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Evaluation of market brands of *Keshar* for its authenticity by pharmacopoeial standards

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Abstract
Background: *Keshar* is very well known plant used since time immemorial in traditional systems of medicine all over the world. When it is related to the authenticity of the samples available in market whether they are genuine or not every person doubtful regarding reliability of the available market samples. To check out this issue we have collected three samples of very well-known brands namely sample 1 from Government shop of Lucknow, sample-2 of Natural brand and sample-3 of baby *Keshar* brand.
Aim: To check the authenticity of three market samples of *keshar* for checking its genuineness. There is rapid adulteration in the *Keshar* due to the high coast for money business hence assessment of correct sample for the use of medicinal purpose is necessary.
Material and Method: for the convenience we have named these samples as K1, K2, K3.
We have followed the standard parameters mentioned in Ayurvedic pharmacopoeia of India Part I, Vol-4 for authenticating all the three samples of *Keshar* like microscopic study, color test by using various chemicals, HPTLC and UV Spectrophotometry analysis.
Result: Test samples K1, K2, K3 all found genuine on the parameters of Ayurvedic pharmacopoeia of India as well as by testing with the HPTLC and UV Spectrophotometry.

Keywords: API, authenticity, keshar, market samples

Introduction
Keshar is well known medicinal plant as well as spice used in Indian traditions. It is also called as *Kumkuma* which consists of dried style and stigma from the flowers of *Crocus sativus* Linn. *Keshar* belongs to the family Iridaceae. It is a small bulbous perennial, 15 to 25 cm high and cultivated by corms in the Kashmir valley, specially in the Pampor plateau, at about 1600 m [1].
It has a light purple colour dioecious flower with three vivid crimson stigmas and three yellow stamens. The three crimson stigmas of it are the most valuable part of the plant. These stigmas are rich in aroma, flavor and colour. Keshar is used as aromatic or coloring agent in various food preparation. also used in pharmaceutical and cosmetic manufacturing.[2]
Keshar has pharmacological attributes like *Katu, Tikta Rasa, Snigdha* in *Guna, Sheeta virya* and *Katu Vipak* It posses *Vatahar, Varnya, Vishaghna, Sleshmahar, Rasayana, Jantuhara* pharmacological actions. *Keshar* posssess various therapeutic applications such as *Chardi, Kasa, Vrana, Vyanga, Shioroga, Drishti Roga, Kantha Roga, Sidhma, Mutrashotha, Udavartta, Mutraghata, Suryavartta, Ardhava Bhedaka.* Therapeutic dose of *Keshar* is 25-50 mg [3].
Nowadays, topics such as food authenticity, genuineness and the detection of adulteration in food products, usually economically motivated, are increasingly important for consumers, regulatory agencies and the food industry [4].
In spite of medicinal use *Keshar* is renowned as the most expensive spice; its market price ranks among the highest in foods, reaching 20,000 €/kg and more for some PDO (Protected Designation of Origin) productions in 2015, and it is the highest priced high value agricultural product (HVAP) in the world. Saffron can be found on the market in the form of entire dried stigmas or as a finely-ground powder. Among the major candidates for adulteration, saffron is one of the most targeted foods and spices. As a consequence, adulteration represents a real and major concern for the saffron market, and such practice is more often performed in ground stigmas.
Presently, within the most-frequently reported plant materials used to adulterate saffron such as cut and/or dyed C. sativus stamens; safflower and calendula petals (*Carthamus tinctorius* L. and *Calendula officinalis* L.); curcuma powdered rhizomes (*Curcuma longa* L.); gardenia yellow from Gardenia jasminoides Ellis fruits; and dye extracted from the flowers of Buddleja officinalis Maxim. Additionally, commercial safflower and curcuma are often mislabeled, using the name "saffron" and the supposed country of origin to mislead consumers [5].

Material and Methods

1 gram each of the *Keshar* sample K1, K2 and K3 were collected by ourselves from various retailer sources of Varansi, UP, India for K2 and K3 were as K1 was collected from market of Aminabad Lucknow, UP during the month of March 2017. All chemicals used for testing were from Merck® Germany.

The parameters given in Ayurvedic pharmacopoeia of India Vol 4, Part I are mentioned as under and analysis on the same parameters is done which are tabulated in table no 1 [6].

Absence of Fixed oil or glycerin: Pressing between clear filter paper, the paper does not display translucent oily spots.

Loss on drying: Loses not more than 14 per cent of its weight, when dried at 100°C.

Ash: Not more than 7.5 per cent.

Acid-insoluble ash: Not more than 1 per cent.

