HortFlora Research Spectrum
Vol. 5, Issue 2; 165-169 (June 2016)
www.hortflorajournal.com
ISSN: 2250-2823

Research Note :

Picrorhiza kurroa Royle ex Benth: A PLANT WITH PHARMACOLOGICAL VALUE

Ritu Mahajan*

School of Biotechnology, University of Jammu, Jammu, (J&K) India
**Corresponding Authorˆs E-mail: ritufeb@gmail.com Tel: +91-191-2456534*

ABSTRACT: Western Himalaya is a reservoir of plants that constitutes a large number of economically important species of both pharmaceutical and medicinal importance. Many of these plant species have become rare and endangered and are in the verge of extinction due to over exploitation. One of such plant is Picrorhiza kurroa which is high altitude plant with a large number of therapeutic properties. Therefore, it is extremely important to explore the different methods of propagation and conservation of *P. kurroa* under in vitro conditions and also in its natural habitat.

Keywords : *Picrorhiza kurroa, in vitro, endangered, conservation, antioxidant*

Picrorhiza kurroa Royle ex Benth also called "kutki", belonging to the family Scrophulariaceae is an important plant in the *Ayurvedic* system of medicine. It is a small perennial herb growing in the North-western Himalayan region at an altitude of 3,000-5,000 meters from Kashmir to Sikkim.

Habitat

The plant grows in rock crevices and also on medium (loamy) and heavy (clay) soils. It mostly prefers moist and sandy soil. It grows from a whorl of radical leaves that arises from rhizome tip. The leaves of the plant are flat, oval and grow close to the roots which are adventitious. They have a pointed apex that narrows into winged petiole base. The flowers are white or light purple, bisexual borne on a tall spike with a few bracts beneath. They appear in June and are present throughout the month of August. The fruit is a capsule which is long ovate, with few lateral grooves. Seeds are oblong and about 1mm in length, with thick seed coat. The plant is self-propagating.

Alkaloids

The underground tuberous part of P. kurroa is rich in iridoid glycoside called as Kutkoside. It gives a crystalline product called as Kutkin. Kutkin is a mixture of picroside-I and picroside-II (Bhandari *et al.,* 4; Patil *et al.,* 16).

Due to presence of these iridoids, a structural link between terpenoides and indole alkaloids is created, due to which the plant possesses spectrum of biological activities. *P. kurroa* show many pharmacological properties like antioxidant, neoplastic, antiulcerogenic, antidiabetic, anti-inflamatory and immune regulatory activities (Rajkumar *et al.,* 17; Sultan *et al.,* 28). A phenolic glycoside of *P. kurroa* "Androsin" has been used to treat infections and allergies of upper respiratory tract like asthma (Dorsch and Wagner, 8) and now is been used extensively in modern medicines. The plant extract is also used against snake bite and scorpion sting (Zargan *et al.,* 30). Rathee *et al.* (18) observed that the plant shows antimicrobial activity against wide range of bacterial and fungal strains.

The plant has reported to have anticancerous activity (Kumar and Ramesh, 11; Mallick *et al.,* 15). The anticancerous activity is due to presence of certain terpene like cucurbitacins (Sindhu *et al.,* 21) and flavonoids like apocynin in its rhizome (Alghasham *et al.,* 1; Aribi *et al.,* 2). The extracts of P. kurroa supports the therapeutic potential of picroside II which enhances the ability of cell proliferation and in treating nervous disorders (Liu *et al.,* 14) and also in treating viral hepatitis (Shetty *et al.,* 22).

Propagation and Extinction

P. kurroa propagates mainly through roots or suckers. Its propagation through seeds is poor due to their low viability (Chandra *et al.,* 7). Indiscriminate collection of its underground parts for active constituents, the plant has been almost depleted from its natural habitat. As a result, the plant is listed as an endangered plant by Red Data Book. Therefore, it is extremely important to explore the different methods to conserve this plant using biotechnological approaches

Article's History:
Received : 06-05-2016 ***Accepted : 15-06-2016***

like in vitro propagation using seeds, apical buds, nodal segments, callus induction (Helena *et al.,* 9).

