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Rapid Detection of Adulteration in Indigenous Saffron of Kashmir Valley, India

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Abstract

Saffron is the important spice of our food. It is the dried stigma of the flower Crocus sativus L., which is the most economic part of the plant. The main aim of the research is to examine the saffron (Kong) of the Kashmir (JandK), India, which is the largest producer in the country. Due to its high price and demand in the world, it has been adulterated in a number of ways to mislead the consumers. The saffron samples were subjected to various chemical and analytic techniques like TLC and Spectroscopy in order to explore the rapid methods of detection and identification of pure and fake saffron.

Keywords: Saffron, thin layer chromatography and spectroscopy.

Introduction

Saffron is the spice which is important recipes of our food. It is the dried stigma of flower Crocus sativus L., also called as saffron crocus; it belongs to the Genus Crocus, Order Lilacs and the Family Iridaceae[1]. It is the perennial herbaceous plant with height of 25cm, which has a fleshy bulb called as the corn of about 3cm in diameter, from which the leaves and the flowers germinate[2]. It is 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[3]. These stigmas are rich in aroma, flavor and colour, used as aromatic or colouring agent in various food preparation[4]. It is also used in pharmaceutical and cosmetic manufacturing [5].

Kashmir (J and K) is the largest producer of high quality Saffron (Kong) in the Country[6]. The high price and large demand often result adulteration of it. Adulteration of the Saffron in the present era is heinous white collar crime as it has a direct effect on the economy of the country and has serious health impacts[7,8]. Forensic examination of adulterated saffron is always a challenge to the forensic scientist to detect and determine the degree of adulteration in it.

Material and Methods

1-5 gram of the Saffron (Kong) are collected from various retailer sources of districts of the Kashmir Division, JandK randomly during the month of May 2014 (figure-1) and were examined in FSL JandK according the guidelines given in the ISO (E) 3632-2 (2010)[9]. All chemicals used are from Merck® Germany.

Chemical colour Tests: Pure saffron gives yellow coloration in the solution of the water and methanol due to Carotenoid pigments-Crocin and Crocetin, but not in benzene, Xylene, ether, chloroform and toluene solution (figure-2). Fake saffron imparts varieties of colour in different solvents (figure-3)[10].

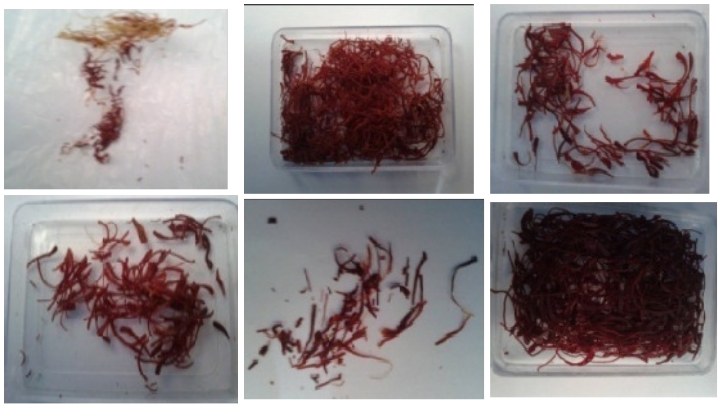


Figure-1
Saffron of Kashmir, JandK, India

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 (figure-4), the reaction is due to the hydrolysis of the Carotenoid esters[11]. The fake saffron produces yellow colour only (figure-5)[12].

Thin Layer Chromatography (TLC): Sample preparation: Methanol extract of equal aliquots of pure and fake saffron (2-stigma thread of saffron in 3ml of methanol).

Stationary phase: A standard Pre-coated TLC plates of Silica gel G 60 (12×5); Merck, Germany. Mobile Phase: 1)1-Butanol, Acetic acid, Water (4, 1, 5 by v/v. Upper phase). 2) Ethyl Acetate, Iso-Propanol, Water (6.5, 2.5,1 by v/v).

