

# Standardization of Storage Conditions and Duration on Picroside-I and Picroside-II in Raw Material of Drug Kutki (*Picrorhiza kurroa* Royle ex Benth.)

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## KEYWORDS

Degradation; *Picrorhiza kurroa*; picroside-I; picroside-II; storage

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## ABSTRACT

The study of the proper storage of medicinal plants after being harvested becomes necessary to minimize the loss of active constituents from microbial reactions, heat, moisture, light and enzymes etc. In the present study, fresh rhizomes of *Picrorhiza kurroa* (Kutki) were collected from 2600 m to 3300 m altitude of Himachal Pradesh (Rohtang area), India. The harvested material was washed to remove soil and other adhering materials, and then cut into small pieces and dried under shade. The air dried samples were packed in the different packing materials (i.e. Gunny bags or poly bags), stored either at room temperature or in refrigerator at 4° to 6 °C or in humid environment (85% humidity and 25 °C) for different storage durations and then analyzed their chemical constituents bimonthly. The study showed that the content of picroside-I and picroside-II decreased with the increase in storage duration irrespective of storage condition. However, the loss in picroside-I and picroside-II with storage was maximum when “Kutki” samples were stored under humid condition (85% humidity, 25 °C) and minimum loss was observed when samples were stored at low temperature (4-6 °C). So, to minimize the loss of picroside-I and picroside-II content in the drug Kutki during storage, Kutki must be stored at low temperature.

## 1. Introduction

Medicinal properties of plants are manifested due to the presence of bioactive compounds present in them and the diverse chemical nature of the active principles present in different plants accounts for their different biological and pharmacological properties. Depending upon the chemical structures, some of the active constituents are found stable, whereas others are unstable. Till the time of drying, storage and processing, the active constituents in the harvested plant/plant part remain in contact with the microbial reactions, heat, moisture, light, solvents and enzymes etc., and some of these compounds undergo degradation. Therefore, after being harvested the medicinal plants at their proper growth and development stage when there is maximum contents of bioactive compounds on them are required to be carefully dried and stored under controlled conditions to minimize the loss of active constituents during drying conditions and storage of dried material till it is finally converted into formulation to be used by the consumers. Storage becomes necessary because many times the herb cannot be immediately processed either due to the processing site being far off or because of heavy rush of the raw material at the processing unit.

In the harvested material, many enzymatic reactions still keep on going in the presence of moisture under normal temperature (Patil and Laloraya 1983), and therefore requiring suitable containers for storage of dried herbs. Light sensitive compounds are required to be stored in dark, whereas, heat sensitive compounds should be stored at low temperature.

Due to lack of knowledge in appropriate storage conditions and time period for *Picrorhiza kurroa* to get optimum benefit, desired compounds from it has been disintegrated in the

present situation. No specific studies on stability of iridoid glycosides (picroside-I and picroside-II) of plant *P. kurroa* under effect of different storage conditions and storage durations have been done, however; some related studies have been made to some extent in other medicinal plants.

Swiatek et al. (1988) analyzed one year old stored and fresh roots of *Gentiana lutea* and observed a higher amount of free phenolic acids in the stored material than in the freshly harvested roots. Brockmann et al. (1974) suggested keeping of the powdered material of *Hypericum perforatum* in dark to avoid decomposition of light sensitive bioactive compounds. Bomme (1997) recommended immediate drying of harvested crop of *H. perforatum* at 40-60 °C to avoid loss of active compounds in the post-harvest period. Franke et al. (1999) observed significant decrease in the content of flavone glycosides at higher drying temperatures of *H. perforatum*, however within the range of 40-80 °C, biapigenins, hypericin and pseudohypericin as well as hyperforin were not substantially influenced by the different drying temperatures.