In Vitro Conservation

Due to high demand of *P. kurroa* for its medicinal value and difficulty of regenerating it by conventional methods, the large scale multiplication of this species can only be met efficiently in a sort span of time by in vitro propagation. The *in vitro* approaches can illucidate its medicinal properties and at the same time can conserve its germplasm. Successful *in vitro* propagation has been achieved in *P. kurroa* using different explants (Jan *et al.,* 10; Rehman *et al.,* 19; Helena *et al.,* 9).

Patil *et al.* (16) used leaves as explants and observed maximum regeneration percent (94.33) and higher shoot number (38.0) in MS medium supplemented with 2.32 ìM of kinetin. Whereas, Helena *et al.* (9) observed maximum shoot frequency in MS medium with 1.0 mg/l BA + 0.75 mg/l Kn from leaf derived callus. Maximum root induction was recorded after 12-15 days in ½ strength MS basal medium supplemented with IAA, IBA (0.4, 0.5, 0.1 mg/l) and NAA (0.3, 0.4, 0.5, 1.0 mg/l) while Jan *et al.* (10) observed maximum root induction per explants in MS basal medium supplemented with 0.4 mg/l NAA.

Bhat *et al.* (5) studied efficient plant regeneration in B5 medium containing 3 mg/l Kn and 1.0 mg/l IBA, with a regeneration frequency of 94% and an average of 10.9±1.3 shoots per explant. They also observed that plant growth regulator Kn was more effective than BAP for shoot regeneration. Increasing the concentration of Kn from 1 to 3 mg/l resulted in enhanced shoot regeneration frequency from 82% to 94%.

Jan *et al.* (10) observed callus formation in MS medium supplemented with 2,4-D (0.1 mgl/l, 0.25 mgl/l and 0.5 mgl/l) and direct shoot regeneration in MS containing BAP . The callus induction was induced in 20% explants in MS medium containing 0.25 mgl/l 2,4-D and direct shoot regeneration rate was recorded in 55 % explants in MS containing 0.25 mgl/l BAP. Helena *et al.* (9) observed maximum callus induction in MS supplemented with 0.5 mg/l TDZ + 0.3 mg/ l IBA and 0.5 mg/l TDZ + 0.5 mg /l IBA in leaf and stem explants, respectively.

Phytochemical and Antioxidant Property

Number of workers have reported the use of HPLC for the quantification of picroside-I and picroside-II in P. kurroa (Sturm and Stuppner, 23; Singh *et al.,* 24). Arsul *et al.* (3) reported that kutkin is responsible for hepatoprotective activity while phenolic and flavonoids are responsible for antioxidant activity in *Picrorhiza kurroa*.

Sharma *et al.* (25) developed HPLC method to determine the picroside-I and picroside-II in rhizome of seven different accessions collected from different altitudinal ranges of North India. They observed that the maximum concentration of picroside-I (3.5%) and picroside-II (2.0%) was in rhizome parts of *P. kurroa* collected from Rohtang area.

Similarly, Sultan *et al.* (28) assessed the quantity of picroside-I and picroside-II in seven different accessions of P. kurroa using HPLC. The highest content of pk-I was found in the accession from Gurez altitude (3750 masl) while the highest content of pk-II was found in accession from Keller (Shopian) altitude (3300 masl). Their results revealed that the picroside content is directly correlated with altitudinal variation.

Sanjay *et al.* (26) measured the stable free radical by DPPH assay in P. kurroa. They observed that effects of different concentrations 3.25 ìg, 6.5 ìg, 9.75 ìg,13 ìg of protein extract on DPPH radical had 59.18%, 61.73%, 69.38%, 74.48% scavenging activity while the hydroxyl scavenging activity at different protein concentration 65 ìg, 130 ìg, 195 ìg, 260 ìg, were found to be 48.84%, 56.34%, 61.12%, 70.45%. Similarly, Chander *et al.* (6) have reported irioids from P. kurroa act as scavengers of superoxide anions by possibly acting like superoxide dismutase, xanthine oxidase inhibitors and metal ion chelators.