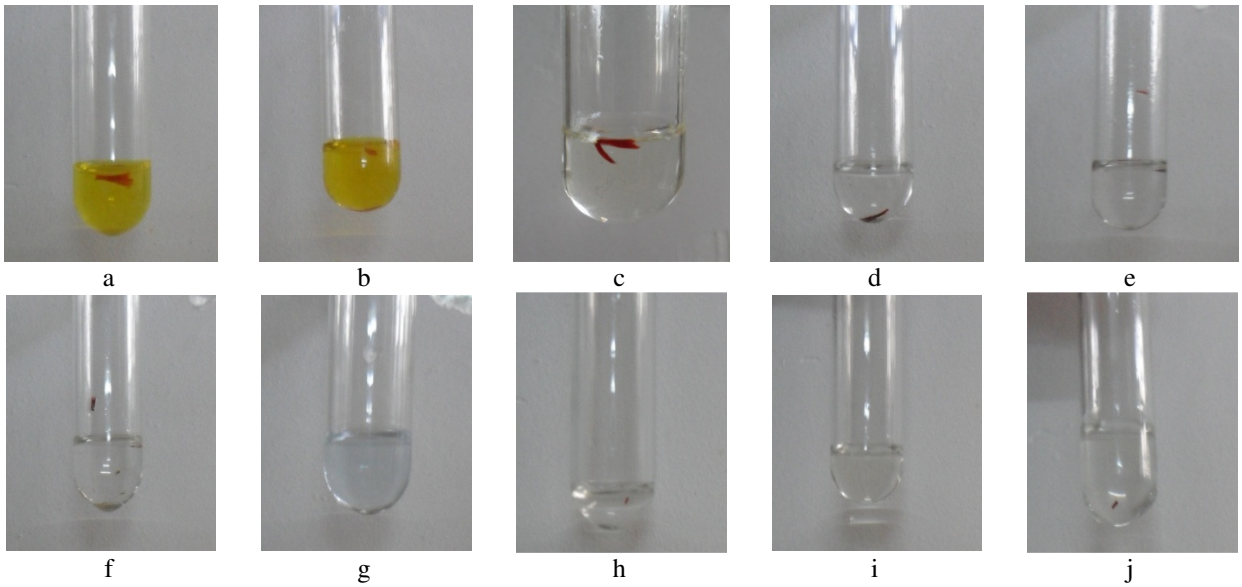


Figure-2

Pure saffron samples imparts yellow colour (a) water, (b) methanol and No coloration (c) benzene, (d) ether, (e) chloroform, (f) Xylene, (g) Toluene, (h) Iso-Propanol, (i) Iso-Butanol, (j) 1-Butanol

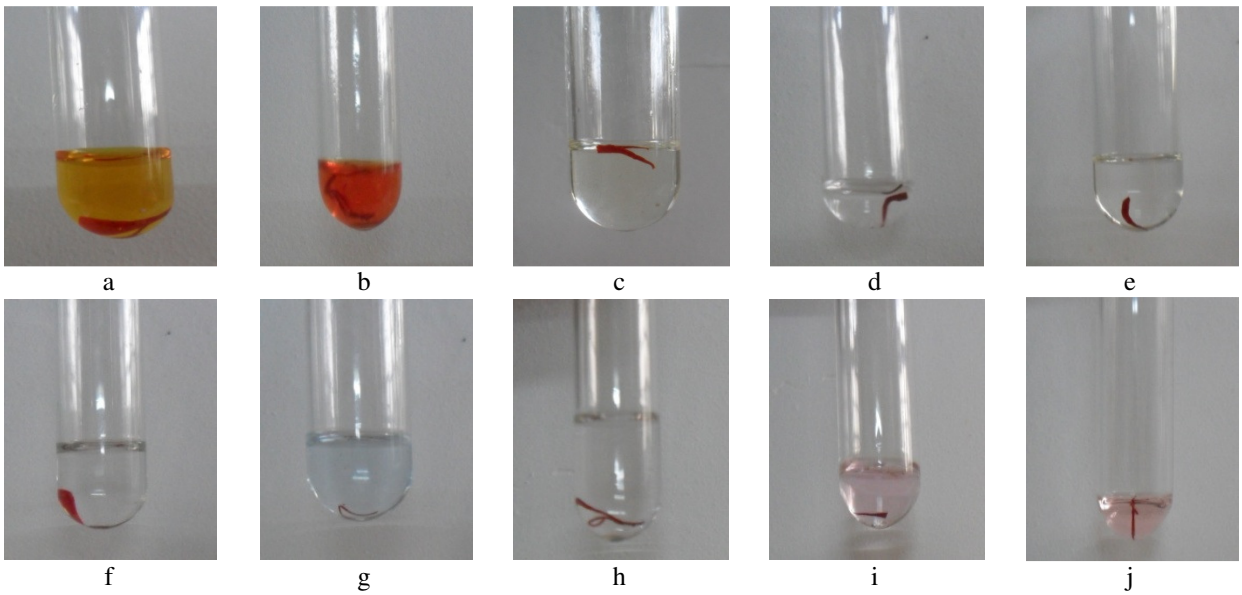


Figure-3

Fake saffron samples imparts light red colour (a) Water, dark red (b) methanol and No coloration (c) Benzene, (d) ether, (e) chloroform, (f) Xylene, (g) Toluene, (h) Iso-Propanol, and light violet color in (i) Iso-Butanol and (j) 1-Butanol

