

■ REVIEW ARTICLE

Crocus Sativus: Comprehensive Pharmalogical Significance and Forensic Identification of Saffron in Illegal Trade

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ABSTRACT

Saffron, a dried stigma derivative of *Crocus sativus*, is tremendously rich in phytochemicals like crocin and safranal. For a long time, the phytochemicals of saffron have remained obscure, and thereby its role in disease treatment remained subsidiary. Of late, researchers have gained momentum in studying its bioactivity because of its numerous beneficial health aspects. The pharmacological activities such as antioxidant, anti-cancer, anti-diabetic, anti-inflammatory, and anti-atherosclerotic of crocin and safranal, have recently been a focus of many researchers. Considering market value and demand, saffron has become the center stage of adulteration and, subsequently, illegal trade. This has prompted researchers to develop advanced, cost-effective analytical tools for rapidly detecting possible adulteration common in illegal saffron trading. This review provides greater insight into methods and counter detection techniques in the adulteration of saffron worldwide. Moreover, this review gives a detailed account of the phytochemicals specific to saffron and their potential role in treatments of various diseases.

KEYWORDS | crocus sativa, saffron, phytochemistry, pharmacological activity

INTRODUCTION

SAFFRON KNOWN AS RED GOLD is one of the most expensive spices in the world. It is obtained from flowers (stigma) of the *Crocus sativa*.¹ Saffron is derived from the Arabic word, 'azaferan' and blooms only in the autumn and is dormant in the summer. *Crocus sativa* belongs to the Asparagales family and is an angiosperm plant. The flower of *Crocus* is solitary and purple with six petals, three stamens, one style, and three red-orange stigmas. The Mediterranean Europe, and West Asia are the leading distributors of saffron. Iran supplies

around 90% of total worldwide saffron output.² Saffron is highly expensive for its golden hue, flavor, and aromatic properties. It is recognized as a food spice and a powerful natural agent with a wide variety of health benefits. The ability of saffron and its key components to guard against natural and chemical toxins has increased the value of this spice. Since saffron is the most expensive spice and is in such high demand by the pharmaceutical industries, illicit trade and adulteration are rampant nowadays.^{3,4}

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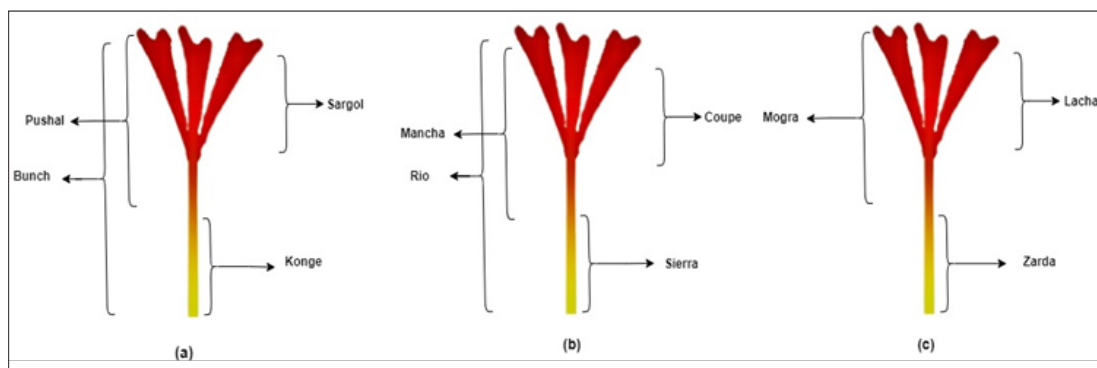


Figure 1: Grades of Saffron: a) Iranian Saffron b) Spanish Saffron c) Kashmiri Saffron. Source: Author self.

Grades of Saffron and ISO 3632

The quality and strength of saffron vary in terms of the amount of style attached to the red stigma. The age of the saffron is also an important factor. Since the color and flavor are concentrated in the red stigmas, the saffron is considered less efficient when more style is present. Saffron from Iran, Spain, and Kashmir are graded according to the proportions of red stigma and yellow styles present (Figure 1). The ISO 3632 certification assures consumers that the saffron they buy is genuine and safe to eat. Based on the following factors, Saffron is classified into grades I, II, and III by ISO 3632: 1. Moisture level (dried) 2. Crocin (color) 3. Picrocrocin (bitterness) 4. Safranal (aroma). The quantities of these key compounds are used to determine the saffron

Characteristic	Category I	Category II	Category III	Test Method
Crocin	>190	150-190	110-150	ISO3632-2, Clause 13
Safranal	20-50	20-50	20-50	ISO3632-2, Clause 13
Picrocrocin	>70	55-70	40-55	ISO3632-2, Clause 13
Moisture & Volatile matter	10	10-12	10-12	ISO3632-2, Clause 13

