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Active Content Enhancement through Different Agrotechnological and Post Harvesting Approaches in *Picrorhiza kurroa* Royle ex Benth: An Endangered Medicinal Plant

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ABSTRACT

High altitude medicinal plants are highly demanding due to their therapeutic use but unfortunately mostly these plants are in threatened stage. So, there is an urgent need to conserve these species by applying appropriate agrotechnological practices. Experimental species was grown in polyhouse and open field conditions and after harvesting, the raw material was dried in different conditions. High Performance Liquid Chromatography (HPLC), was applied to separate and quantify iridoid glycosides (picrotin and picrotoxin) which are naturally present in *Picrorhiza kurroa*. The major active ingredients were quantitatively estimated in field and polyhouse grown plants. Good linear response over the range of 0.4 to 3.5% on the basis of dry weight was observed for each component. Both picrotin and picrotoxin were recorded in higher quantity respectively 3.1 and 1.2% of dry weight in polyhouse grown plants, While low 2.5 and 0.99% of dry weight of picrotin and picrotoxin, respectively in field grown plants. It was interesting to observe that the samples dried in room condition or in shade showed higher quantity of both the active contents in contrast to oven dried sample. It was also found that the broad leaf plants showed higher quantity of active ingredients in comparison to narrow leaf. On the basis of this study cultivation of the broad leaf variant inside the polyhouse and drying in room condition can be implemented for good quality and quantity of *P. kurroa*.

Key words: *Picrorhiza kurroa*, picrotin, picrotoxin, post harvesting techniques, morphological variants

INTRODUCTION

At present, almost half of all prescribed drugs are synthesized from natural products in industrialized nations. In developing world, WHO has estimated that 3.4 billion peoples used the plants as a primary source of medicine (Benamar *et al*., 2010). The Uttarakhand state of India is a store house of highly valuable medicinal and aromatic plants. One third of higher plants are used in medicine and collected from their natural sources (Gaur and Kaushik, 2011a, b).

Picrorhiza kurroa Royle ex Benth of family Scrophulariaceae is an important herb of Himalayan region growing at an altitude from 3000 to 5000 m elevation in alpine belts and also considered as an medicinally important endangered plant species (Nayar and Shastry, 1987). Information on taxonomy (Naithani, 1985), ecophysiology and seed germination (Nautiyal, 1985) of *P. kurroa* is available and recently (Nautiyal *et al*., 2001), gave detail agro-technology at lower altitude. In Indian system of medicine it is known as kutki and constitutes an important drug of 2000 drug items derived from vegetable sources (Chopra *et al*., 1956). Secondary metabolites are important source of pharmaceuticals and also related with the adaptation of plants in their environment (Rao and Ravishankar, 2002).

P. kurroa furnishes the drug picrotin obtained from dried stolons and roots. It is considered to be a valuable tonic, antiperiodic, cholagogue, stomatic, laxative an small doses and cathartic in large doses (Kirtikar, 1988). It is also used in fever and stomach pain by local inhabitants and its medicinal properties are well described in Indian system of medicine. *P. kurroa* is among those that are facing serious threat of extinction in its natural habitat because the underground rhizome of the species are collected from wild and are used for extraction of the picrotin and used in traditional products. The wild population of the species is decreasing day by day due to its over exploitation by legal or illegal means. The cultivation of the species is still not in practice so we are losing this prestigious herb.

Keeping in view the above problem, increasing demand of this species as a herbal drug and its assessment as an endangered species in nature, the present study is an attempt to examine the presence of active ingredients in the plants collected form nature, cultivated in field nursery at lower altitude as well as in polyhouse conditions with special reference to post harvesting drying technique. Difference in active ingredients in two morphological variants like broad and narrow leaf of *P. kurroa* was also observed.

