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HPLC STUDY OF GENUINE SAMPLE OF KATUKI (*PICRORHIZA KURROA* ROYLE EX BENTH) AND IT'S MARKET SAMPLE

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ABSTRACT

Introduction The importance of this drug *Katuki* 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 the prime obstacle in excellence of the Ayurveda in this present Era. So it is mandatory to study the market samples to check the adulteration. Although chromatographic evaluation is overpriced criteria for the identification of drugs and developing countries like India may not afford these costly identification techniques. But, it may be essential sometimes in the competitive world. Hence chromatographic study is proposed to satisfy the needs of the country as well as the scientific society. **Materials and methods** Markets samples from six major markets of India and genuine sample of *Katuki* i.e. rhizomes of *Picrorhiza kurroa* from hills of Bhaderwa, dist. Kathua, State- Jammu and Kashmir were collected. Genuine sample of *Katuki*, lateral roots of Katuki and the most inferior quality sample from all the six markets were chosen for HPLC study. HPLC study was done in Amol Pharmaceuticals Private Limited Sitapura, Jaipur. Picroside II % in all the samples was calculated with the help of standard Picroside II. **Results** Genuine sample was having Picroside II 2.90% whereas Kolkatta sample and roots of *Katuki* had 1.47% and 0.54% respectively. **Discussion** HPLC results clearly shows that the genuine sample had almost double the amount of Picroside II as compared to the sample from kolkatta and 5.5 times more in comparison with the roots (Table-2). It also clearly shows that the roots are the adulterant and it will surely reduce the therapeutic potential of *Katuki*.

KEYWODS: Market study of Katuki, Adulteration, Picroside II, HPLC Study.

INTRODUCTION

Due to excessive deforestation and extreme weather conditions adulteration of Himalaya herbs is rampant. There are about 50 medicinal plants from the Himalaya which are extensively used in Ayurvedic formulations out of which 32 plants are scarce and found adulterated in the market study.[1] It is well known fact that today in the open market different plant parts are used in place of *Pushkarmool, Kutki, Kustha, Ativisha* etc. and like this so many other drugs are being adulterated and as such used by both physicians and pharmaceutical industries. Hence, the need for identification of these herbs through botanical surveys, pharmacognostic studies and the assessment of the quality of the material available in a particular area or market is essential. *Katuki* has been used in the indigenous system of medicine since a long time. The authentic source of the drug is rhizomes of *Picrorhiza kurroa* Royle ex Benth belongs to family Scrophulariaceae.[2] The plant is native of North-West Himalayas. 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.[3] 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 the prime obstacle in 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. *Gentiana kurroo* are used as substitute for *katuki*. Some other species of Gentiana as *Gentiana tenella, Gentiana decumbens, Gentiana lutea*, roots of *Actaea spicata, Cimcifuga foetida, Coptis teeta, Swertia chirata, Helleborus niger* are sold in the drug market under the name of *Katuki*.[4]

NEED OF STUDY

In ancient days Vaidyas usually go to forest to collect medicinal plants and prepare the medicines by themselves. Therefore there was not much documented information with regard to morphology and identification of medicinal plants in Ayurvedic texts. However due to extensive industrialization and urbanization it has become almost impractical for Ayurvedic physician to personally procure the authentic drugs and therefore totally dependent on raw

drug sellers and the middle men for procurement of medicinal plant raw materials. This exclusive dependence on traders has created serious malpractice of adulteration and selling of substandard medicinal plant raw materials in the market. So it is mandatory to study the market samples to check the adulteration. The chief methods employed in evaluating drugs are Organoleptic i.e. practical & on the spot tool, Physical & Chromatographic – Lab based tools, Experimental and Clinical-final confirmative tools. Practical, physiochemical studies are inadequate and chromatographic as well as finger prints are more beneficial in identification. Although chromatographic evaluation is overpriced criteria for the identification of drugs and developing countries like India may not afford these costly identification techniques. But, it may be essential sometimes in the competitive world. Hence chromatographic study is proposed to satisfy the needs of the country as well as the scientific society.

