Impact Factor 3.025

**Refereed And Indexed Journal** 

# AAYUSHI INTERNATIONAL INTERDISCIPLINARY RESEARCH JOURNAL (AIIRJ)

**UGC Approved Monthly Journal** 





**CHIEF EDITOR – PRAMOD PRAKASHRAO TANDALE** 

Vol - IV	Issue-XI	NOVEMBER	2017	ISSN 2349-638x	Impact Factor 3.025

# Chromatographic Study between Katuki (Picrorhizakurroa Royle ex Benth) **Rhizomes and Roots.**

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### Abstract

Introduction- Katuki, PicrorhizakurroaRoyle ex Benth, is considered to be a valuable bitter tonic and a propitious remedy in bilious dyspepsia accompanied with fever. The importance of this drug has create a demand in the market but due to excessive deforestation and extreme weather conditions Himalaya is not able to meet the demand there by leading to unethical and dangerous practice of adulteration. The official part of use of Katuki is rhizome, the rhizomes are commonly adulterated with other parts such as the roots, stems, dried leaves of the same plant.

Aim of Study- To check the quantitatively and qualitatively difference between the rhizomes and roots of the Katuki.

*Materials and Methods-Samples of Katuki were collected from their natural habitat and from major raw* drug markets of India. Analytic tests like extractive values, total glycoside contentand TLC were done in Dravyaguna laboratory of National Institute of Ayurveda, Jaipur. HPLC study was done in Amol Pharmaceuticals Private Limited Sitapura, Jaipur.

**Results**- Market samples were found Adulterated (7 to 47%) much above the standard value of 2% as per API. Significant differences were found in the extractive values and total glycoside content between rhizomes and roots of Katuki. Thin Layer Chromatography of genuine katuki and its roots revealed difference in the number of spots and also difference in Rf values. The rhizomes of genuine sample of Katuki has more than 5 times amount of Picroside II in comparison with the roots.

**Conclusion**- From the above results it clearly shows that the roots are the adulterant and it will surely reduce the therapeutic potential of Katuki. V 2349-60

KeywordsKatuki, HPLC, Picroside, Adulteration.

#### Introduction

WWW : Katuki has been used in the indigenous system of medicine since a long time. The authentic source of the drug is rhizomes of *Picrorhizakurroa*Royle ex Benthbelongs to family Scrophulariaceae.<sup>1</sup> The plant is native of North-West Himalayas from Kashmir to Sikkim. It grows on bare hill sides as well as on the edges of rocks. Its rhizome is used in many Ayurvedic medicines. Katuki is considered to be a valuable bitter tonic and a propitious remedy in bilious dyspepsia accompanied with fever. It is antipyretic, anthelmintic, laxative and is useful in asthma, blood troubles, burning sensation, piles, inflammations, ringworm.<sup>2</sup>The importance of this drug has create a demand in the market but due to excessive deforestation and extreme weather conditions Himalaya is not able to meet the demand there by leading to unethical and dangerous practice of adulteration which seems to be he prime obstacle in

#### Aayushi International Interdisciplinary Research Journal (AIIRJ) UGC Approved Sr.No.64259 Vol - IV **Issue-XI NOVEMBER** ISSN 2349-638x 2017 **Impact Factor 3.025**

excellence of the Ayurveda in this present Era. The rhizomes are commonly adulterated with other parts such as the roots, stems, dried leaves of the same plant.

### **Materials and Methods**

To check the degree of adulteration market study was done along with the collection of genuine samples from the native habitat. Genuinesample of Katuki i.e. rhizomes of Picrorhizakurroa from hills of Bhaderwa, dist. Kathua, State- Jammu and Kashmir were collected. After collection Herbarium sheet was made and authenticated at IIIM Jammuwith herbarium sheet no17772. After collection of all the market samples, it had been observed that, the samples taken from all the major Ayurvedic drugs whole sale markets were having all diagnostic characters & same appearance as that of genuine sample of rhizome of *Picrorhizakurroa*. Only difference was the quality of the samples, difference in the size of rhizomes and amount of mixing of adulterant in the form of roots and other materials from the same plant.



Fig 2- Katukilateral roots

S.no	Sample	Total weight	Foreign matter	Percentage
01	Genuine	350 gms	4 gms	01.142%
02	Kullu	502 gms	92 gms	<mark>18.326%</mark>
03	Amritsar	501 gms	45.701 gms	09.140%
04	Jaipur	500 gms // 22	38.808 gms	07.761%
05	Mumbai	500 gms	100 gms	20.000 %
06	Kolkata	510 gms	240 gms	47.058%
07	Kochin	505 gms airi	63.420 gms	12.558%

# Table 1: Foreign matter of Katuki and its market samples

Standard- Not more than 2 % (API Part I Vol. II Page no. 92)

Percentages of Foreign matter from all the market samples were found much above the standard i.e. 2 %. On examination of foreign matter, lateral roots of Katukiwere found in large quantity. Qualitatively there was no difference in the presence of secondary metabolites. But differences in the extractive values were found.