Chapelle and Denoel (1972) observed rapid degradation of valtrate (a bioactive compound) in valerian drug. However under usual preservative conditions, degradation was found minimum when powdered root was preserved under vacuum, low temperature, low humidity and under exposure to light. Lutomski and Turowska (1973) observed significant effect of storage periods and relative humidity on contents of essential oil and valepotrianes (bioactive compounds) in valerian drug samples. The essential oil content was reduced to 50% after six months of storage, whereas, degradation degrees of total valepotriates at relative humidities of 100, 90, 80, 70 and 60% were 100, 100, 50, 32.9 and 15.7%, respectively

when the powdered roots of *Valeriana officinalis* were stored for six months. Lutomski et al. (1976) observed decrease in valepotriates content in proportion to increased drying temperature when *Valeriana* rhizomes were dried from 20-100 °C in laboratory without forced air flow. The optimum drying temperature was found to be 32-35 °C. Highest content of valepotriates was obtained at drying temperature of 60 °C with the air flow reaching 0.05 m<sup>3</sup>/min. Storage of crude drug resulted in significant loss in valepotriates. Verbist and Monnet (1975) have recommended immediate processing of fruits of *Solanum xanthocarpum* to avoid loss of solasodine during storage period.

Keeping in view of finding a best method of storage condition and storage duration for the raw material of *P. kurroa*, the present investigation was carried out.

## 2. Materials and Methods

### 2.1. Collection and storage of raw material

Fresh roots and rhizomes of *P. kurroa* were collected from 2600 m to 3300 m altitude of Himachal Pradesh (Rohtang area), India in 2012. The specimen of the plant material was submitted and authenticated at Department of Forest Products, Dr. Y. S. Parmar University of Forestry and Horticulture, Solan, India. The harvested material was washed with water to remove soil and other adhering materials and then cut into small pieces and dried under shade. The air dried samples (20 g each) were packed in the different packing materials (i.e. gunny bags or poly bags) and stored either at room temperature or in a refrigerator at 4° to 6 °C or in humid environment (85% humidity and 25 °C) for different storage durations as stated below:

(A) Storage conditions: 5

(1) Gunny bag and storage at room temperature; (2) Gunny bag and storage in refrigerator at 4° to 6 °C; (3) Gunny bag and storage under humid condition (85% humidity and 25 °C); (4) Transparent polybag (closed) and storage at room temperature; and (5) Black polybag (closed) and storage at room temperature.

(B) Storage durations: 8

(1) Zero day (at the time of storage); (2) Two months; (3) Four months; (4) Six months; (5) Eight months; (6) Ten months; (7) Twelve months; and (8) Fourteen months.

### 2.2. Extract preparation

After completion of each storage period, stored material was taken out of packing, dried to a constant weight in an oven at 50 °C and then ground to form a uniform powder. Accurately weighed powdered plant material (2 g) was subjected to Soxhlet extraction for 24 hrs using methanol. After extraction, the solvent was distilled off and obtained residue was dried to a constant weight under vacuum.

### 2.3. Quantitative estimation of picroside-I and picroside-II in the extracts using High Performance Liquid Chromatography (HPLC)

Picroside-I and picroside-II were quantified in the extracts using HPLC (Water's binary HPLC unit with Waters HPLC pump 515 and dual λ absorbance detector 2487) on column Sunfire C-18 (4.6 × 250 mm, 5 μm). Mobile phase consisted of methanol:water (40:60) with the flow rate of 0.9 ml/min. Temperature was maintained at 24 ± 1 °C.

Each dried extract residue was 1000 times diluted with mobile phase, filtered through the membrane filter and 20 μl of each sample was injected in HPLC. Area under curve of peaks of picroside-I and picroside-II was recorded at 270 nm wavelength on the basis of retention time comparing with that of standard picroside-I and picroside-II. Their contents in the extracts were quantified on the basis of area of the peak.

Percentage of picroside-I and picroside-II in each sample was calculated by using the following formula where percent purity of standard compound was taken as 95%:

$$\text{Picrosides content (\%)} = \frac{\text{Test Area}}{\text{Standard Area}} \times \frac{\text{Weight of Standard}}{\text{Standard Dilution}} \times \frac{\text{Test Dilution}}{\text{Test Weight}} \times 100 \times \% \text{ purity}$$

The experiment was conducted under factorial Complete Randomized Design with two replications. Analysis of variance was worked out and critical difference at 5% of significance was calculated. The data was analyzed by using OPSTAT software.