MATERIALS AND METHODS

Collection of Samples

Study was done in the year 2012-13 in National Institute of Ayurveda, Jaipur. For the market study six major markets were selected from all over India and markets samples were collected by scholar or by contacts and genuine sample of *Katuki* i.e. rhizomes of *Picrorhiza kurroa* was collected from hills of Bhaderwa, dist. Kathua, State- Jammu and Kashmir dated 11-09-2012. After collection Herbarium sheet was made and authenticated at IIIM Jammu with herbarium sheet no 17772. Markets samples were collected as such and not verified on spot. All the available grades were collected with the simple order method samples purchased or received from contacts were properly labeled, stored and subjected to investigation.

Fig 1: *Katuki* Rhizomes

Fig 2: Herbarium sheet.

FOREIGN MATTER

Method: Genuine and market sample of plant material was weighed by the electronic monopan balance and a thin layer of sample was spread on a white color sheet. By bull lens, the layer was examined for foreign matter. Foreign matter was separated and collected in to another paper. Plant material was recollected and weight again. Percentage of foreign matter in relation to the total quantity of plant material was calculated.

Fig 3: *Katuki* lateral roots.

HIGH PREFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Samples chosen for HPLC study were the lateral roots of *Katuki* (K 0) which found admixture in large amounts with the rhizomes, genuine samples of *Katuki* (K 1) and Kolkata market sample (K 5). From all the market samples, Kolkata sample was chosen because during organoleptic study it is found to be the most inferior quality sample. HPLC study was done in Amol Pharmaceuticals Private Limited Sitapura, Jaipur. Crude samples were given to the Amol pharmaceuticals in an air tight poly bag. Picroside II % in all the samples was calculated with the help of standard Picroside II.

Process followed by Amol Pharmaceuticals

Preparation of Mobile phase: 150 ml of acetonitril, 100 ml methanol and 750 ml of aqueous phosphoric acid solution pH 3 were mixed properly and filtered through 0.45 µm membrane filter and degas. **Preparation of Standard solution**: Standard equivalent to 5 mg of Picroside II mixed with 25 ml of mobile phase in a 100 ml volumetric flask. Sonicate the solution to dissolve the content. Make up 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 µl) of standard and sample preparation. Record the chromatograms calculate the average area and finally calculate the percentage of Picroside II.

Instrument setup conditions

Instrume: High Performance Liquid Chromatography equipped with UV- Visible Detector.

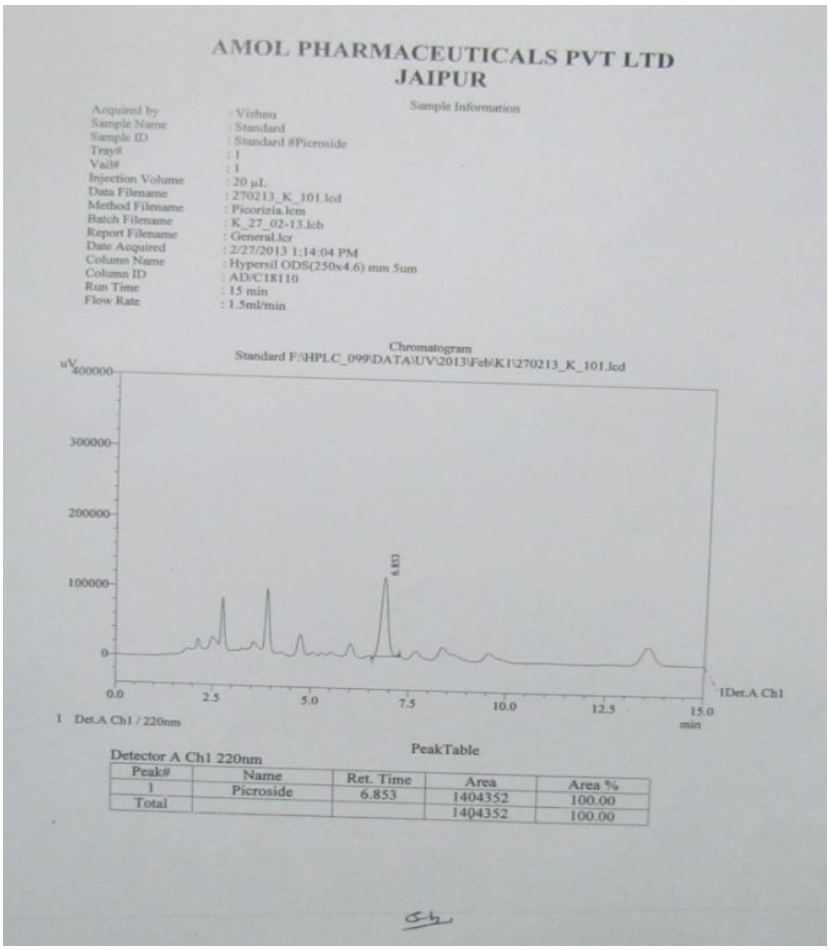
Column: Hypersil Octadecyl Silane 5 µm (4.6 mm × 250 mm).