Kant *et al.* (12) explored antioxidant activity in P. kurroa in different solvent extracts using DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonicacid) assay. Butanol and ethyl acetate extract showed greater antioxidant activity as compare to ethanol extract. The IC50 values for DPPH and ABTS ranged from 0.81 to 29.48 ìg.

Antimicrobial Property

Rani and Khullar (19) studied and observed that the antimicrobial activities of alcoholic extracts of *P. Kurroa* roots were active against *Micrococcus pyogenes* var. aureus, *Escherichia coli, Staphyloco ccus aureus* and *Salmonella typhi* while the aqueous extract of the roots showed moderate activity against *Staphylococcus* aureus and *Staphtyphi* and marked inhibition against *E. coli.* Also, the extract of this plant and its major constituents exhibited significant activity against fungi.

Rathee *et al.* (18) observed the effects of methanolic and aqueous extracts on some pathogenic bacterial and fungal strains *viz. Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, E. coli* and *Candida albicans, Aspergillus niger,* respectively. The aqueous and methanolic extracts showed antibacterial activity against *P. aeruginosa* and *S. aureus* while a moderate activity against *E. coli, B. subtilis* and *M. luteus*. They also observed that the aqueous extract was less effective against the microbial strains and showed no activity against fungal strains.

Usman *et al.* (29) investigated antimicrobial activity of ethanolic extract of *Picrorhiza kurroa* against different strains of Gram-positive, Gram-negative bacteria and fungi. The extract was tested against Gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus,* Gram-negative bacteria like Pseudomonas aeruginosa and *Escherichia coli* and three fungal species *Aspergillus niger, Candida albicans* and *Malasseiza furfur*. They observed that the extract was active against all assayed organisms with minimal inhibitory concentration (MIC) values ranging from 65 to 260 mg /ml.

Marker Analysis

Genetic diversity in twenty five accessions of *P. kurroa* using simple sequence repeats (SSR) and cytochrome P-450 markers was reported by Katoch *et al.* (13) . They reported that out of 22 SSR markers, 13 SSR markers showed a mean 5.037 alleles with a mean polymorphic information content (PIC) of 0.7718, whereas eight cytochrome P-450 markers detected mean 5.0 alleles with a mean PIC of 0.7596. The dendrogram showed a clear consistency between SSR and cytochrome P-450 trees in terms of positioning of most Picrorhiza accessions. SSR markers could cluster various *P. kurroa* accessions based on their geographical locations whereas cytochrome P-450 markers could cluster few accessions as per their geographical locations. They found a high degree of genetic variation among the accessions of each eco-geographic region.

Shitiza *et al.* (27) the genetic profiling in P. kurroa using SSR primers. Out of 361 primers tested, only 35 primers showed polymorphism. Highest PIC value of 0.55 was observed with SSR marker PKSTS-P9. Mean allele number was 2.97 and mean observed and expected heterozygosity was 0.597 and 0.452 respectively. Cluster analysis revealed a low genetic diversity among the accessions with Nei's genetic diversity and Shannon's information index as 0.39 was 0.58, respectively.

CONCLUSION

P. kurroa a pharmacologically important herb has small population and large economic value. The plant has been affirmed as endangered medicinal plant due to indiscriminate collection of its underground parts for extraction of active constituents. The biological importance of this plant is attributed due to presence of irioid glycosides known as picroside I and picroside II. Conservation using in vitro cultures can be used for large scale propagation and also in selecting the cell lines with enhanced secondary metabolite production.

Acknowledgement

The author is thankful to National Medicinal Plant Board (NMPB), New Delhi for their financial support for research project on *P. kurroa.*