Table 1 ISO classification for various grades of saffron

quality (Table 1). A higher concentration of these compounds implies greater quality of saffron. The highest quality saffron is graded in Group I by ISO 3632, which means direct readings of absorbance of E1% aqueous solution of saffron at 440 nm, 330 nm, and 257 nm for crocin, safranal, and picrocrocin are greater than 190, 20, and 70, respectively.⁵

Phytochemistry of Saffron

The primary composition of saffron contains 14 to 16% water, 11 to 13% nitrogenous matter, 12–15% sugars, 41 to 44% soluble extract, 0.6 to 0.9% volatile oil, 4 to 5% fiber, and 4-6% overall ash. Two essential vitamins are found in Saffron: riboflavin and thiamin and a small amount of β -carotene. Saffron contains many volatile and non-volatile metabolites. Volatile elements constitute terpene alcohols, terpenes, and their esters. Non-volatile compounds of saffron include Crocin, picrocrocin, crocetin, and safranal (Figure 2).⁶

The orange-yellow color imparted to the saffron is due to the presence of α -crocin. The chemical nature of crocin is trans-crocetin di- β -D gentiobiosyl with an IUPAC name of 8,8

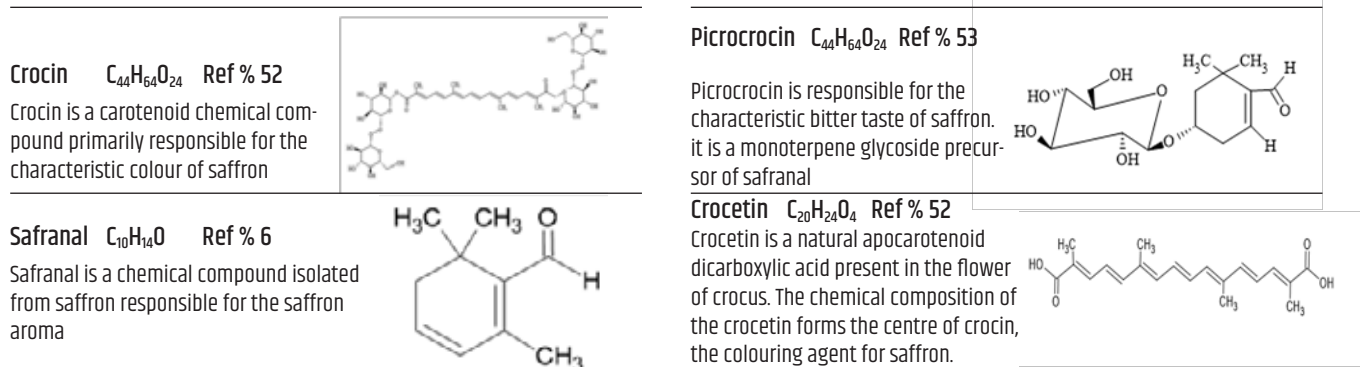


Figure 2: Chemical Structure, formula, properties of Saffron metabolites Source: Author self.

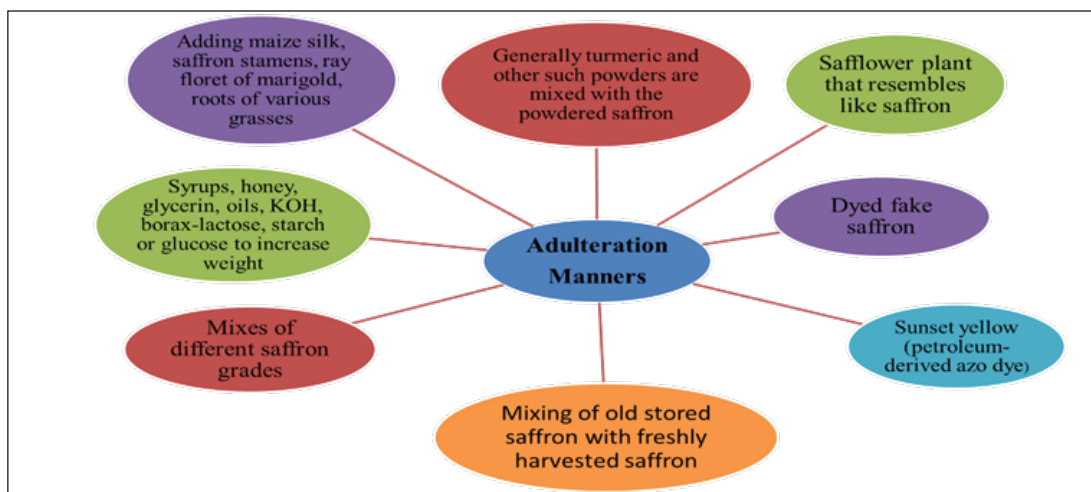


Figure 3: Various ways of Adulterating Saffron Source: Author self.