Wavelength: 220 nm

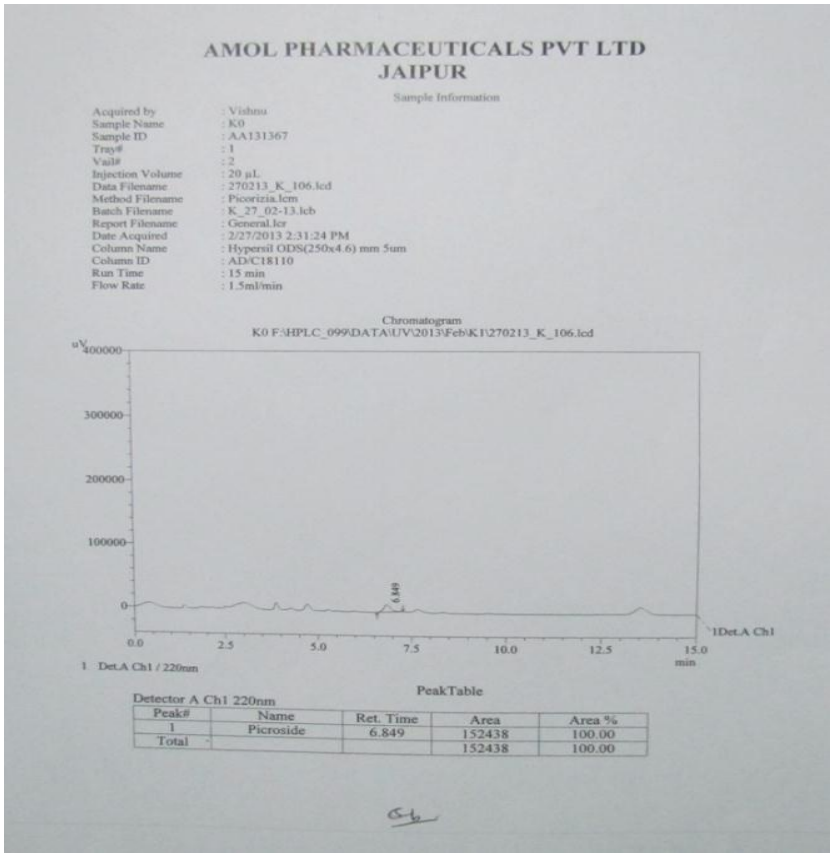
Flow rate: 1.5 ml per minute.

Run Time 15 minutes.