REFERENCES

1. Alghasham, A.A. (2013). Cucurbitacins - a promising target for cancer therapy. *Intern. J. Health Sci.,* **7**: 77–89.
2. Aribi, A., Gery, S., Lee, D.H., Thoennissen, N.H., Thoennissen, G.B., ALvarez, R., Ho Q., Lee K., Doan, N.B., Chan, K.T., Toh M., Said, J.W. and Koeffler H.P. (2013). The triterpenoid cucurbitacin B augments the antiproliferative activity of chemotherapy in human breast cancer. *Intern. J. Cancer,* **132** : 2730–2737.
3. Arsul, A., Ganjiwale, R.O. and Yeole, P.G. (2010). Phytochemical and Pharmacological Standardisation of polyherbal tablets for hepatoprotective activity against carbon tetrachloride induced hepatotoxicity vilas. *Intern. J. Pharmaceu. Sci. and Drug Res.,* **2** : 265-268.
4. Bhandari, P., Singh, B. and Kumar, N. (2010). Online HPLC-DPPH method for antioxidant activity of *Picrorhiza kurroa* Royle ex Benth. and characterization of kutkoside by Ultra-Performance LC-electrospray ionization quadrupole time-of-flight mass spectrometry. *Indian J. Exp. Biol.,* **48** : 323-328.
5. Bhat, W.W., Lattoo, S.K., Rana, S., Razdan, S., Dhar, R.S. and Vishwakarma, R.A. (2012). Efficient plant regeneration via direct organogenesis and *Agrobacterium tumefaciens* mediated genetic transformation of *Picrorhiza kurroa*: an endangered medicinal herb of the alpine Himalayas. *In vitro Cell Dev Biol.—Plant,* **48** : 295–303.

6. Chander, R., Kapoor, N.K. and Dhawan, B.N. (1992). Picroliv, picroside-I and kutkoside from *Picrorhiza kurroa* are scavengers of superoxide anions. *Biochem. Pharmacol.,* **44** : 180-183.
7. Chandra, S., Bandopadhyay, R., Kumar, V., Chandra, R.O. (2010). Acclimatization of tissue cultured plantlets: from laboratory to land. *Biotechnol Lett,* (DOI: 10.1007/s10529-010 -0290-0).
8. Dorsch, W. and Wagner, H. (1991). New antiasthmatic drugs from traditional medicine. *Int. Arch. Allergy Appl. Immunol.,* **94** : 262.
9. Helena, D.S., Kannojia, G. and Gaur, A.K. (2015). *In vitro* regeneration of Picrorhiza kurroa Royal ex benth for ex situ conservation and sustainable utilization. *Ann. Biol. Res.,* **6** : 7-14.
10. Jan, A., Thomas, G., Shawl, A.S., Jabeen, N., and Kozgar, M.I. (2010). Improved Micropropagation Protocol of an Endangered Medicinal Plant-*Picrorhiza Kurroa* Royle ex Benth. Promptly Through Auxin Treatments. *Chiang Mai J. Sci.,* **37** : 304-313.
11. Kumar, M.H. and Ramesh,. C (2014). Anticancer activity of nano encapsulated formulation from the extracts of *Picrorhiza kurroa* against human cancer cell lines. *J. Pharmaco. Phytochem.* **2** : 182-185 M.
12. Kant, K., Walia, M., Agnihotri, V.K., Pathania, V., and Singh, B. (2013). Evaluation of Antioxidant Activity of *Picrorhiza kurroa* (Leaves) Extracts. *Indian J. Pharmaceu. Sci.,* **75** : 324–329.
13. Katoch, M., Hussain, M.A. and Ahuja, A. (2013) Comparison of SSR and cytochrome P-450 markers for estimating genetic diversity in *Picrorhiza kurrooa* L. *Plant System. Evolution,* **299** : 1637-1643.
14. Liu, J.W., Yu, Y., Zheng, P., Zhang, X., Li, T., Cao, Y., Guo, M. (2007). Synergistic protective effect of picroside II and NGF on PC12 cells against oxidative stress induced by H_2O_2. Pharmacol. Reports, **59** : 573-579.
15. Mallick, M.N., Singh, M., Parveen, R., Khan, W., Ahmad, S., Najim, M.Z .and Hussain, S.A. (2015). HPTLC Analysis of bioactivity guided anticancer enriched fraction of hydroalcoholic extract of *Picrorhiza kurroa. BioMed Res. Intern.,:* Article ID 513875.
16. Patil, V., Devi, K., Sharma, M., Bhattacharya, A. and Ahuja, P.S. (2012). Propagation of *Picrorhiza kurroa* Royle ex Benth: An important medicinal plant of Western Himalaya. *J. Med. Pl. Res.,* **6** : 4848-4860.
17. Rajkumar, V., Guha, G., and Kumar R.A. (2011). Antioxidant and anti-neoplastic activities of *Picrorhiza kurroa* extracts. *Food Chem Toxicol.,* **49** : 363–369.
18. Rathee, P., Rathee, S. and Rathee, D. (2012). Phytochemical screening and antimicrobial activity of *Picrorrhiza kurroa*, an Indian traditional plant used to treat chronic diarrhea. *Arabian J. Chemistry,* http://dx.doi.org/ 10.1016/j. arabjc. 2012.02.0 09
19. Rehman, H.U., Ahmad, J., Chester, K., Tajuddin, T.E. and Ahmad, S. (2014). Chemoprofiling and tissue culture studies on *Picrorhiza kurroa* Royle ex Benth. for production of picroside II. Ann. *Phytomed.,* **3** : 70-77.
20. Rani, P. and Khullar, N. (2004). Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drugs resistant *Salmonella typhi. Phytother. Res.,* **18** : 670-673.
21. Sindhu, N., Pratima, T. and Ashwini, W. (2011). Isolation of a cucurbitacin from Picrorhiza Kurroa by column chromatography and its characterization. *Res. J. Pharmaceu., Biol. and Chem. Sci.,* **2**: 149–156.
22. Shetty, S. N., Mengi, S., Vaidya, R., and Vaidya, A. (2010). A study of standardized extracts of Picrorhiza kurroa Royle ex Benth in experimental nonalcoholic fatty liver disease. *J. Ayur. and Integrative Medicine,* **1** : 203–210.
23. Sturm, S., and Stuppner, H. (2001). Analysis of iridoids glycosides from Picrorhiza kurroa by capillary electrophoresis and high performance liquid chromatography-Mass spectrometry. Chromatogr., 53: 612– 618.
24. Singh, B., Bhandari, P., Kumar, N., and Kaul, V.K. (2008). Simultaneous determination of sugars and picrosides in Picrorhiza species using extraction and high performance liquid chromatography with evaporative light scattering detection. *J. Chromatogr. A.,* **1194** : 257– 261.
25. Sharma, N., Pathania, V., Singh, B. and Gupta, R.C. (2012). Intraspecific variability of main phytochemical compounds in *Picrorhiza kurroa* Royle ex Benth. from North Indian higher altitude Himalayas using reversedphase high-performance liquid chroma- tography. *J. Medicinal Pl. Res.,* **6** : 3181-3187.
26. Sanjay, S., Banu, S.H., and Chetankumar, M. (2015). The study of potentiality of *Picrorhiza*