diapo-8,8 cartenoic acid. This is a testimony of the fact that crocin giving color to saffron is an ester of carotenoid crocetin (Digentiobiose)⁷. Crocins also belong to the hydrophilic series of carotenoids, either monoglycosyl polyene or diglycosyl polyene ester of the crocetin. Crocetin is a hydrophobic compound that is polyene conjugated dicarboxylic acid and also soluble in oil. The esterification of crocetin with two gentibioses that are water-soluble and yields a product that is water-soluble.⁸ The carotenoid pigment, i.e., alpha crocin, makes 10 % mass of the dry saffron. Thus, the presence of esterified gentibioses enables crocin to be an ideal coloring agent in various foods and dishes. Crocin is a water-soluble carotenoid and is responsible for the characteristic golden-yellow color of saffron. 'Crocine' is the most significant carotenoid glycoside, giving distinctive color to saffron. In addition to these, it is a crocetin-digentiobiose ester (C₂₀H₂₄O₄) which can hydrolyze with emulsion (β -glucosidase) with a beta-shaped glycosidic bond.

The pungent flavor of saffron is attributed to the presence of a bitter glucoside known as picrocrocin. The chemical formula of picrocrocin is C₁₆H₂₆O₇ with a systematic name of 4-(β -D-glucopyranosyloxy)-2,6,6 trimethyl-cyclohexene carbaldehyde. Picrocrocin is a blend of carbohydrate and safranal (2,6,6 trimethylcyclohexaene carbaldehyde).⁹ It is known to have pesticidal and insecticidal properties comprising 4% of saffron when dried. Picrocrocin is docked compound of zeaxanthin with a glycoside nature of aldehydic

terpene safranal formed with oxidative cleavage. Post harvesting, when saffron is dried, the enzymatic action along with controlled heat breaks picrocrocin into glucose and safranal. Picrocrocin was discovered by Kajser and it is a glycoside that cracks into a glucose molecule and 4-hydroxy-b-cyclocitral (aglycon) because of acids and alkali. The aglycon loses a water molecule and becomes the safranal compound.

Safranal, a less bitter molecule, is a volatile oil that gives an aroma to the saffron.^{10,11} The Safranal metabolite of saffron is a volatile oil and is mainly meant for the characteristic aroma of the spice. Safranal comprises up to 70% volatile fraction of saffron. The fresh stigma is not having any smell. After harvest, saffron undergoes processing and drying. The combined enzymatic action and the heat split picrocrocin to obtain D-glucose along with a free molecule of safranal in yield.⁶ It has a distinctive fragrance that gives a characteristic aroma to the spice. Safranal is a cyclic terpenic aldehyde with brute formula C₁₀H₁₄O (m.w.= 150; e.p.70 OC/1 mm), IUPAC (2,6,6-trimethyl 1,3-cyclohexadiene-1-carboxaldehyde). The name derives from the first researchers Kuhn and Winterstein to obtain it through the hydrolyzing of picrocrocin.

Crocetin is hydrophobic in nature and therefore is oil soluble. It is a conjugated polyene dicarboxylic acid that is meant for the unique pleasant smell of the spice. Crocetin belongs to the broad natural dye family known as carotenoids, but the

provitamin function is lacking in this compound. Hydrocarbons with a general formulation of $C_{40}H_{56}$ or oxygenated derivatives are the main elements of this class.

Pharmaceutical significance of saffron derived phytochemicals

For the last couple of decades, phytochemicals of plant origin have been regarded as potential pharmaceutical agents with minimum side effects as compared to chemically formulated drugs.¹² *Crocus sativus*, as a popular spice agent, has also been found essential in treatment of various diseases including asthma¹³, depression¹⁴, menstruation disorders¹⁵, cardiovascular disease¹⁶, digestive ailments¹⁷, cancer¹⁸, insomnia¹⁹, and many others. The therapeutic properties associated with saffron are attributed to its phytochemicals like crocetin, safranal, and crocins.²⁰ Even though the biomedical studies of the saffron-derived compounds have been experimented long ago, however, those early reports were more observational and the molecular relevance has remained in doubt for a long period.²¹ One of the studies has shown saffron as a potent gastrointestinal modifier to prevent gastrointestinal atonia²² and a significant therapeutic agent on female genitals.²³ Safranal is useful in treating respiratory ailments like chronic bronchitis. It acts on the alveoli through vagal nerves, thus sedating coughing.²⁴ Crocin, an analgesic, has been recommended for painful dysmenorrhea, thereby helping in reducing uterine contractions.²⁵ On the other hand, picrocrocin has been reported with tranquilizing properties, which induces a sedative effect on lumbar and spasm pains.²⁶

