The co-administration of *PK* (50 mg/kg/day for 45 days) along with isoniazid and rifampicin, significantly prevented induced hepatitis, induced by the antitubercular drugs’ treatments where *Picrorhiza kurroa* reduced the induced alterations and maintained normality in the rats [34].

**Anti-anticholestatic activity**

Picroliv is an iridoid glycosides mixture containing 60% picroside-1 and kutkoside in ratio of 1:1.5. It showed efficacy comparable to silymarin in rodent models of galactosamine, paracetamol, thiacetamide and CCl₄ induced hepatic damage. It showed cholericetic and anti- anticholestatic effect in rats, guinea pigs and cats, when treated with paracetamol and ethinyl estradiol [35].

**Anti-oxidant activity**

*Picrorhiza kurroa* showed the free radical scavenging effect on line HPLC-DPPH and colorimetric DPPH methods. The anti-oxidant activity of *P. kurroa* extract by comparison of both methods revealed that colorimetric method showed very less free radical scavenging effect while HPLC-DPPH method showed very high activity. *Picrorhiza kurroa* methanolic extracts showed free radical scavenging action and also affected DNA cleavage induced by H₂O₂-UV photolysis. In addition, it was also investigated whether the plant extract was capable of reducing the hydrogen peroxide (H₂O₂) induced cytotoxicity and DNA damage in non- immortalized fibroblasts. Methanolic extract of plant is reported for its dose dependent free radical scavenging capacity [36, 37].

**Anti-ulcer Activity**

*Picrorhiza kurroa* was used in healing of indomethacin-induced acute stomach ulceration in mice and its capacity to modulate oxidative stress was examined along with the levels of prostaglandin and EGF process. The macroscopic indices revealed maximum ulceration on the 3rd day after indomethacin administration, which was effectively healed by *P. kurroa*. Under the optimized treatment regime, *P. kurroa* and omeprazole reduced the ulcer indices by 45.1% (P < 0.01) and 76.3% respectively (P < 0.001) when compared to the untreated ulcerated mice [38].

The ethanolic extract of *Picrorhiza kurroa* rhizome accelerated the healing of stomach wall which was ulcerated by using indomethacin in rats by in vivo free radical scavenging action at the dose of 20 mg/ kg body weight. Ethanolic extract of *Picrorhiza kurroa* rhizomes, at a dose of 20mg/ kg
body weight, when administered orally for 10 consecutive days, enhanced the rate of healing in indomethacin-induced gastric ulcer in rats, when compared to the ulcerated group without treatment [39]. The effect of *P. kurroa* rhizomes and roots was studied on HCl/ethanol induced ulceration in rats and acid/pepsin, peroxidation status, proteins and glycoproteins in the gastric mucosa were investigated. Oral pre-treatment with *P. kurroa* (100mg/kg/day) for 10 days significantly prevented the induced ulceration and maintained the rats at near normal status [40].

**Anti-cytochrome action**

In these studies the effects of picroside-II have been observed on CYP450 by cocktail method using rats. The five probe drugs (diclofenac, midazolam, dextromethorphan, chlorzoxazone and omeprazole) were simultaneously given to rats after single and multiple dosing of picroside-II. The plasma concentrations of the five drugs and their corresponding pharmacokinetic parameters in rats after single dosing of picroside-II (10 mg /kg) were measured by LCMS. The single administration of picroside-II had very little impact on the activities of CYP450 while multiple administration of picroside-II inhibited CYP2C19 activity [41].

**Immunomodulator Action**

*Picrorhiza kurroa* was studied for the effects of biopolymeric fraction RLJ-NE-205 on in vivo immune function of the mice. Mice were treated with the biopolymeric fraction RLJ-NE-205 (12.5, 25 and 50 mg/kg body weight) for 14 days using sheep red blood cell (SRBC) as an antigen. The haemagglutination antibody (HA) titre, plaque forming cell (PFC) assay, delayed type hypersensitivity (DTH) reaction, phagocytic index, proliferation of lymphocytes, analysis of cytokines in serum and CD4/CD8 population in spleen (determined by flowcytometry) were studied. At the dose of 50 mg/kg, a significant increase in proliferation of lymphocytes and cytokinin level in serum was observed. Hence these results show the immunomodulator action [42].

