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Productivity and picroside contents of *Picrorhiza kurroa* Royle ex Benth. cultivated at multi-locations in Uttarakhand, India

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Cultivation is cost effective for conservation and sustainable supply of rare and high value medicinal plants. Assessment of productivity and quality testing validates cultivation and improves trade prospective. *Picrorhiza kurroa* is a recently domesticated Himalayan medicinal herb. Despite, specific habitat preference above 3000 m in natural habitats, *P. kurroa* is cultivated successfully below 3000 m under diverse cultivation conditions. In addition to roots/rhizomes, the leaves of *P. kurroa* are known to contain picrosides (I & II). Therefore, it is inevitable to assess production (roots/rhizomes and leaves), and picroside content (in roots/rhizomes and leaves) of cultivated *P. kurroa*. A total 12 locations having different age group crop were selected for assessing the production of roots/rhizomes and leaves, and HPLC method was used for estimation of picrosides (I & II) content. Production of roots/rhizomes and leaves (on dry weight basis) of less than two years old crop was 1146.67±95.04 to 1583.33±420.63kg/ha and 1146.67±298.72 to 1396.67±110.15kg/ha respectively. Crop having more than two years, but less than three years age, produced 1760.00±79.37 to 2316.67±330.05 kg/ha roots/rhizomes and 1256.67±11.55 to 2180.00±208.81 kg/ha leaves. Productivity of roots/rhizomes and leaves was 2996.67±90.18 to 3546.67±173.88 kg/ha and 3046.67±56.86 to 3423.33±299.56 kg/ha, respectively for the crop that has completed three years. Irrespective of age of crop and variability in cultivation conditions, picroside I content in roots/rhizomes was from 0.54 to 2.43%, while it was 1.42 to 4.42% in leaves. Picroside II content was from 4.72 to 8.62% in root/rhizomes and from 1.93 to 7.03% in leaves. Production of roots/rhizomes and leaves of *P. kurroa* under cultivation (*ex-situ)* is encouraging and based on picroside content in different plant parts, the quality of cultivated *P. kurroa* is comparable to naturally growing (*in-situ*) plants.

Keywords: *Picrorhiza kurroa,* cultivation, roots/rhizomes, leaves production, picrosides (I&II).

INTRODUCTION

Different species of *Picrorhiza* (*Picrorhiza kurroa* Royle ex Benth. or *P. kurrooa, Picrorhiza tungnathii* Pusalkar and *Neopicrorhiza scrophulariiflora* D. Y. Hong (Pennell) D. Y. Hong, English name–Picrorhiza, Hellebore or Yellow Gentian, etc., Family - Plantaginaceae), growing from 3000 to 5000 m asl in the Indian Himalayan Region (IHR) are low volume-high value medicinal plants (The Wealth of India, 2003; Pusalkar, 2014; Paudeyal et al.

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2019). In the indigenous health care system and Ayurveda, roots/rhizomes of *P. kurroa* are used for relieving fever and as a laxative, and it is termed *Katuka* or *Kadwi* (bitter medicine). *P. kurroa* is also used in the preparation of herbal medicines useful in jaundice, fever, asthma, liver protection and enhancing immunity (Bhattacharjee et al., 2013; Das, 2022). Medicines developed from Picrorhiza are administrated orally and no toxic effects are known; however, prolonged consumption may lead to vomiting, rash, anorexia, diarrhea and itching. Ever-increasing demand and trade-driven overharvesting have posed threat to the survival of *P. kurroa* and other *Picrorhiza* species in the IHR (Olsen, 2005; Uniyal et al., 2011; Chandra et al., 2021). *P. kurroa* is categorized as 'vulnerable' in the Red Data Book (RDB) of Indian Plants and enlisted on appendix II of CITES (Chowdhery, 1987; CITES, 2019), though, the species is yet to be assessed on new International Union for Conservation of Nature and Natural Resources (IUCN) criteria of threat. Cultivation of high value medicinal plants is practical for sustainable supply of authentic raw material of threatened species, and generating economic options for rural livelihood (Kuniyal and Negi, 2016).