kurroa root proteins to inhibit free radicals and A-amylase enzyme. *Asian J. Pharmaceu. Clinical Res.,* **8** : 220-225.

27. Shitiza, K., Pandita, S., Chanumolua, S., Sooda, H., Singha, H., Singha, J. and Chauhan, R.S. (2015). Mining simple sequence repeats in *Picrorhiza kurroa* transcriptomes for assessing genetic diversity among accessions varying for picrosides contents. *Plant Genetic Resour.: Characterization and Utilization*, **1**–10.
28. Sultan, P., Jan, A. and Pervaiz, Q. (2016). Phytochemical studies for quantitative estimation of iridoid glycosides in *Picrorhiza kurroa* Royle. *Botanical Studies : An Intern. J.,* **57** : 7.
29. Usman, M., Surekha, Y., Chhaya, G. and Devendra, S. (2012). Preliminary screening and antimicrobial activity of *Picrorhiza kurroa* Royle ethanolic extracts. *Int. J. Pharm. Sci. Rev. Res.,* **14** : 73-76.
30. Zargan, J., Sajad, M., Umar, S., Naime, M., Ali, S. and Khan, H.A. (2011). Scorpion (*Androctonus crassicauda*) venom limits growth of transformed cells (SH-SY5Y and MCF-7) by cytotoxicity and cell cycle arrest. *Exper. Mol. Pathol.,* **90** : 447–454.

Citation : Mahajan R. (2016). *Picrorhiza kurroa* Royle ex Benth : A plant with pharmacological value. *HortFlora Res. Spectrum,* **5**(2) : 165-169.