MATERIALS AND METHODS

Cultivation and post harvesting approaches: The rhizomes of *P. kurroa*, grown in open field and polyhouse conditions, were collected from experimental site situated at Pothibasa (2200 m.a.s.l.) district Rudraprayag, Uttarakhand, India. Plants were raised through vegetative part (stolon cuttings) and harvested after the maturation of plants (after third year of transplantation) during September. Collected plant material was dried in three different conditions viz., oven (30-35°C) sun (20-35°C) and room (12-20°C).

Quantitative estimation of active contents: Powdered plant material was extracted by using 70% ethanol in a Soxhelt apparatus for an hour on water bath maintained at 60°C temperature. Extract was filtered and dried in vacuum pressure and stored in desiccators until analyzed. The dry crude extract (1 mg) was dissolved in 100 mL solution (water:methanol:isopropanol:acetonitrile in the ratio of 60:30:5:5) and from this stock solution 10, 20, 40, 80 and 100 ppm solutions were prepared. Picrotin and picrotoxin (sigma chemical) were used for establishing the calibration curve. Before running the sample, solutions were filtered through a 0.45 μ millipore filter. Beckman system Gold HPLC consisting of two pumps, a 20 μL loop injector reveres phase ODS ultrasphere column (4.5×250 mm) was used. A mixture of water:methanol:isopropanol:acetonitrile (60:30:5:5) as a solvent for an elution at a flow rate of mL min^{-1} was used. Twenty micro liter of sample was injected and components were detected at λ_{max} 220 nm using variable wavelength UV

detector. Components were identified by simultaneous ran of standard compared with their retention time and quantified by standard peak area method.

Data analysis: Data were analyzed from High Performance Liquid Chromatography (HPLC) and the concentration of active contents in the entire experimental material is presented in tabular form in result and discussion.

RESULTS AND DISCUSSION

Table 1 shows the quantitative variations in active content of *P. kurroa* on the basis of different drying methods. The quantity of both the picrotin and picrotoxin was recorded higher respectively 2.9 and 1.7% on the basis of dry weight in the room dried samples followed by sundry and lowest in oven dried samples. The decrease in active contents in oven dried samples can be attributed to the decomposition of active contents due to overheating of the raw material. Since, the oven dried samples contain low quantity of picrotin and picrotoxin, it is considered that the iridoide glycoside is highly temperature sensitive.

As the drying/semidrying processes affect the drug quality, it might be suggested that the room drying processes would be suitable for the best yield of drug and also the cost effective technique and thus farmers could be benefited by adopting this appropriate technology. Drying process employed significantly decreased losses in quality and quantity of agriculture produce in most of the developing countries of tropical region (Hassan *et al*., 2007; Agoreyo *et al*., 2011). Alkaloids are a chemically diverse group of organic nitrogen compounds, rank among the most efficient and therapeutically significant plant substances (Chew *et al*., 2011). Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents all over the world for their analgesic, antispasmodic and bactericidal effects (Hussain *et al*., 2010). In recent years, attention has been focused on alkaloids with anti-tumourous effects, but it is documented that the plants containing alkaloids should never be self-administered (Mazzio and Soliman, 2009).

Table 2 shows that the polyhouse grown plant yielded higher quantity (3.1% of dry wt.) of picrotin while it was found low (2.5%) in field grown plants. The total biomass of the species was also recorded higher under polyhouse conditions when compared with the field grown plants. These results indicated that *P. kurroa* could be cultivated in polyhouse/semi-natural condition at the appropriate altitude in the Garhwal Himalaya. Secondary metabolites, represent the chemical

Table 1: Concentration of active ingredients in different drying methods

	Conc. (% dry wt.)		
Active Content	Room or shade dry	Sun dry	Oven dry
Picrotin	2.90±0.22	2.10±0.17	1.50±0.35
Picrotoxin	1.70±0.13	0.90±0.28	0.80±0.23

Table 2: Concentration of active ingredients in polyhouse and field grown plants

	Conc. (% dry wt.)	
Active content	Polyhouse grown	Field grown
Picrotin	3.10±0.29	2.50±0.24
Picrotoxin	1.20±0.18	0.99±0.11