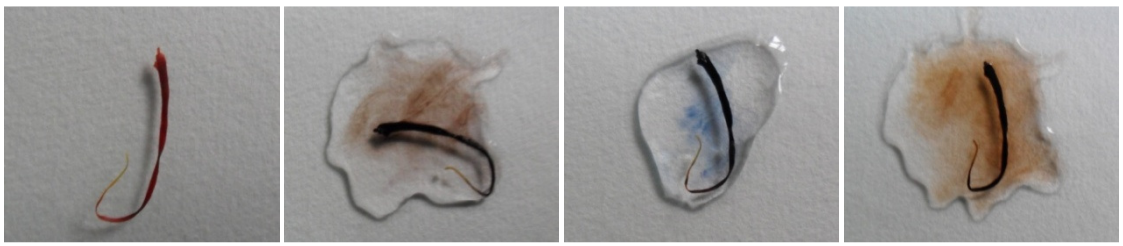


Figure 4

Series of reaction of pure saffron with sulphuric acid

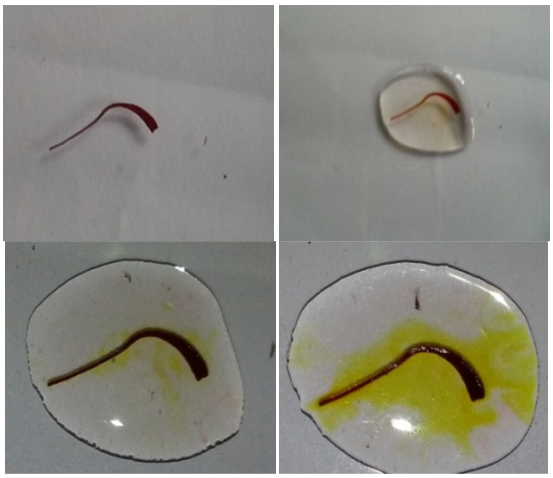
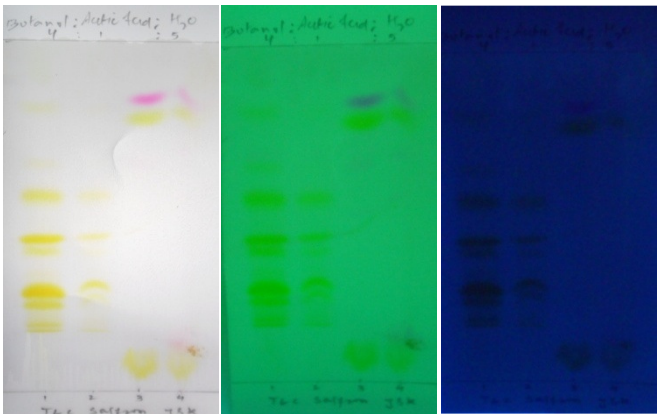
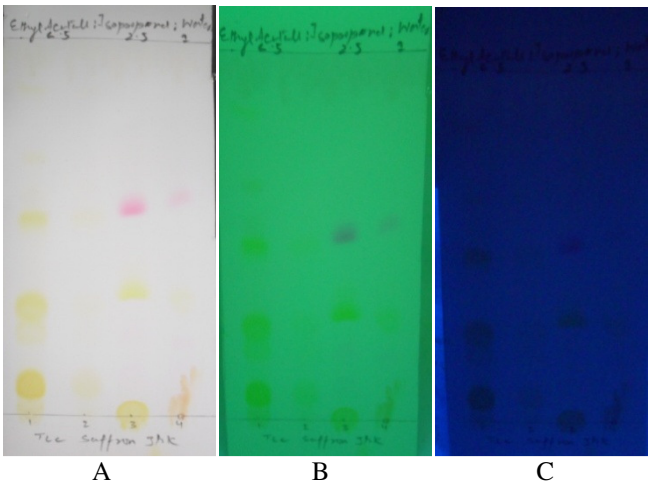


Figure-5
Series of reaction of fake saffron with sulphuric acid



A B C

Figure-6
TLC analysis of pure and fake saffron using solvent system 1-Butanol, Acetic acid, Water (4.1.5)



A B C

Figure-7
TLC analysis of pure and fake saffron using solvent system Ethyl Acetate, Iso-Propanol, Water (6.5, 2.5, 1)

A. Normal daylight visualization, trace 1-shows 9 yellow spots of Crocin and crocetin of pure saffron-I, trace 2 shows 6 yellow spots of pure saffron-II, trace 3 and 4 shows 4 violet and 2 yellow spots of fake saffron. B. Short wave (254nm) visualization of TLC plate, trace 1-2 gives light green fluorescence; trace 3-4 gives yellowish and pink fluorescence C. Long wave (365nm) visualization of TLC plate.

