



iJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 3

Issue: IV

Month of publication: April 2015

DOI:

www.ijraset.com

Call:  08813907089

E-mail ID: ijraset@gmail.com

Forensic Analysis of the Saffron: Rapid Authenticity Testing

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Abstract-Saffron is the valuable spice of our food. It is the dried stigma of the flower *Crocus sativus* Linnaeus, the most economic part of the plant. Due to its high price and demand throughout world, it became frequent object of adulteration and gained attention to dishonest producers. The main aim of the research is forensic analysis of saffron. The various chemicals methods, microscopic and analytical techniques like TLC & UV-Visible Spectrophotometry were performed on the saffron samples to explore rapid authenticity testing of genuine saffron.

Keywords: Forensic, saffron, adulteration, frauds, testing

I. INTRODUCTION

Saffron is the processed stigma of the flower of *Crocus sativus* Linnaeus. It is having tremendous value in Spices as of its antiquity for colour, flavor and medicinal properties [1]. Saffron is a native of the southern Europe and is now produced by many countries like Spain, France, Italy, Germany, Iran and India [2]. In India Saffron producing state are Gujarat, Himachal Pradesh and Jammu and Kashmir [3]. In Jammu and Kashmir, Pampore is famous for production of high quality mogra saffron [5]. The high price and demand in the world, the frauds and the adulteration of saffron are very common [6]. The Forensic examination of the saffron and its products are a challenge to the examiners for determination of authenticity and detection of frauds.

II. MATERIAL AND METHODS

A. Sampling

3-gram of high quality mogra saffron (Genuine Sample) collected from the saffron market of the Pampore district Pulwama, while as the fake samples (assumed to be fake) are collected from the local market of Kashmir (fig. 1).



Fig. 1 Genuine (left) and Fake (right) saffron samples

B. Chemicals

All the chemical are analytical and laboratory grade and obtained from the Merck® Germany.

C. Preliminary Examination

The methodology adopted for Forensic analysis of the saffron are according to ISO (IEC) 3632(2) 2010,

1) *Physical Examination:* The physical examination includes colour, condition and texture of the saffron thread (table-1) [7].

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Saffron	Colour	Condition	Texture
Genuine	Crimson Red	Dry	Smooth
Fake	Crimson Red	Moist	Smooth

Table-1 Physical examination of saffron

- 2) *Colour Test*: Genuine saffron impart pale yellow colour in water, while as fake saffron imparts yellow colour quickly in water (fig. 2) [8].



Fig. 2 Colour test of saffron samples

- 3) *Cotton Colour Test*: Genuine saffron yields pale yellow-orange colour, while fake saffron imparts yellow colour in cotton soaked with polar solvent like water, methanol etc. (fig. 3).



Fig. 3 Cotton Color Test of genuine (left) and fake (right) saffron

- 4) *Whatman paper Test*: Rubbing the fake saffron thread between whatman Paper No.1 soaked with polar solvents like water etc. yields yellow colour quickly as compared to genuine saffron (fig. 4).

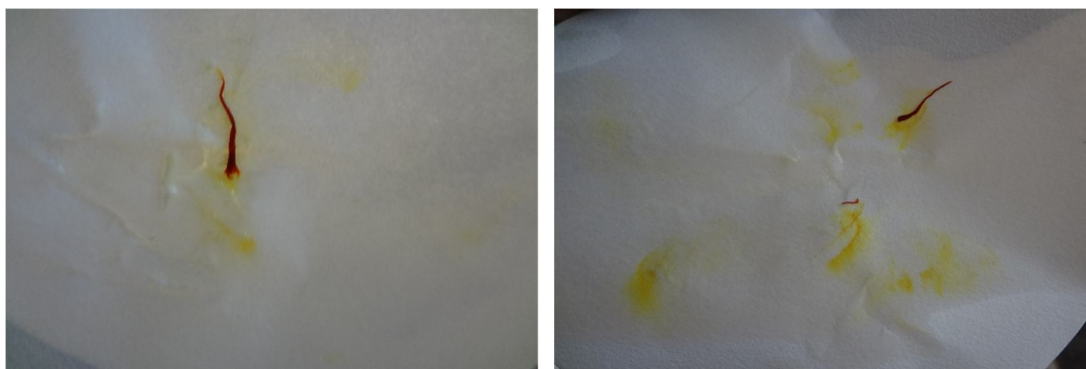
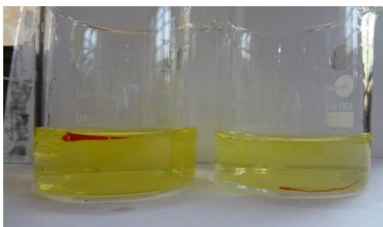