Table 3: Concentration of active ingredients in broad and narrow leaf variants

	Conc. (% dry wt.)	
Active content	Broad leaf	Narrow leaf
Picrotin	2.25±0.21	1.05±0.20
Picrotoxin	1.65±0.13	0.83±0.11

interface between the plants and the environment and therefore their synthesis is often affected by nutrient and water availability (Alsafar and Al-Hassan, 2009; Azizi and Kahrizi, 2008; Supanjani *et al*., 2005) and growing habitat (Azizi and Kahrizi, 2008; Mosayebi *et al*., 2008). The synthesis of phenolic compounds (secondary metabolites) can be stimulated by acting on different parameters like environmental factors, use of precursors of the targeted molecules, use of elicitors and genetic transformation of the plants (Jovancevic *et al*., 2011). It is well known that the response of plants to climate depends on their life history characteristics and ecophysiology and largely differs between species. There has been little focus on investigating the effects of climate conditions on secondary metabolite production in medicinal plants (Gairola *et al*., 2010). Table 3 shows the difference in active ingredients in two morphological variants of *P. kurroa* i.e., broad and narrow leaf. The value of both picrotin and picrotoxin were observed high (2.25 and 1.65%, respectively) in broad leaf plants and low (1.05 and 0.83%, respectively) in narrow leaf plants. This indicates the superiority of broad leaf plants over the narrow leaf and it can be taken as elite or superior morphovariant by the grower for cultivation programmes. It has been shown that the content of secondary metabolites in roots varies depending upon growing conditions (Kovalenko *et al*., 2004). The content of the other important compound in liquorice root and glycyrrhizic acid varies between 2 and 15 w/w depending on the species, geographic location, climate conditions and season of harvest (Hennell *et al*., 2008).

CONCLUSION

By practicing cultivation, the demand of pharmaceutical companies and ethnic medicine can be fulfilled. On the basis of the study, it was concluded that *P. kurroa* can be cultivated throughout the year at temperate zone near the vicinity of the villages to fulfill the demand of pharmaceutical companies and also reduce the pressure on natural populations. To enhance the quantity of active ingredients (Picrotin and Picrotoxin), *P. kurroa* can be cultivated inside the polyhouse instead of open field condition. Among the post harvest drying methods, room dried samples showed the higher quantity of medicinally important components. Cultivation of suitable morphological variants (broad leaf) also played major role to enhance the active content of the experimental species. Cultivators should grow the best variants of *P. kurroa* at best site and follow the adequate process of drying to enhance the quantity of active contents.

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REFERENCES

Agoreyo, B.O., O. Akpiroroh, O.A. Orukpe, O.R. Osaweren and C.N. Owabor, 2011. The effects of various drying methods on the nutritional composition of *Musa paradisiacal*, *Dioscorea rotundata* and *Colocasia esculenta*. Asian J. Biochem., 6: 458-464.

Alsafar, M.S. and Y.M. Al-Hassan, 2009. Effect of nitrogen and phosphorus fertilizers on growth and oil yield of indigenous mint (*Mentha longifolia* L.). Biotechnology, 8: 380-384.

Azizi, K. and D. Kahrizi, 2008. Effect of nitrogen levels, plant density and climate on yield quantity and quality in cumin (*Cuminum cyminum* L.) under the conditions of Iran. Asian J. Plant Sci., 7: 710-716.

Benamar, H., W. Rached, A. Derdour and A. Marouf, 2010. Screening of Algerian medicinal plants for acetylcholinesterase inhibitory activity. J. Biol. Sci., 10: 1-9.

Chew, Y.L., E.W.L. Chan, P.L. Tan, Y.Y. Lim, J. Stanslas and J.K. Goh, 2011. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. Biol. Med. Chem. Complement. Alternative Med., Vol 11.

Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. CSIR, PID, New Delhi, India.