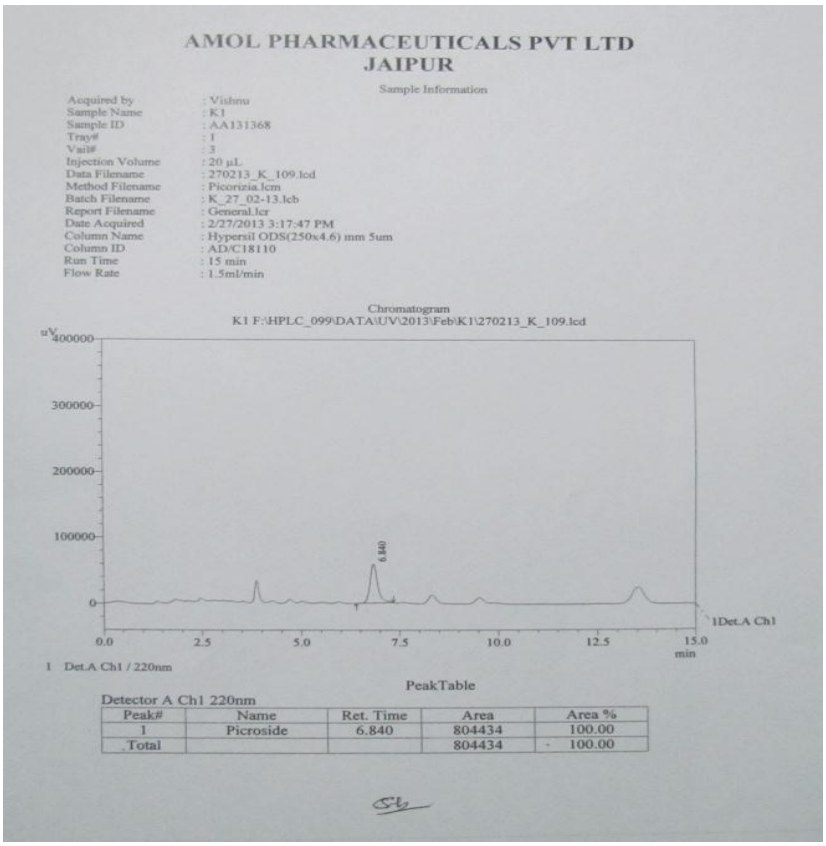
Injection Volume: 20 µl.



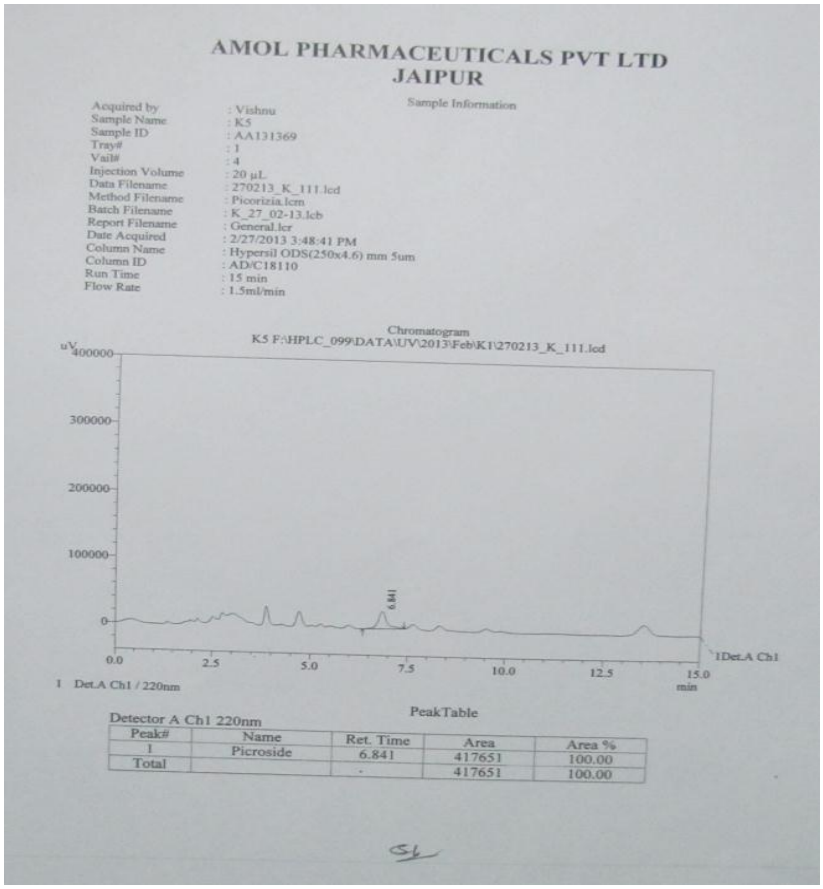
Chromatogram No 1 Standard Picroside II.