Fig.4
Whatman
colour test
of genuine
(left) and
fake (right)
saffron

- 5) *Flotation Test*

The genuine saffron floats in water while the fake saffron sinks (fig. 5).



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FIG. 5 FLOTATION TEST OF SAFFRON

6) *Chemical Examination:*

Pigment Extraction: Pigments were extracted from the saffron using water, followed by water bath in watch glass to get dried pigments.

a) *Reaction with Sulphuric acid:* Genuine saffron yields Indigo-blue colour immediately on application of sulphuric acid to the extracted dried pigments (fig. 6) [9].

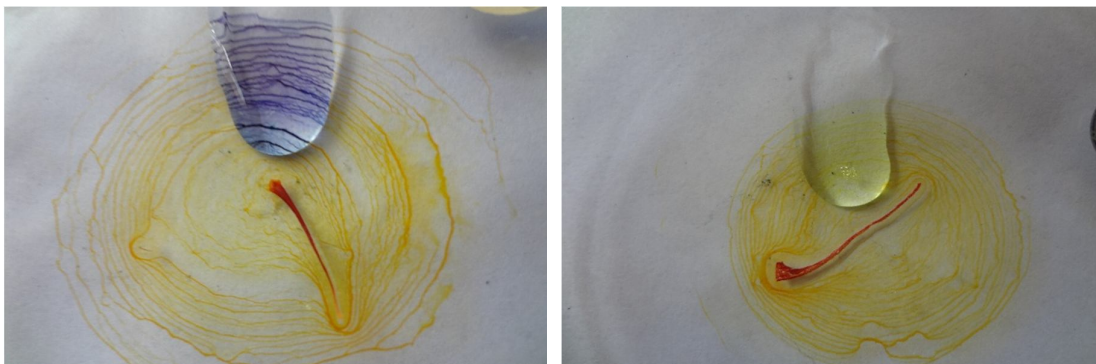


Fig. 6 Reaction of saffron pigment with sulphuric acid

1. *Reaction with Nitric acid*

The pigment of genuine saffron imparts light blue colour on reaction with nitric acid (fig. 7)

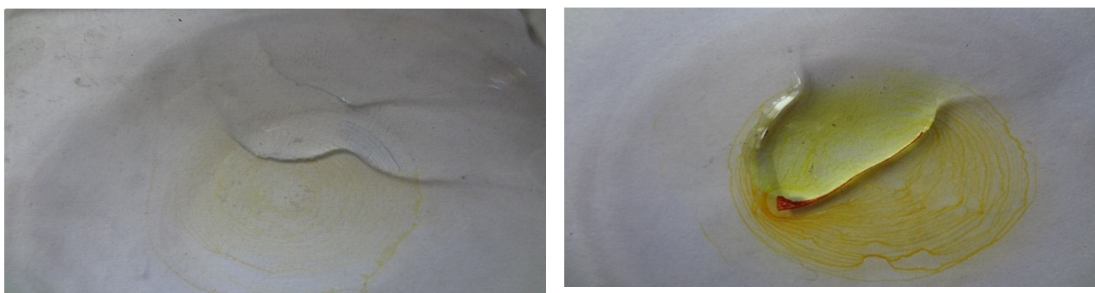


Fig. 7 Reaction of saffron pigment with Nitric acid

2. *Reaction with Ammonia*

Pigments of genuine saffron imparts yellow orange colour with ammonia while as the fake one imparts light brown colour (fig. 8).