## 3. Results and Discussion

All the samples stored under five different storage conditions were analyzed at bimonthly interval to check the effect on picroside-I and picroside-II contents with storage duration. The results were found statistically significant and are presented in Tables 1-4. Significant differences were observed under different storage conditions and durations on

Table 1. Effect of different storage conditions of rhizomes of drug "Kutki" on picroside-I content (%)

Storage Durations	0 day	2 months	4 months	6 months	8 months	10 months	12 months	14 months	Mean A
<b>Storage Conditions</b>									
Gunny bag (room temperature)	6.827	6.67	6.462	6.362	6.255	6.08	5.887	5.65	6.274
	-2.798	-2.77	-2.732	-2.713	-2.694	-2.661	-2.624	-2.579	-2.696
Gunny bag (maximum humidity)	6.827	0.638	0.353	0.108	0.1	0.015	0.006	0	1.006
	-2.798	-1.28	-1.163	-1.053	-1.049	-1.008	-1.003	-1	-1.294
Black sealed poly bag	6.827	6.801	6.782	6.657	6.57	6.467	6.349	6.163	6.577
	-2.798	-2.793	-2.79	-2.767	-2.751	-2.733	-2.711	-2.676	-2.752
Transparent sealed poly bag	6.827	6.788	6.672	6.537	6.525	6.361	6.171	5.962	6.48
	-2.798	-2.791	-2.77	-2.745	-2.743	-2.713	-2.678	-2.639	-2.735
Gunny bag (refrigerated condition)	6.827	6.811	6.799	6.781	6.777	6.759	6.748	6.726	6.779
	-2.798	-2.795	-2.793	-2.789	-2.789	-2.786	-2.784	-2.78	-2.789
Mean B	6.827	5.541	5.414	5.289	5.245	5.136	5.032	4.9	
	-2.798	-2.485	-2.449	-2.414	-2.405	-2.38	-2.36	-2.335	

Values in parentheses are transformed values using transformed values.

Table 2. Effect of different storage conditions of rhizomes of drug "Kutki" on picroside-II content (%)

Storage Durations	0 day	2 months	4 months	6 months	8 months	10 months	12 months	14 months	Mean A
<b>Storage Conditions</b>									
Gunny bag (room temperature)	5.021	4.93	4.83	4.676	4.529	4.449	4.326	4.235	4.624
	-2.454	-2.435	-2.415	-2.382	-2.351	-2.334	-2.308	-2.288	-2.371
Gunny bag (maximum humidity)	5.021	0.289	0.177	0	0	0	0	0	0.686
	-2.454	-1.135	-1.085	-1	-1	-1	-1	-1	-1.209
Black sealed poly bag	5.021	4.962	4.895	4.793	4.643	4.531	4.417	4.353	4.702
	-2.454	-2.442	-2.428	-2.407	-2.376	-2.352	-2.328	-2.314	-2.387
Transparent sealed poly bag	5.021	4.935	4.863	4.645	4.57	4.493	4.382	4.272	4.648
	-2.454	-2.436	-2.421	-2.376	-2.36	-2.344	-2.32	-2.296	-2.376
Gunny bag (refrigerated condition)	5.021	4.995	4.957	4.934	4.905	4.884	4.852	4.835	4.923
	-2.454	-2.448	-2.441	-2.436	-2.43	-2.426	-2.419	-2.415	-2.434
Mean B	5.021	4.022	3.944	3.809	3.729	3.672	3.595	3.539	
	-2.454	-2.179	-2.158	-2.12	-2.103	-2.091	-2.075	-2.063	

Values in parentheses are transformed values using transformed values.

Table 3. Loss in picroside-I content (%) during storage under different storage conditions

Storage Durations	0 day	2 months	4 months	6 months	8 months	10 months	12 months	14 months	Mean A
<b>Storage Conditions</b>									
Gunny bag (room temperature)	5.021	4.93	4.83	4.676	4.529	4.449	4.326	4.235	4.624
	-2.454	-2.435	-2.415	-2.382	-2.351	-2.334	-2.308	-2.288	-2.371
Gunny bag (maximum humidity)	5.021	0.289	0.177	0	0	0	0	0	0.686
	-2.454	-1.135	-1.085	-1	-1	-1	-1	-1	-1.209
Black sealed poly bag	5.021	4.962	4.895	4.793	4.643	4.531	4.417	4.353	4.702
	-2.454	-2.442	-2.428	-2.407	-2.376	-2.352	-2.328	-2.314	-2.387
Transparent sealed poly bag	5.021	4.935	4.863	4.645	4.57	4.493	4.382	4.272	4.648
	-2.454	-2.436	-2.421	-2.376	-2.36	-2.344	-2.32	-2.296	-2.376
Gunny bag (refrigerated condition)	5.021	4.995	4.957	4.934	4.905	4.884	4.852	4.835	4.923
	-2.454	-2.448	-2.441	-2.436	-2.43	-2.426	-2.419	-2.415	-2.434
Mean B	5.021	4.022	3.944	3.809	3.729	3.672	3.595	3.539	
	-2.454	-2.179	-2.158	-2.12	-2.103	-2.091	-2.075	-2.063	