Table 1: Physico- chemical analysis of *Keshar* samples KI, K2, K3

S. No	Sample	Loss on drying	Ash value	Acid-insoluble ash
1.	K1	3.3%	1.3%	1.02%
2.	K2	4.09%	1.23%	1.1%
3.	K3	4.23%	1.56%	1.04%

Chemical colour Tests

On Macerat ing 10 mg sample in 5 ml of alcohol (95 per cent) a distinct greenish yellow colour is imparted to the liquid; with corresponding quantities of *Keshar* (fig. no. 1). In ether or chloroform the solvents remain almost colorless (fig. no.2); so also with xylene, benzene (fig. no.3) [6].

Sulphuric Acid Test: The Carotenoid pigments like Crocin, Crocetin and Picocrocin reacts with the sulphuric acid to give bluish colour immediately, which finally changes to Violet to red (fig. no 4), the reaction is due to the hydrolysis of the Carotenoid esters [7, 8].

Macroscopic Study

Yellowish style, broken or intact along with trifid stigma; stigma is dark red or reddish-brown, cornucopia shaped, with fimbriate margin, and about 25 mm long; broken style are very thin, upto about 10 mm long; odour was strongly aromatic with taste slightly bitter [6].

Microscopic Study

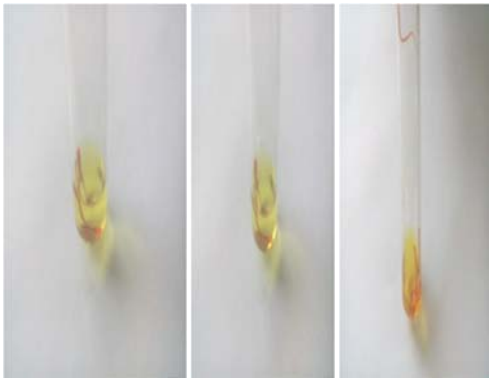
Stigma composed mostly of elongated, thin-walled, parenchyma cells containing colouring matter; at the upper end numerous cylindrical papillae or trichomes up to 150 microns long present; pollen grains, a few, spherical, nearly smooth, from 40 to 120 microns in dia; occasionally germinated and exhibiting pollen tubes. soaked drug is examined under a lens or microscope, the stigmas found either separate or united in three to the apex of yellowish styles. Each stigma is about 25-mm long and has the shape of a slender funnel, the rim of which is dentate or fimbricate (fig.no.5a.i- fig.no.5c.ii) [9].

Assay: Weighted accurately 0.1 gm *Keshar* of each batch in moderately fine powder and macerated at room temperature in 100 ml of water for 3 hours with frequent shaking. It was Filter immediately, sufficient water was added through the filter to make 100 ml. 10 ml of this filtrate, Was diluted with 100 ml with water. Immediately colors were compared of this solution in Nessler tubes or in a colorimeter, with the colour of N/100 potassium dichromate. The color of the solution approximates that of the N /100 potassium dichromate, and the strength of the color is not less that of an equal depth in mm of the N /100 potassium dichromate (fig.no. 6) [6].

High Performance Thin Layer Chromatography (HPTLC)

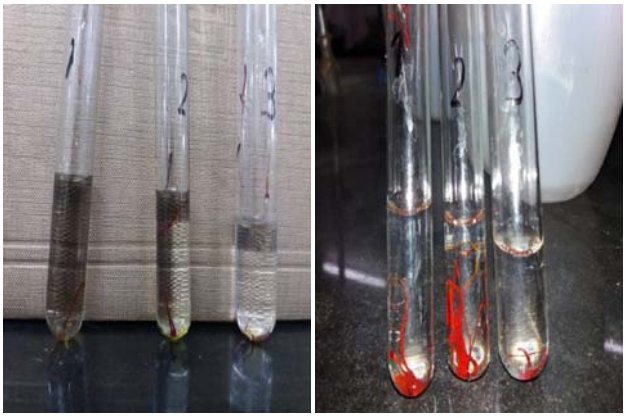
High Performance Thin Layer Chromatography (HPTLC)-Chromatography was performed on 10x 10cm silica gel 60 F254 HPTLC plate. 10 mg of each samples K1,K2,K3 were moisten with few drop of water and taken in 1 ml of AR methanol and 10 mg/ml samples was prepared 10µl of samples were spotted on TLC plate. The TLC tank Saturated with solvent system of Ethyl acetate: methanol 8:2 for about 30 minutes the plate was run in this tank for about 10cm and spots were clearly visible at Rf about 0.2, 0.5 and 0.7 were observed (fig.no. 7). 3

Crocin and crocetin are the glycoside compounds of saffron. Crocin has maximum absorbance around 450 and 433nm in methanol media. All the three samples K1.K2, K3 eluted at above different Rf values from preparative TLC Plate were taken in methanol and UV absorption was recorded.