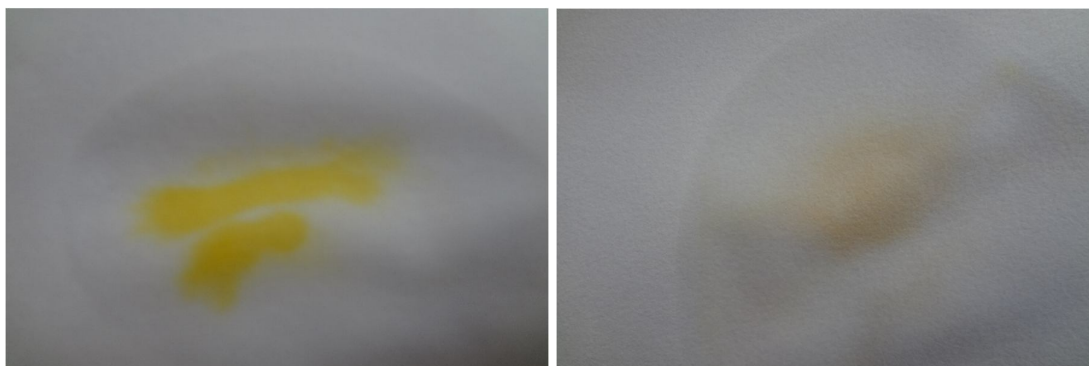


Fig. 8 Reaction of saffron pigment with Ammonia

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b) Instrumentation Analysis

1) Thin Layer Chromatography

A Pre-coated TLC silica gel G of Merck Germany developed in Chamber containing solvent system n-Butanol, Acetic acid, Water (4, 1, 5 by v/v upper phase) [10]. For analysis each lane received 1µl spot of alcoholic extract (methanol) of the saffron. After 2 hour development spots were visualized in daylight and UV chamber (fig. 9). The genuine saffron produces continuous series of yellow spots (9), while as the fake saffron produces coloured spots (yellow-2 and violet with pinkish shade-7).



Fig. 9 TLC of saffron pigments

A. Spectroscopy

Calculation of the maximum wave length of alcoholic extract of saffron samples using Spectrophotometer Specord® 100 (fig. 10) [11].The result obtained are in table-2.

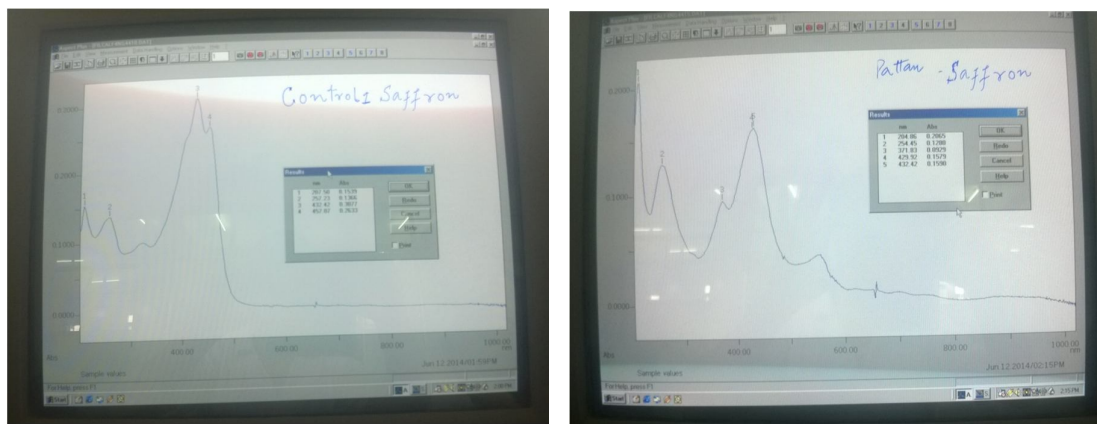


Fig. 10 UV-Visible spectrograph of genuine (left) and fake (right)

Saffron	Wavelength	Absorbance
Genuine	255.57	0.2832
	433.74	0.9608
	458.93	0.835
Fake	204.86	0.2065
	254.85	0.1286
	371.83	0.929
	429.29	0.1579
	432.42	0.159

Table-3 Calculation of maximum wavelength

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B. Microscopy

Observation of anatomical characteristics in saffron samples after depigmentation in polar solvents like methanol, under compound light microscope (fig. 11) [12].