The presence of iridoide glycosides, picrosides I and II in Picrorhiza have much pharmacological importance. Earlier, it was known that only roots/rhizomes of *P. kurroa* contain picroside, however, some recent studies on naturally growing plants have confirmed, in addition to roots/rhizomes, leaves and inflorescence of this species also contain picrosides I and II (Katoch et al., 2011; Singh et al., 2011; Attri et al., 2021). The biosynthesis of picrosides I and II occurs through non-mevalonate (MEP), mevalonate (MVA), phenylpropanoid, and iridoid pathways (Shitiz et al., 2015). Phytotherapy has received acceptance, therefore, quantifying active ingredients and testing the quality of herbal medicines has become mandatory (Efferth and Greten, 2012; Brito et al., 2016). As per the ICH (International Conference on Harmonization) of the technical requirement for registration of pharmaceuticals for human use, guidelines, HPLC technique is suitable for quantification of picroside (Sharma et al., 2018).

Herbal Research and Development Institute (HRDI), the state-level nodal agency of Uttarakhand government (India) is authorized for capacity building, promoting nursery enterprises, cultivation of Rare, Endangered, and Threatened (RET) and other Medicinal and Aromatic Plants (MAPs), extending financial support, providing facilitation for marketing and addressing policies and legal issues related to the MAPs (Kuniyal et al., 2014, 2015). In addition to other CITES and RET species, *P. kurroa* is also prioritized for mass scale cultivation in Uttarakhand. Each year and preferably in July, free planting material (one-year old rooted stolon cutting) of *P. kurroa* is distributed to the farmers from authentic sources. Production of *P. kurroa* is estimated under experimental conditions and assumptions are made based on quantity of roots/rhizomes sold by farmers (Nautiyal et al., 2001; Kuniyal et al., 2021). Information is available on picrosides I and II content of different accessions and market samples collected from herbal market (Thani et al., 2018; Attri et al., 2021). However, information on productivity (production of roots/rhizomes and leaves) and picroside content of cultivated material is not available. Therefore, a study was conducted for assessing productivity (production of roots/rhizomes and leaves) and estimation of picroside content (in roots/rhizomes and leaves) of cultivated material in relation to crop age and plant's part.

MATERIALS AND METHODS

Cultivation sites and weather conditions

A total of 12 locations, having different age group crops (less than two years; two to three years, and more than three years); namely, Urgam (URG), Kalsir (KAL), Ruisan (RUI), Parsari (PAR. experimental farm of HRDI), Ramni (RAM), Paderganv (PAD), Waduk (WAD), Ghes (GHE), Himni (HIM), and Wan (WAN) in Chamoli, and Ghangasu (GHA) and Syalmi (SYA) in Rudraprayag districts of Uttarakhand, India, were selected (Table 1). Planting material was distributed to farmers in July 2017 at GHE, HIM, and WAN in Chamoli and SYA in Rudraprayag. At RAM, PAD, WAD in Chamoli, July 2018. Also for experimental purpose, similar numbers of plants were planted at PAR experimental site of HRDI (July 2018). At URG, KAL and RUI in Chamoli and GHA in Rudraprayag planting material was distributed in July 2019. Randomized Block Design (RBD) was followed for planting and 2200 saplings/plants were planted in 10×20 m area at three different places, located apart 12.0±3.0 m distance.

In general, July and August are key months, when, these areas receive main rainfall and from January to March, snow mixed rainfall is common. Annually, a total 1136.40 to 1937.30 mm rainfall is received in Chamoli and Rudraprayag districts of Uttarakhand. Mean maximum temperature during January to February is reported to be around 12°C and from June to August it may reach up to ~26°C. Mean minimum temperature remains below 4°C from December to February (IMD, 2014).

Collection of soil samples

Subsequently, with the planting, soil samples (in triplicate) from the fields selected for biomass estimation were collected for analysis of pH, total organic carbon, available nitrogen, phosphorus and potassium contents. Soil samples were collected from 15 cm depth from ground level by following standard procedure. pH, total organic carbon, available nitrogen, phosphorus and potassium contents were estimated as per the methods suggested by Olsen et al. (1954), Jackson (1967), and Page et al. (1982).

Assessment of productivity

Generally, from last week of September and onward, farmer starts harvesting *P. kurroa*; therefore, from last week of September to first week of October 2020, samples were collected for estimation of productivity (roots/rhizomes and leaves biomass) and picroside content in the roots/rhizomes and leaves. Destructive sampling approach (harvesting of total herbal biomass), as suggested by

Table 1. Description of the sites selected for assessing the production of roots/rhizomes and leaves of *P. kurroa* and collection of samples for estimation of Picrosides (I and II).