Values in parentheses are transformed values using transformed values

Table 4. Loss in picroside-II content (%) during storage under different storage conditions

Storage Durations	0 day	2 months	4 months	6 months	8 months	10 months	12 months	14 months	Mean A
<b>Storage Conditions</b>									
Gunny bag (room temperature)	0	1.805	3.797	6.858	9.801	11.378	13.833	15.644	7.889
	-1	-1.673	-2.189	-2.798	-3.286	-3.518	-3.85	-4.079	-2.799
Gunny bag (maximum humidity)	0	94.25	96.482	100	100	100	100	100	86.341
	-1	-9.76	-9.873	-10.05	-10.05	-10.05	-10.05	-10.05	-8.86
Black sealed poly bag	0	1.156	2.509	4.527	7.511	9.751	12.012	13.307	6.347
	-1	-1.429	-1.873	-2.35	-2.917	-3.279	-3.605	-3.782	-2.529
Transparent sealed poly bag	0	1.708	3.131	7.485	8.976	10.506	12.715	14.913	7.429
	-1	-1.643	-2.032	-2.909	-3.156	-3.392	-3.702	-3.989	-2.728
Gunny bag (refrigerated condition)	0	.513	1.274	1.735	2.305	2.71	3.365	3.703	1.951
	-1	-1.158	-1.505	-1.653	-1.8	-1.917	-2.089	-2.162	-1.661
Mean B	0	19.886	21.439	24.121	25.718	26.869	28.385	29.513	
	-1	-3.132	-3.495	-3.952	-4.242	-4.431	-4.659	-4.812	

picroside-I content (Table 1). The initial value of picroside-I content (6.827%) recorded at the time of storage of the material decreased after 14 months of storage to 5.650% in gunny bag (room temperature), nil in gunny bag (85% relative humidity), 6.163% in black sealed poly bag, 5.962% in transparent sealed poly bag and 6.726% in gunny bag (refrigerated condition). Among different storage conditions, maximum mean value of picroside-I content (6.779%) was found in gunny bag (refrigerated condition) and minimum value (1.006%) was recorded in gunny bag (85% relative humidity).

Under all storage conditions, picroside-II content

decreased with storage durations (Table 2). The initial value of picroside-II content recorded as 5.021% at the time of storage of material, decreased after 14 months to 4.235% in gunny bag (room temperature), nil in gunny bag (85% relative humidity), 4.353% in black sealed poly bag, 4.272% in transparent sealed poly bag and 4.835% in gunny bag (refrigerated condition). Among different storage conditions, maximum mean value of picroside-II content (4.923%) was found in gunny bag (refrigerated condition) and minimum value (0.686%) was recorded in gunny bag (maximum humidity).

The analysis of stored samples showed the loss in picroside-I and picroside-II contents increased with increase in storage duration but the quantum of loss varied under different storage conditions (Tables 3 and 4). The results show that though both picroside-I and picroside-II contents decreased with storage duration under all the storage conditions, however, storage of Kutki rhizome at maximum humidity (85%) and at temperature 25 °C recorded complete loss of picroside-I (in 14 months) and picroside-II (in 6 months) suggesting that raw material of Kutki should not be kept under humid condition after drying. The loss in picroside-I and picroside-II contents was minimum when raw material was stored at low temperature which suggests that if raw material of Kutki is required to be stored for long duration then it should be stored at low temperature.

#### 4. Conclusion

During storage, picroside-I and picroside-II contents decrease with different storage conditions and durations. Therefore, to minimize the loss of these bioactive compounds in the drug "Kutki" during storage, it must be stored at low temperature.

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