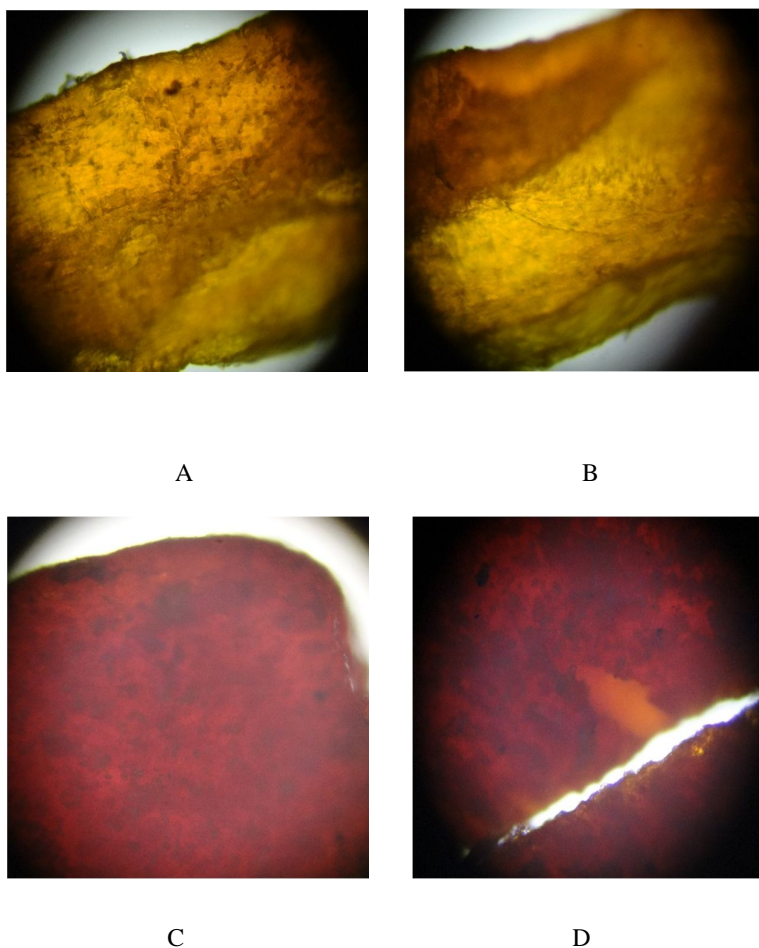


Fig. 11 Microscopic examination of saffron samples. A & B photomicrographs of genuine saffron at magnification 10x, B & D photomicrographs of fake saffron at magnification 10x.

III. RESULT AND DISCUSSION

The Forensic examination of the saffron samples (genuine and fake) involves various chemical tests to find the preliminary authenticity of the saffron (fig. 2, fig. 3). However reaction of components of the saffron (crocin, crocetin, picrocrocin) with acids like sulphuric acid and nitric acid yields indigo blue colour which forms rapid primary authenticity test (fig. 6, fig. 7). The instrumentation analysis provides a significant information of genuine and fake saffron. The separation and identification of genuine saffron by TLC yields continuous yellow spots in contrast to its fake (fig. 9). In addition the microscopy also provides a crucial information about the various anatomical characteristic features of genuine saffron and its adulterated form.

IV. CONCLUSION

The saffron samples were analysed using physical, chemical and instrumentation methods in order to find the rapid authenticity testing methods. The various colour tests performed provides preliminary authenticity of the genuine saffron in short span of time, however further analysis of saffron using instrumentation provides rapid authenticity testing of the saffron.

V. ACKNOWLEDGMENT

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Authors are very thankful to Amity University, Noida, Uttar Pradesh.

CONFLICT OF INTEREST

None

FUNDING

None

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