A Normal daylight visualization, trace 1-shows yellow spots of Crocin and crocetin of pure saffron-I, trace 2 shows 5 yellow spots of pure saffron-II, trace 3 and 4 shows violet and two yellow of spots fake saffron. B. Short wave (254nm) visualization of TLC plate, trace 1-2 gives light green fluorescence; trace 3-4 gives yellowish and pink fluorescence C. Long wave (365nm) visualization of TLC plate.

Spectroscopy: The saffron samples were examined under UV-Visible Spectrophotometer SPECORD® 100 and the maximum Wavelength and the absorbance were calculated.

Result and Discussion

The saffron are examine according to the ISO (E) 3636-29 (2010), and their nature of originality determined. The main colouring part of the saffron are Carotenoid (Crocin, Crocetin) which are responsible for imparting yellow colour in various Polar and Non-polar solvents[14]. Hydrolysis of Carotenoids by sulphuric acid yields blue colour immediately. The chromatography separation of saffron produces continuous 6-10 yellow spots.

In contrast, the fake saffron fails the solubility test and yield yellow colour instead of blue when hydrolyzed by sulphuric acid and its chromatography separation by TLC produces combination of yellow (2) and violet (2-4) spots.

Table-2
Pre-treatment of saffron sample[13]

Saffron	Quantity	Solvent (methanol)
Pure	1µ gram	500 µL
Fake	1µ gram	500 µL

Table-3
Calculation of maximum wavelength (λmax)

Saffron	wavelength	Absorbance
Pure	255.57	0.2832
	433.74	0.9608
	458.93(λmax)	0.8535
Fake	204.86	0.2065
	254.45	0.1286
	371.83	0.929
	429.29	0.1579
	432.42(λmax)	0.159

Table -1
Rf and hRf of the pure and fake Saffron samples in different TLC Solvent system

S. No	Solvent System	Saffron	No. of Spots	Colour of Spots	Intensity	Rf	hRf
1	1-Butanol, Acetic acid, Water (4,1,5) Upper	Pure-I	10	1.yellow	Significant	0.19	19
				2.yellow	Significant	0.21	21
				3.yellow	Significant	0.26	26
				4.yellow	Significant	0.3	30
				5 yellow	Significant	0.42	42
				6.yellow	Significant	0.46	46
				7.yellow	Significant	0.58	58
				8.yellow	Fade	0.68	68
				9.yellow	Fade	0.83	83
				10.yellow	Fade	0.94	94
		Pure-II	6	1.yellow	Significant	0.19	19
				2.yellow	Significant	0.21	21
				3.yellow	Significant	0.26	26
				4.yellow	Significant	0.3	30
				5 yellow	Significant	0.42	42
				6.yellow	Significant	0.46	46
		Fake-I	6	1.yellow	Significant	0.01	0.8
				2.violet	Fade	0.15	15
				3.violet	Fade	0.6	6
				4.violet	Fade	0.7	7
				5.yellow	Significant	0.81	81
				6.violet	Significant	0.87	87
		Fake-II	6	1.yellow	Significant	0.01	0.8
				2.violet	Fade	0.15	15
				3.violet	Fade	0.6	6
				4.violet	Fade	0.7	7
				5.yellow	Significant	0.81	81
				6.violet	Significant	0.87	87
2	Ethyl Acetate, Iso-Propanol, Water (6.5,2.5,1)	Pure-I	6	1.yellow	Significant	0.01	0.8
				2.yellow	Fade	0.21	21
				3.yellow	Significant	0.28	28
				4.yellow	Significant	0.51	51
				5 yellow	Fade	0.66	66
				6.yellow	Fade	0.85	85
		Pure-II	4	1.yellow	Significant	0.01	0.8
				2.yellow	Fade	0.21	21
				3.yellow	Significant	0.28	28
				4.yellow	Significant	0.51	51
		Fake-I	4	1.violet	Fade	0.18	18
				2.violet	Fade	0.21	21
				2.yellow	Significant	0.32	32
				3.yellow	Significant	0.54	54
		Fake-II	4	1.violet	Fade	0.18	18
				2.violet	Fade	0.21	21
				2.yellow	Significant	0.32	32
				3.yellow	Significant	0.54	54

Conclusion

In the present research the saffron of Kashmir J and K are examined to explore the rapid method for identification of pure and fake saffron. The forensic examinations of adulterated saffron encounter are often a challenge to forensic examiner. Thus having various chemical examinations for saffron, the sulphuric acid chemical test is reliable, rapid and sensitive method to find originality of the saffron in quick time. Further, Thin Layer Chromatography serves as best techniques to decide purity of saffron to highest degree.

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