S/N	Sites, District (Abbreviation)[a]	Elevation (masl) and approximate location	Soil characteristics (± SD)[b]					
			Soil type and color	*p*H	Organic carbon (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)
1	Urgam, Chamoli (URG)[A]	2200; 30.541410, 79.447422	Clay, black	5.60±0.08	2.84±0.07	0.61±0.03	125.57±12.45	22.33±0.11
2	Kalsir, Chamoli (KAL)[A]	2160; 30.446183, 79.215519	Mold, brown	5.40±0.03	3.29±0.07	0.61±0.16	118.77±17.73	19.01±0.05
3	Ghangasu, Rudraprayag (GHA)[A]	1950; 30.49215, 78.969304	Mold, brown	4.96±0.02	3.08±0.15	0.61±0.15	106.33±12.80	11.74±0.20
4	Ruisan, Chamoli (RUI)[A]	2250; 30.109687, 79.525068	Sandy, black	5.60±0.02	3.68±0.44	0.65±0.20	218.10±19.07	19.27±0.03
5	Parsari, Chamoli (PAR)[B,#]	2650; 30.528479, 79.573374	Sandy, brown	5.82±0.04	2.61±0.06	0.48±0.09	264.33±40.49	17.94±0.15
6	Ramni, Chamoli (RAM)[B]	2550; 30.319433, 79.507012	Sandy, black	6.11±0.03	3.69±0.16	0.55±0.04	199.67±43.66	20.16±0.11
7	Paderganv, Chamoli (PAD)[B]	2580; 30.311068, 79.532504	Sandy, brown	5.19±0.03	3.60±0.30	0.39±0.03	111.87±8.85	16.03±0.02
8	Waduk, Chamoli (WAD)[B]	2200; 30.259392, 79.498878	Sandy, black	5.05±0.02	3.30±0.38	0.42±0.09	103.09±5.25	16.86±0.08
9	Ghes, Chamoli (GHE)[C]	2350; 30.119845, 79.692419	Clay, Sandy	6.37±0.03	3.26±0.11	0.55±0.13	182.63±20.03	23.49±0.22
10	Himni, Chamoli (HIM)[C]	2450; 30.143901, 79.729004	Sandy, black	5.67±0.04	4.03±0.07	0.89±0.17	321.40±46.81	19.09±0.17
11	Wan, Chamoli (WAN)[C]	2500; 30.208298, 79.621257	Mold, Brown	6.84±0.05	3.03±0.18	0.46±0.13	131.93±7.75	17.11±0.21
12	Syalmi, Rudraprayag (SYA)[C]	2280; 30.505688, 79.174353	Clay, black	5.39±0.03	4.04±0.29	0.74±0.10	204.13±30.34	15.99±0.16

[a]Age of the crop etc., [A]< 2 years, [B]>2 years but < 3 years, and [C] >3 years, [#]experimental farm of Herbal Research and Development Institute (HRDI), [b]± SD = standard deviation.

Salunkhe et al. (2014), was followed for the estimation of total biomass (roots/rhizomes and leaves production or productivity). 1×1 m area at three places and nearly 15 m apart was marked. The numbers of plants in the marked area were counted and total biomass (roots/rhizomes and leaves) was harvested. Harvested material was washed thoroughly with running water. After blotting, harvested material was segregated into roots/rhizomes (stolon) and leaves, and rotten or dead plant material was removed. Fresh weight of roots/rhizomes and leaves was noted and samples were brought to the laboratory. All the samples were dried at 60±5°C in a hot air drier (LabPro), till the dry weight reached the constant values. Finally, dry weight of samples was noted with the help of analytical balance (Contech CAI – 234).