Gairola, S., N.M. Shariff, A. Bhatt and C.P. Kala, 2010. Influence of climate change on production of secondary chemicals in high altitude medicinal plants: Issues needs immediate attention. J. Med. Plants Res., 4: 1825-1829.

Gaur, S. and P. Kaushik, 2011a. Biodiversity of vesicular arbuscular mycorrhiza associated with *Catharanthus roseus*, *Ocimum* spp. and *Asparagus racemosus* in Uttarakhand State of Indian Central Himalaya. Int. J. Bot., 7: 31-41.

Gaur, S. and P. Kaushik, 2011b. Influence of edaphic factors on distribution of mycorrhiza associated with medicinal plants in Indian central Himalayas. J. Biol. Sci., 11: 349-358.

Hassan, S.W., R.A. Umar, I.K. Matazu, H.M. Maishanu, A.Y. Abbas and A.A. Sani, 2007. The effect of drying method on the nutrients and non-nutrients composition of leaves of *Leptadenia hastata* (Asclipiadaceae). Asian J. Biochem., 2: 188-192.

Hennell, J.R., S. Lee, C.S. Khoo, M.J. Gray and A. Bensoussan, 2008. The determination of glycyrrhizic acid in *Glycyrrhiza uralensis* Fisch. ex DC. (Zhi Gan Cao) root and the dried aqueous extract by LC-DAD. J. Pharm. Biomed. Anal., 47: 494-500.

Hussain, J., Z. Muhammad, R. Ullah, F.U. Khan and I.U. Khan *et al.*, 2010. Evaluation of the chemical composition of *Sonchus eruca* and *Sonchus asper*. J. Am. Sci., 6: 231-235.

Jovancevic, M., J. Balijagic, N. Menkovic, K. Savikin, G. Zadunic, T. Jankovic and M. Dekic-Ivankovic, 2011. Analysis of phenolic compounds in wild populations of bilberry (*Vaccinium myrtillus* L.) from Montenegro. J. Med. Plants Res., 5: 910-914.

Kirtikar, K.R., 1988. Indian Medicinal Plants. Scientific Publishers, New York, USA.

Kovalenko, P.G., V.P. Antonjuk and S.S. Maliuta, 2004. Secondary metabolites synthesis in transformed cells of *Glycyrrhiza glabra* L. and *Potentilla alba* L. as producents of radioprotective compounds. Ukrainica Bioorganica Acta, 1/2: 13-22.

Mazzio, E.A. and K.F.A. Soliman, 2009. *In vitro* screening for the tumoricidal properties of international medicinal herbs. Phytother. Res., 23: 385-398.

Mosayebi, M., G. Amin, H. Arzani, H. Azarnivand, M. Maleki and A. Shafaghat, 2008. Effect of habitat on essential oil of *Achillea filipendulina* L. in Iran. Asian J. Plant Sci., 7: 779-781.

Naithani, B.D., 1985. Flora of Chamoli Volume 2, Botanical Survey of India. Government of India, New Delhi, India.

Nautiyal, B.P., V. Parkash, R.S. Chauhan, H. Purohit and M.C. Nautiyal, 2001. Assessment of germinability, productivity and cost benefit analysis of *Picrorhiza kurrooa* cultivated at lower altitude. Curr. Sci., 81: 579-585.

Nautiyal, M.C., 1985. In Indegenious Medicinal Plant. Today Tomorrow's Printers and Publishers, New Delhi, pp: 107-112.

Nayar, M.P. and A.R.K. Shastry, 1987. Red Data Book of Indian Plants. 2nd Edn., Botanical Survey of India, India .

Rao, S.R. and G.A. Ravishankar, 2002. Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol. Adv., 20: 101-153.

Supanjani, A.R.M. Tawaha, M.S. Yang and K.D. Lee, 2005. Calcium effects on yield, mineral uptake and terpene components of hydroponic *Chrysanthemum coronarium* L. Int. J. Bot., 1: 196-200.