**Neuromuscular action**

It is reported that apocynin is used to treat degree of NADPH oxidase by experimental diabetic neuropathy methods. Diabetes causes a 20% reduction in nerve motor conduction velocity and a 14% deficit for sensory saphenous nerve. The apocynin treatment corrected defects by 32% and 48% respectively. The experimental data showed that NADPH oxidase contributes to the neuromuscular deficits in diabetic rats. Apocynin treatment elevated the reactive oxygen production in diabetes hence this compound could provide a therapeutic action in diabetic neuropathy and vasculopathy system [43].

**Anti-cancer activity**

Picroliv showed the inhibition of cancer induced by chemicals in mice when administrated by
doses of 100 and 200 mg/kg, (p.o.). An inhibition of sarcoma development by 47% and 53% on day was observed after 20-MC administration. The control animals started dying of by tumor burden after 76 days of administration, and all animals were dead by the day after 170. Picroliv treated animal’s survived 60% and 66% at the dose of 100 and 200 mg/kg respectively [44]. The extract of *Picrorhiza kurroa* significantly inhibited hepatocarcinogenesis induced by N-nitrosodiethylamine in a dose dependant manner [45, 46].

**Anti-leishmanial activity**

Effect of picroliv (12.5mg/kgx7days oral) alone and in combination with sodium stigluconate was tested on parasitemia, lipid peroxidation and heptic, during *Leishmania donovani* infection. The results indicated a significant anti-leishmanial activity. It has also been utilized as an adjunct to chemotherapy or in combination therapy of kala azar along with sodium stibogluconate [45].

**Anti-inflammatory activity**

Apocynin was isolated from root extract of *P. kurroa* and its effect was studied on production of arachidonic acid-derived inflammatory mediators by guinea pig pulmonary macrophages. Apocynin concentration dependently inhibited the formation of thromboxane A2, whereas the release of prostagladins E2 and F2 were stimulated. Apocynin potentially inhibited arachidonic acid-induced aggregation of bovine platelets by inhibition of thromboxane formation. Hence this study showed new anti inflammatory or anti-thrombic activity [47]. Another studies showed that iridoid pikuroside isolated from the root of *Picrorhiza kurroa*, together with three known iridoids like picroside-I, picroside-II and 6-feruloyl catalpol had has no anti inflammatory activity, although the picroside-II demonstrated moderate activity [23]. Apocynin selectively inhibits reactive oxygen species production by activated human neutrophils. Apocynin proved to be effective in the experimental treatment of several inflammatory diseases such as arthritis, colitis and atherosclerosis. Apocynin blocks NADPH oxidase dependent reactive oxygen species generated by neutrophil which suggests that it could be a prototype of a novel series of non-steroidal anti inflammatory drugs (NAISD) [48]. The alcoholic extract of powdered root of *Picrorhiza kurroa* contains active constituents like kutkin, picroside-I and kutkoside. These constituents were examined for anti-inflammatory action by using animal model and kutkin showed significant activity [49].

**Bioavailability studies**

The bioavailability studies of apocynin were performed in plasma, liver and brain tissue by intraperitoneal administration. It was found that apocynin is converted to diapocynin *in vivo*. Apocynin (5 mg/kg b.w.) was injected intraperitoneal to adult mice and Sprague-Dawley rats. Plasma, liver and brain were collected at different time (30 min, 1 and 2 hr) after injection and
samples were treated with B-glucuronidase to hydrolyse the glycosyl linkage. These were analyzed by HPLC/MS at 30 min and 1 hr after injection. Approximately 50% of apocynin was converted to its glycosyl derivative and distributed in plasma, liver and brain. Diapocynin was chemically synthesized and characterized by NMR and IR. It was not detected in any of the samples. Hence these results indicate rapid glycosylation of apocynin and transport to blood and other organs, but no apparent conversion to diapocynin in vivo [50].

**Hypolipemic effect**

Hypolipemic effect of the water extract of *Picrorhiza kurroa* was observed in a high fat diet fed hyperlipemic mice at doses of 50,100 and 200 mg/kg, orally, once a day for 12 weeks. Liver weight, serum aspartate transferase, alanine transferase, low density lipoprotein, triglyceride and total cholesterol levels were significantly reduced by the treatment. On the contrary, serum HDL level were not affected by *P. kurroa* water extract [51,52].