Preparation of samples and standards (picrosides I and II)

Sharma et al. (2012), with minor modification was followed for the preparation of samples. Dried plant material used for assessment of productivity was grinded with the help of electric grinders (Bajaj, India). The ground material was sieved (mesh size BSS 36, pore size 400 µ). 200 mg powdered material (in triplicate, roots/rhizome and leaves of each sample) was extracted with 20 ml 80% methanol by the reflux extraction method for 6 to 8 h. The extraction process was repeated thrice for each sample. Extracted samples were filtered separately through Whatman No. 1 filter paper and filtrates were pooled. Pooled samples were concentrated with the help of a rotatory evaporator at reduced pressure, at ~ 45°C. Concentrated extracts were diluted to 20 ml with 80% methanol. Diluted samples were filtered through 0.45 µm syringe filter and were used for analysis. Stock solutions (1000 µg/ml) of picroside I and picroside II (Sigma chemicals) were prepared in absolute methanol (99.9% purity). Stock solutions were stored at 4°C. From stock solution equal volumes of picrosides I and II were mixed to make a standard solution of 500 µg/ml. From this standard mixture, working solutions of 15.62, 31.25, 62.50, 125, and 250 µg/ml were prepared for calibration (Figure 1A and B).

HPLC analysis

The marker compounds were separated and quantified by DIONEX, a semi-preparative HPLC system (Ultimate 3000 series, equipped with SRD 3200 degasser, HPG 3200 P binary pump, WPS 3000 SL auto-sampler, TCC 3000 SD column compartment, and DAD 3000 UV detector). Chromeleon software, version 6.80, (DIONEX, Germany) was used for data processing. Quantification of picrosides I and II were carried out by reverse phase HPLC through C-18 (5 µm), 250 × 4.6 mm column (Beckman USA). Two solvents system was used as mobile phase for analyzing the samples, that is, solvent A (0.05% Trifluroacetic acid) and solvent B (1:1, methanol and acetonitrile acetic acid). Solvents A and B were used in the ratio of 70:30 and were delivered at the flow rate of 1.0 ml/min and the retention time was 30 min for each sample. Picrosides I and II were detected at 270 nm wavelength. 10 µl sample and standards volume were injected. The retention/cycle time was 30 min and the temperature was 30±2°C. Picrosides I and II were identified based on retention time and comparison with standard UV spectra (Figure 1C and D). The methods suggested by Katoch et al. (2011) and Pandit et al. (2012) with minor modifications were followed for analysis of samples. All chemicals, solvents, and water used for analysis were HPLC grade (Thermo Fisher Scientific, India, Limited).