Chromatogram No 2 lateral roots (K 0).



Chromatogram No 3 Genuine *Katuki* (K 1).



Chromatogram No 4 Market Sample (K 5).

RESULTS

According to the API, in samples of *Katuki*, foreign matter should not be more than 2%. Only genuine collected sample was having foreign matter less than the standard value. All the markets samples were having foreign matter much more than the standard value. Results are given in Table 1. Maximum was found in the sample of Kolkatta (47.058%) (). HPLC study- Genuine sample was having Picroside II 2.90% whereas Kolkatta sample and roots of *Katuki* had 1.47% and 0.54% respectively.

Table 1: Foreign matter of *Katuki* and its market samples.

S.no	Sample	Total weight	Foreign matter	Percentage
01	Genuine	350 gms	4 gms	01.142%
02	Kullu	502 gms	92 gms	18.326%
03	Amritsar	501 gms	45.70 gms	09.140%
04	Jaipur	500 gms	38.80 gms	07.761%
05	Mumbai	500 gms	100 gms	20.000 %
06	Kolkata	510 gms	240 gms	47.058%
07	Kochin	505 gms	63.42 gms	12.558%

Foreign matter- Standard- Not more than 2%.[2]

Table 2: Percentage of Picroside II [Chromatogram No. 1-4].

Peak	Name of sample	Retention Time	Area	Picroside II Percentage
1	Root K 0	6.859	147927	0.54 % w/w
1	Market sample (Kolkata K 5)	6.841	417651	1.47 % w/w
1	Genuine K 1	6.840	804434	2.90 % w/w

DISCUSSION

After collection of samples it had been observed that all the samples, taken from major whole sale markets of Ayurvedic drugs, were having all diagnostic characters & same appearance as that of genuine sample of rhizome of *Picrorhiza kurroa*. Only difference was the quality of the samples, difference in the size of rhizomes and amount of mixing of adulterant. During the market study we had found the roots, stems pieces, some dried leaves of *Katuki* i.e. *Picrorhiza kurroa* mixed along the official part i.e. rhizome of the same plant. Only in Mumbai sample we had found pedicles of other plant which could be the part of *Cinnamomum tamala.* According to the API, in case of *Katuki,* foreign matter should not be more than 2%. Only genuine collected sample was having foreign matter less than the standard value. All the markets samples were having foreign matter much more than the standard value. On examination lateral roots of *Katuki* were found in large quantity as a foreign matter. For HPLC study three samples were chosen viz. roots of the *Katuki* (K 0) commonly admixture in *Katuki* as an adulterant, rhizomes of genuine samples (K 1) and rhizomes of Kolkatta (K 5). All the samples were compared with the standard Picroside II as per the protocol of APPL. HPLC results clearly shows that the genuine sample had almost double the amount of Picroside II as compared to the sample from kolkatta and 5.5 times more in comparison with the roots (Table-2). The differences in the Picroside II content between the genuine and Kolkatta market sample may be due to the difference in species or might be due to difference in cultivation or harvest practices.

CONCLUSION

The demand of *Katuki* has been increased remarkably in the last decade and also adulteration. All the market samples had high amount of foreign matter mostly the roots and leaves of the same plant. HPLC reports showed that its roots contain Picroside II six times less than the genuine samples. It also clearly shows that the roots are the adulterant and it will surely reduce the therapeutic potential of *Katuki.* So the sample should be free from these root part.

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