# Table 2:

### Quantitative values between Rhizomes and Roots of KatukiofAmritsarmarketsample.

S.No	Values	Katuki rhizomes	Katuki roots
01	Water extractive %	54.344 %	25 %
02	Alcoholic Extractive %	37.189 %	23 %
03	Total glycoside content	29.647 %	10.081 %

### Method of isolation of glycosides<sup>3</sup>:

- Powdered drug was extracted with alcohol in soxhlet extractor.
- Alcoholic extract was then treated with lead acetate solution to precipitate tannins, proteins, coloring matter and other non-glycosidal part.
- $\bullet$  The precipitate formed was filtered and to the filtrate H<sub>2</sub>S gas was passed to precipitate excess lead as lead sulphide and removed by filtration.
- Filtrate was evaporated to dryness on water bath and dried residue was collected and weighed to get total glycoside content.

### Thin Layer Chromatography of Katuki Rhizomes and Katuki Roots

The official useful part of Katuki is its rhizomes and in the study roots were found adulterated heavily in rhizomes. Roots are inferior to rhizomes and reduce the quality and potential medicinal value of Katuki, so comparative TLC between rhizomes and roots were done.

#### Sample Preparations

Total Glycoside content of Katuki rhizomes and Katuki roots (extraction as per above method).

#### **TLC chamber**

Mobile Phase:	Chloroform: Methanol (95:5)
<b>Stationary Phase:</b>	Thin layer chromatographic plates (Silica gel, 60 F254)
	Merck, Germany
Distance Travelled:	7.5 cm. V 2349-638

#### **Distance Travelled:**

# Table 3:

### Rf values of Katuki rhizomes and Katuki roots.

Samples		Iodine Vapour	Vanillinsulphuric acid reagent		
Rhizome	09	Rf. 0.05, 0.07, 0.16, 0.21, 0.26, 0.47, 0.70, 0.88, 0.96.	10	Rf. 0.05, 0.07, 0.01, 0.16, 0.21, 0.26, 0.47, 0.70, 0.88, 0.96.	
Roots	03	0.24, 0.34, 0.61	04	0.24, 0.34, 0.46, 0.58.	

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# Fig 3- TLC Plate Rhizome (Left) Roots (Right)

# High Preformance Liquid Chromatography (HPLC)

HPLC study was done in Amol Pharmaceuticals Private Limited Sitapura, Jaipur. Crude samples were given to the Amol pharmaceuticals in a air tight poly bag.

#### Katuki

Samples chosen for HPLC study was the root part of Katuki (K 0) which found admixture in large amounts with the rhizomes, genuine samples of Katuki (K 1).Picroside II % in both the samples was calculated with the help of standard Picroside II.

# Process fallowed by Amol Pharmaceuticals: 2349-638

**Preparation of Mobile phase:** Mix properly 150 ml of acetonitril, 100 ml methanol and 750 ml of aqueous phosphoric acid solution pH 3, filter through 0.45 µm membrane filter and degas.

**Preparation of Standard solution**: Weigh accurately working standard equivalent to 5 mg of picroside II in a 100 ml volumetric flask. Add 25 ml of mobile phase. Sonicate the solution to dissolve the content. Makeup volume with mobile phase. Mix properly and filter.

**Preparation of sample solution:**Weigh accurately about 100 mg sample in a 100 ml volumetric flask. Add 25 ml of mobile phase, sonicate the solution to dissolve the content. Make up volume with mobile phase. Mix properly and filter.

**System suitability:** Inject 5 replicate of reference standard, calculate the % RSD, it should not be more than 2%.

**Procedure:**Inject equal volume (20  $\mu$ l) of standard and sample preparation. Record the chromatograms, calculate the average area and finally calculate the percentage of picroside II.

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<u>Aayushi International Interdisciplinary Research Journal (AIIRJ)</u>						
UGC Approved Sr.No.64259						
Vol - IV	Issue-XI	NOVEMBER	2017	ISSN 2349-638x	Impact Factor 3.025	

#### Instrument setup conditions:

High Performance Liquid Chromatography equipped with UV- Visible Detector.

Column		:	HypersilOctadecylSilane 5 $\mu$ m (4.6 mm × 250 mm)
Wavelangth	:	220 nm	
Flow rate		:	1.5 ml per minute
Run Time		:	15 minutes
Injection Volume		:	20 µl

# Table 4: Percentage of Picroside II [Chromatogram No. 1-4]

Peak	Name of sample	Ret. Time	Area	Picroside II %
1	Root K 0	6.859	147927	0.54 % w/w
1	Genuine K 1	6.840	804434	2.90 % w/w





Chromatogram No 2 lateral roots (K 0)

# **Results and Conclusion**

The official part of use of *Katuki*is rhizome of *Picrorhizakurroa*Royle ex Benth. belongs to family Scrophulariaceae (API Part I Vol. II Page no. 91). During the market study it was found adulterated (7 to 47% table no 1), major part of adulteration was its lateral roots. Significant differences were found in the extractive values and total glycoside content between rhizomes and roots of *Katuki*(Table no 2). Thin Layer Chromatography of genuine katuki and its roots revealed difference in the number of spots and also

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difference in Rf values (Table no 3). The rhizomes of genuine sample of Katuki has more than 5 times amount of Picroside II in comparison with the roots (Table no 4). From the above results it clearly shows that the roots are the adulterant and it will surely reduce the therapeutic potential of Katuki. So the sample of Katukishould be free from these lateral roots.

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