K1 K2 K3

Fig 1: Chemical color test of ethanol

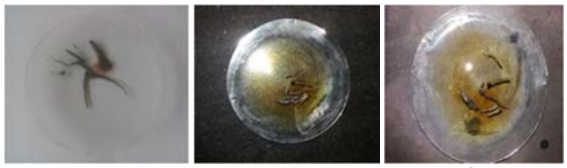


Samples KI, K2 and K3 in ether | Samples KI, K2 and K3 in chloroform

Fig 2: Chemical color test in ether and chloroform

Samples KI, K2 and K3 in Benzene Samples KI, K2 and K3 in Zylene

Fig 3: Chemical color test in Benezene and Zylene



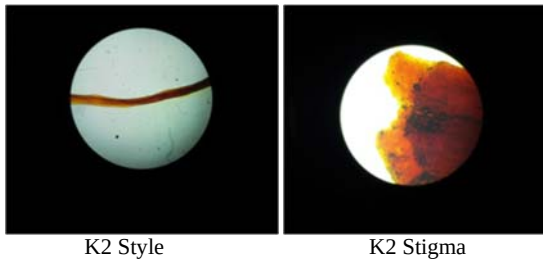
K1 K2 K3

Fig 4: Sulphuric acid test

Microscopic study

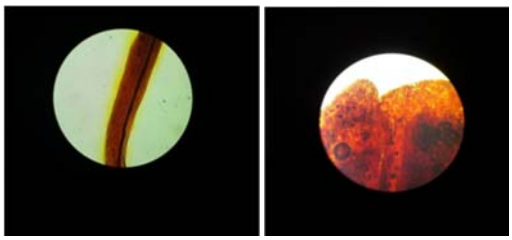
K1 Style K1 Stigma

Fig 5a.i **Fig 5a.ii**



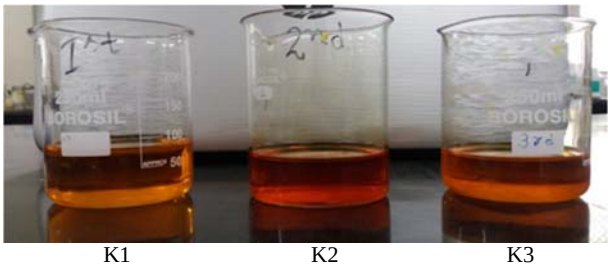
K2 Style K2 Stigma

Fig 5b.i **Fig 5b.ii**



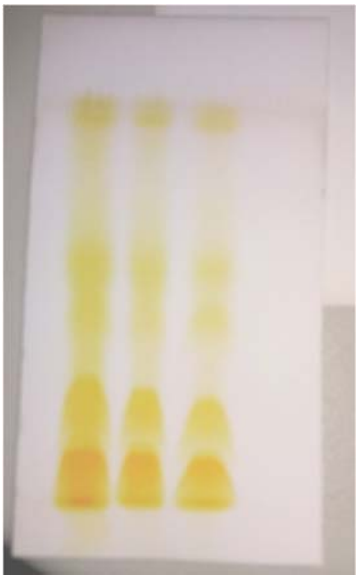
K3- style K3- stigma

Fig 5c.i **Fig 5c.ii**



K1 K2 K3

Fig 6: Color Assay with potassium dichromate



K1 K2 K3

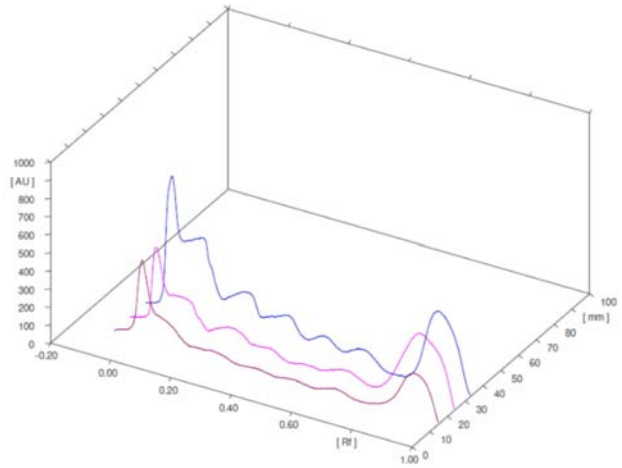


Fig 7: High Performance Thin Layer Chromatography for sample K1, K2, K3

Result: All the test parameters followed to check the authenticity of the three samples of the keshar on the line of physico-chemical tests, colour tests, microscopic and macroscopic observations, HPTLC and spectroscopic study are found approximately same regarding the observation in colour change, values of tests and structures. In the present study the market samples of Keshar K1, K2 and K3 all are found authentic on the parameters of Ayurvedic Pharmacopoeia of India, part I Volume 4.

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