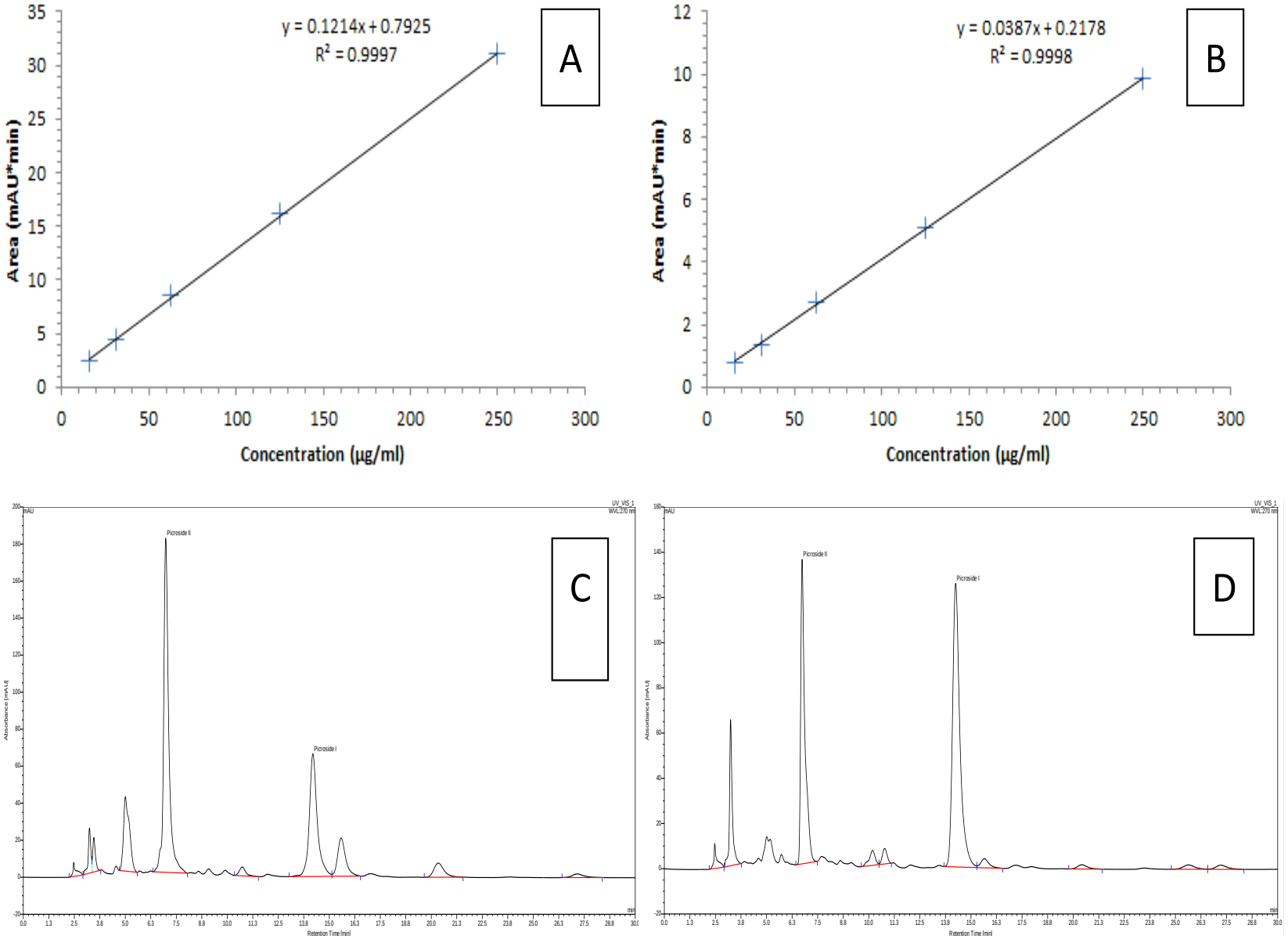

Figure 1. (A) Calibration curve of picroside I, (B) Calibration curve of picroside II, (C) HPLC analysis of picroside I and II in the roots/rhizomes of *Picrorhiza kurroa.* (D). HPLC analysis of picroside I and II in the leaves of *Picrorhiza kurroa* (C and D are representative figures).

Data analysis

Obtained data were analyzed suitably for assessing the density of plants, production of roots/rhizomes, and leaves in a hectare area (100 × 100 m) area. For comparison purpose, single factor analysis of variance (ANOVA) and correlation analysis were applied with the help of XLSTAT data analysis tools on the averages of obtained data on production of roots/rhizomes and leaves, and picrosides I & II content in roots/rhizomes and leaves. Also, Tukey's Honestly Significant Difference (HSD) of means was tested (https://astatsa.com/OneWay_Anova_with_TukeyHSD/_result/) for the averages of obtained data.

RESULTS AND DISCUSSION

Soil characteristics

The soil of cultivation sites in majority was clay, mold, and sandy with black color. The pH was 4.96 ± 0.02 to 6.84 ± 0.05, while the organic carbon content (%) was from 2.61 ± 0.06 to 4.04 ± 0.29. Total available nitrogen (%) was 0.39 ± 0.03 to 0.89 ± 0.17, and the availability of phosphorus (ppm) and potassium (ppm) contents were 103.09 ± 5.25 to 321.40 ± 46.81 and 11.74 ± 0.20 to 23.49 ± 0.22, respectively (Table 1). The pH of the soil of selected sites was acidic and soils nutrient status of cultivation sites was better than the natural (subalpine and alpine) habitats (Bisht et al., 2014a).