**Cardioprotective effects**

The cardioprotective effects of the ethanol extract of *Picrorhiza kurroa* rhizome and roots on isoproterenol induced myocardial infarction in rats with respect to lipid metabolism in serum and heart tissue has been investigated. Oral pre-treatment with PK 80 mg/kg/day for 15 days, significantly prevented the isoproterenol-induced myocardial infarction and maintained the rats at near normal status [53].

**Anti-diabetic activity**

An alcoholic extract of *Picrorhiza kurroa* was found to lower blood glucose in basal conditions and after a heavy glucose load in normal rats. Maximum reduction in serum glucose was observed after 2 h at a dose level of 75 mg extract/kg of body weight. *P. kurroa* extract was also found to reduce the increased blood sugar in alloxan-induced diabetic rats (43% at 75 mg/kg body weight and 60% at 150 mg/kg body weight). Chronic administration of the extract significantly reduced the blood sugar in alloxan-induced diabetic rats for several days (10 days). The extract was also found to reduce the increased blood urea nitrogen and serum lipid peroxides in alloxan-induced diabetic animals and to inhibit the body weight reduction and leukopenia induced by alloxan administration. These results indicate that *P. kurroa* extracts are able to ameliorate biochemical damages induced by alloxan in diabetic rats [54].

**In vitro Anti-malarial activity**

In vitro antimalarial evaluation for the activity of plant *Picrorhiza kurroa* Royle ex Benth rhizomes has been performed by serial dilution technique. The different extracts were tested by using Plasmodium falciparum parasite. Out of four extract the methanol extract of IC50 of 26.6 µg/ml revealed significant results against the parasite, by in vitro method. Hence this study authenticates the traditional use of the plant [58].
FORMATION OF CALLUS
Established callus cultures from different explants such as leaf discs, nodal and root segments of *P. kurroa* for the optimization of *in vitro* conditions for callusing and regeneration of *P. kurroa* not only for the selection of cell lines with enhanced content of phytopharmaceutical or in the genetic transformation of *P. kurroa* as it has been listed as an endangered herb. Moreover, the regeneration holds a great promise in the production of metabolites in cell cultures. Callus induction was highest (70%) in root segment followed by leaf discs (56.3%) and nodal segment (38.3%) on MS medium supplemented with 2,4-D (2mg/l) +IBA (0.5 mg/l) +sucrose 3% (w/v) + agar- agar 0.8% (w/v). The callus cultures derived from different explants were differentiated into multiple shoots on MS medium containing different concentrations and combination of BA, KN and IBA. Regeneration was highest in the calli derived from root segments and leaf discs on MS + BA (2 mg/l) +KN (3 mg /l) + sucrose 3% (w/v) + agar-agar 0.8% (w/v) with 76.7 and 72.2% calli forming shoot primordial respectively. Most of the nodal segment derived calli got differentiated into roots rather shoots. Comparative callusing and shoot regeneration from different explants revealed that root segments are the best explants for *in vitro* studies in *P. kurroa* [13].

DOSAGE AND TOXICITY
*Picrorhiza* is not readily water soluble and soluble in ethanol, the bitter taste makes tinctures unpalatable and so it is usually administered as a standard encapsulated powder (4% kutkin) extract. The typical adult dosage is 400 to 1500 mg/kg/day with dosage upto 3-3.5 g/day being recommended as antiperiodic and 0.6 -1.2 g to 4 g/day for malaria is recommended. The LD 50 of kutkin is greater than 2600mg/kg in rats with no data available for humans. *Picrorhiza* rhizome extracts are widely used and no adverse effects have been reported. Its bitterness is intolerable sometimes.

CONCLUSION
The genus *Picrorhiza* comprises of important traditional medicinal plants which contain many chemical constituents that stabilise metabolic function of body and treat many disorders. In this review, authors have listed the phytochemical and pharmacological profile of the genus. This provides a further opportunity to elaborate the phytochemical study so as to authenticate the traditional profile of the genus.

REFERENCES


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