Roots/rhizomes and leaves productivity

Productivity (on a dry weight basis) of the roots/rhizomes of less than two years *P. kurroa* crop, at Urgam (URG), Kalsir (KAL), and Ruisan (RUI) in Chamoli and Ghangasu (GHA) in Rudraprayag, was from 1146.67 to 1583.33 kg/ha (kg/ha, 1.0 kg = 1000 g and 1 ha =100×100 m), (Table 2). Roots/Rhizomes productivity of crop having the age of more than two years but less than three years at Parsari (PAR; experimental site of Herbal Research and Development Institute, HRDI), Ramni (RAM), Paderganv (PAD), and Waduk (WAD) in Chamoli was estimated from 1760.00 to 2316.67 kg/ha. Production of roots/rhizomes of the crop that has completed three years at Ghes (GHE), Himani (HIM), and Wan (WAN) in Chamoli and Syalmi (SYA) in Rudraprayag was 2996.67 to 3546.67 kg/ha (Table 2). Production of leaves of less than two year crop in URG, GHA, and RUI was 1146.67 to 1396.67 kg/ha, while the productivity of leaves of more than two, but less than three years crop at PAR, RAM, PAD, RAM, and WAD was 1256.67 to 2180.00 kg/ha and from 3046.67 to 3530.00 kg/ha leaves were produced in the crop that has completed three years at GHE, HIM, WAN, and SYA (Table 2). Probably, *lower* productivity at PAR in the identical age group crop was due to the fact, that, the routine maintenance activities at this experimental farm were stopped in third year, and only some places were retained for assessment of productivity and sample collection *P. kurroa* is an endangered Himalayan medicinal herb thus, in nature it occurs randomly in specific habitats and restricted pockets (Bisht et al., 2014b). Frequency of *P. kurroa* under natural habitat in the part of the Indian Himalayan Region (IHR) is reported to be 42.86% (Ghimire et al., 1999). Density of this species in Indian trans-Himalaya was approximately 70.6 individuals/m^2 (Kala, 2000). Also, the density of *P. kurroa* was noted approximately 38972.83 ha^{-1} in a part of western Himalaya and estimations on available biomass (dry weight, kg/ha, roots and rhizomes) indicated that around 1.42 to 28.31 kg/ha roots/rhizomes biomass was available in nature (Uniyal et al., 2002). Production of *P. kurroa* roots/rhizomes after three years under experimental cultivation is supposed to be 450.00 to 612.00 kg/ha from seedlings and 864.00 to 1092.00 kg/ha from vegetative cuttings (Nautiyal et al., 2001). Based on quantity marketed by farmers, the production of roots/rhizomes is estimated to be approximately 796.71 kg/ha (Kuniyal et al., 2021). Assessments on the production of roots/rhizomes and leaves indicate, the productivity of roots/rhizomes of *P. kurroa* is far better (1146.67 to 3546.67 kg/ha, for different age group crop) than experimental conditions or the assumptions made on the basis of produce marketed by farmers in past years. The productivity of roots/rhizomes varies in different locations, instead of soil characteristics; the age of crop seems a key factor for accumulation of biomass. Our field observations have also indicated that, better productivity under cultivation depends on high density of plants and farmers' efforts or better care.

The general gestation period for *P. kurroa* is two and a half to three years and flowering in vegetatively propagated plants was common after two years of planting. Sometimes, farmers may retain the crop for more than three years for vegetative multiplication/ production of cuttings. Approximately 110,000 cuttings/ha of *P. kurroa* are planted for mass scale cultivation. The density of *P. kurroa* plants in the fields having less than two years of plantation was estimated at optimum, that is 550,000 to 600,000 plants/ha, while the density of plants in more than two years and less than three years crop was near about 650,000 plants/ha. The density of plants, despite the mortality, etc., reaches the best possible within two years of planting thus, the crop that has completed three years was noted to have near about comparable plants (650,000 to 700,000), as to the previous year's crop. Under experimental-field conditions, it was observed that, before two years, stolon, leaves and roots remain delicate and after two and a half to three years of plantation, thickening/hardening of roots/ rhizomes occurs. It is observed, that the cultivation sites of *P. kurroa* looks like compact green area and any other plant species hardly finds enough space for usual growth.

Picrosides I and II content in the roots/rhizomes and leaves

Picroside I content in the roots/rhizomes of all samples

Table 2. Production of roots/rhizomes and leaves (kg/ha., on a dry weight basis), and picroside I and II content (%) in the roots/rhizomes and leaves of *Picrorhiza kurroa* cultivated at different locations.

S/N	Sites, District (Abbreviation)[a]	Productivity[b] (on dry weight basis, Kg./ha., ± = SD[c])		Picroside			
				Picroside I, (%, ± SD)		Picroside II, (%, ± SD)	
		Roots/Rhizomes	Leaves[d]	Roots/Rhizomes	Leaves	Roots/Rhizomes	Leaves
1	Urgam, Chamoli (URG)[A]	1146.67±95.04	1316.67± 30.55	1.45±0.35	2.37±0.81	7.43±1.26	2.53±0.55
2	Kalsir, Chamoli (KAL)[A]	1193.33±136.50	-	2.10±0.31	-	8.62±0.26	-
3	Ghangasu, Rudraprayag (GHA)[A]	1276.67±283.08	1146.67±298.72	1.11±0.15	4.11±0.53	7.13±0.42	3.26±0.62
4	Ruisan, Chamoli (RUI)[A]	1583.33±420.63	1396.67±110.15	0.54±0.11	1.42±0.22	4.98±0.53	3.53±0.55
5	Parsari, Chamoli (PAR)[B,#]	1760.00±79.37	1256.67±11.55	1.05±0.04	2.11±0.08	7.23±0.30	4.07±0.15
6	Ramni, Chamoli (RAM)[B]	2206.67±353.70	1720.00±361.66	1.86±0.41	4.42±0.61	7.00±1.20	7.03±0.49
7	Paderganv, Chamoli (PAD)[B]	2316.67±330.05	1836.67±258.13	2.43±0.35	2.83±0.64	7.39±0.34	5.34±0.94
8	Waduk, Chamoli (WAD)[B]	2270.00±261.53	2180.00±208.81	1.25±0.20	2.68±0.13	7.08±0.45	1.93±0.20
9	Ghes, Chamoli (GHE)[C]	3540.00±175.78	3243.33±340.78	1.36±0.43	1.51±0.40	5.10±0.73	2.99±1.11
10	Himni, Chamoli (HIM)[C]	2996.67±90.18	3530.00±144.22	1.48±0.18	2.03±0.84	5.84±0.75	4.34±0.86
11	Wan, Chamoli (WAN)[C]	3096.67±110.15	3046.67±56.86	1.63±0.38	1.93±0.88	4.72±1.12	4.60±1.86
12	Syalmi, Rudraprayag (SYA)[C]	3546.67±173.88	3423.33±299.56	1.18±0.09	3.75±0.95	5.72±1.45	6.93±1.22
ANOVA	Among sites	LSD[e] (P<0.05) = 399.61	LSD (P<0.05) = 368.36	LSD (P<0.05) = 0.47	LSD (P<0.05) = 1.01	LSD (P<0.05) = 1.41	LSD (P<0.05) = 1.46
	Among age groups	LSD (P<0.001) = 725.19	LSD (P<0.001) = 605.64	LSD (P<0.05) = 0.17	LSD (P<0.05) = 0.42	LSD (P<0.05) = 1.06	LSD (P<0.05)= 0.74

Tukey's HSD[f] (n =4 in triplicate)	Treatments pair (age group)	Q Stat	p-value	Inter ference	Q Stat	p-value	Inter ference	Q Stat	p-value	Inter ference	Q Stat	p-value	Inter ference	Q Stat	p-value	Inter ference	Q Stat	p-value	Inter ference
	A vs B	6.696	0.003	**p<0.01	3.459	0.085	ns	1.304	0.636	ns	1.614	0.515	ns	0.290	0.900	ns	2.492	0.236	ns
	A vs C	15.934	0.001	**p<0.01	10.360	0.001	**p<0.01	0.422	0.900	ns	0.515	0.900	ns	3.636	0.070	ns	2.627	0.206	ns
	B vs C	9.238	0.001	**p<0.01	6.900	0.002	**p<0.01	0.882	0.799	ns	1.100	0.715	ns	3.925	0.051	ns	0.135	0.900	ns
Correlation among age group		r^2 = 0.991			r^2 = 0.964			r^2 = 0.095			r^2 = 0.096			r^2 = 0.690			r^2 = 0.788		

[a]Age of the crop etc., [A]< 2 years, [B]>2 years but < 3 years, and [C]>3 years, [#]experimental farm of Herbal Research and Development Institute (HRDI), [b]Kg. = kilogram, ha. = hectare and [c]± SD = standard deviation, and [d]sample was damaged. [e]LSD = least significant difference, [f]**p<0.01 = significantly different among different age groups, ns = not significant, r^2 = coefficient of determination.

was from 0.54 to 2.43% and picroside II was 4.72 to 8.62%. Picroside I in leaves (excluding KAL, the sample was damaged) was estimated 1.42 to 4.42%, and picroside II (excluding KAL) was 1.93 to 7.03% (Table 2). Picroside II in the roots/rhizomes and leaves of RAM samples was 7.00 and 7.03% (Table 2). Syalmi (SYA) was the only site where picroside II was slightly higher (6.93%) in leaves as compared to roots/rhizomes (5.72%). Picroside I in the leaves of plants cultivated at GHA, RAM and SYA, was 4.11, 4.42, and 3.75%. Picroside I was comparatively higher in leaves of all samples as compared to the roots/ rhizomes, while picroside II was comparatively higher in roots/rhizomes (Table 2). Comparatively, a higher amount of picroside I (3.5 to 4.5%) was estimated in the root/rhizomes and leaves of naturally growing plants of *P. kurroa* collected from higher altitude or natural habitats (viz. ~2750 to 4500 m); however, the samples collected from 1900 to 2000 m were noted to have 0.28 to 1.51 picroside I in the roots and 1.71% picroside II in the leaves (Katoch et al., 2011; Sharma et al., 2012). A recent study has confirmed that around 0.74 to 2.50% picroside I and 3.25 to 5.62% picroside II was available in the roots/rhizomes samples of *P. kurroa* procured from market (Thani et al., 2018). Also, considerable variations were observed in the picrosides I and II content in the roots/rhizomes (stolon) and leaves of *P. kurroa* accessions maintained under experimental conditions at 2200 m altitude in Uttarakhand (Attri et al., 2021).

Picrosides I and II in the roots/rhizome and leaves of cultivated *P. kurroa* was comparable to the natural population or accessions maintained under experimental conditions at an almost similar altitude (Singh et al., 2005; Katoch et al., 2011; Sultan et al., 2016; Thani et al., 2018; Attri et al., 2021). The average altitude of all cultivation sites was approximately 2400 m (1950 to 2600 m), but the variations in available picrosides I and II in relation to altitudes, age of crop and cultivation sites do not show any definite trend either in roots/rhizomes or in leaves. Generally, high altitude medicinal plants are known to have important active ingredients in their specific plant's parts, but some studies have confirmed, in addition to routinely used parts, that is, roots or rhizomes, other plants parts such as stem and leaves also contain medicinally important compounds (Bhadula et al., 1996; Singh et al., 2011; Pandey et al., 2013; Attri et al., 2021). The availability of picrosides I and II content in leaves is encouraging, because, after harvesting and primary processing of *P. kurroa* at village level, farmer discards the leaves. If the cultivators are aware, the leaves also contain picroside; it will help them to sell the produce with self-assurance.

In addition to estimation of active ingredients, encouraging farmers for adopting Good Agriculture Practices (GAP) is necessary (NMPB, 2009). Information on productivity and estimation of active ingredients in cultivated produce will help in checking wild harvesting, the authenticity of quantity marketed, and purity of the material in terms of chemical diversity (Verma et al., 2016). In addition to conservation and resources management aspects, information on the active ingredients in high value medicinal plants has pharmaceutical and nutraceutical significance (Mohd et al., 2018; Kumar et al., 2021). Genes responsible for biosynthesis of Picrosides are identified, further, exploring the complete pathway for Picroside biosynthesis and genetic improvement of cultivated material will be useful approach (Shitiz et al., 2015).

Conclusion

P. kurroa is a newly domesticated medicinal herb in different villages of Chamoli and Rudraprayag districts, and some other temperate areas of Uttarakhand, India. Despite, habitat specificity in natural habitat, this herb is thriving well under diverse cultivation conditions and has achieved the status of optional cash crop. The findings of this study suggest, the productivity of *P. kurroa* under cultivation at lower elevation is far better and available picrosides I and II contents are comparable to naturally growing plants. Therefore, *ex-situ* cultivation does not have negative impact on biosynthesis of picrosides I and II. Field information on productivity of *P. kurroa* will help in real-time assessments and comparative analysis of different age group crops. Information on picrosides I and II will add assurance to the quality of produce and the traders will have confidence in buying produce from cultivated sources. Better productivity and acceptable quality will also encourage other farmers for further area extension or large-scale cultivation of *P. kurroa*. Preliminary information on production and quality of cultivated produce will help in establishment of regional herbal growth centers. This is a preliminary study on productivity and picroside content of cultivated *P. kurroa*, further studies on impact of microclimate, soil conditions, and pathological instances will be important. Based on better productivity and disease resistance, selection of improved cultivars may help in the development of new variety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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