Several *in vitro* and *in vivo* studies have been carried out to explore saffron as an excellent anti-cancer agent. Bathaie *et al.*, studied the importance of saffron extract (SE) in rats after being subjected to MNNG (1-methyl-3-nitro-1-nitrosoguanidine) gastric cancer induction. The administration of SE showed cancer progression inhibition and more than 15% of rats were found to be normal after treating them with higher SE doses.²⁷ Saffron has also been reported to cause the reduction of DEN (diethylnitrosamine) induced increase of hepatic dyschromatic nodules while carrying out studies on rats. It is known to counteract DEN-induced stress in rats as analyzed by catalase, superoxide mutase, and myeloperoxidase levels.²⁸

The phosphorylation of acetyl-CoA carboxylase (AMPK/ACC) and mitogenic activated protein kinases in saffron greatly enhance the peripheral insulin sensitivity; however, they could not enhance PI3-kinase/Akt. Crocetin therapies in diabetic rats have revealed an increase in insulin sensitivity by lowering adiponectin's protein and messenger RNA (mRNA) levels, tumor necrosis factor (TNF), and leptin in white adipocytes.²⁹ Crocetin also prevents insulin resistance induced by dexamethasone by reducing free fatty acids and triglycerides in plasma and downregulating TNF- α regulators.³⁰ Crocetin could also inhibit the expression of adiponectin contributing to improved sensitivity for insulin.³¹ Examination of insulin susceptibility in HOMA-IR diabetic animals has shown that crocin significantly decreases glycosylated hemoglobin level and enhances insulin sensitivity by preventing oxidative stress and improving plasma lipid profile.³² Safranal functions as a potent inducer of Insulin signal transduction (IST) by inhibiting protein tyrosine phosphatase 1B (PTP1B).³³ The crocin can improve insulin sensitivity by suppressing TNF- α and interleukin-1 β (IL-1 β) levels in plasma and the TNF- α and interferon- γ (IFN- γ) levels in pancreatic tissues.³⁴ It has been found that saffron can enhance the plasma glycemic profile by upregulation of the GLUT4/AMPK molecular pathway.³⁵ Thus, saffron's active metabolites display their hypoglycemic effects by enhancing IST and inducing insulin sensitivity.

Methods of Saffron adulteration and its detection:

Saffron is very costly and there is a great demand. Due to less production and high demand for this spice, it is highly prone to illicit trade and adulteration.³⁶ Adulteration of saffron is mainly done by adding safflower, maize silk, marigold floret, horsehair, grassroots, stamens of saffron, red dried silk fiber, etc. These kinds of adulterants resemble saffron in color and texture. Sugar, potassium hydroxide, borax lactose, glycerin, fats, glucose, starch, etc., are mainly added to increase weight. Adulteration is not only done by adding a foreign substance to the saffron but also mixing different grades is one of the common methods of adulteration. The powdered form of saffron is more likely to be adulterated. Usually, turmeric and other powders resembling saffron are blended and sold in the markets with powdered saffron (Figure 2).³⁷

The ongoing adulteration of saffron has badly affected the production and economy of saffron cultivators. Various instrumentation methods are available to detect adulterations in saffron. Lozano *et al.*, (1999) carried HPLC analysis on Mancha, Rio, and Sierra grades of Iranian saffron, and ten different metabolites were found in the saffron extract. Each chromatograph was depicted at three wavelengths (250, 310, 440), and it was concluded that Mancha showed the highest concentration for all sec-metabolites followed by Rio and Siera.³⁸ Sujata *et al.*, (1992) used TLC, HPLC, and Gas chromatography to check the authenticity of saffron. Crocin, picrocrocin, and crocetin were resolved using TLC and HPLC analysis. However, safranal was evaluated using Gas chromatography. In TLC analysis n-butanol, Acetic acid, and water (4:1:1) were used as mobile phase and Rf (Retention factor) was calculated as 0.63, 0.32, and 0.98 for crocin, picrocrocin, and crocetin, respectively. In HPLC analysis, the extract of pure saffron in 80% ethanol was passed through a cartridge eluted with 100% acetonitrile and Rt was obtained. It was found that Rt of crocin, picrocrocin, and crocetin was 13.5, 14-18, and 18 respectively. Gas chromatography was used to resolve safranal. Nitrogen at a flow rate of 30ml/min was used and it was found that safranal could be resolved into a sharp peak at a rate of 3.6 minutes.³⁹ Semiond *et al.*, (1996) studied the isotopic analysis and identification of saffron using Supercritical fluid extraction (SFE). Safranal metabolites obtained from saffron of different origins were analyzed and a difference was found between synthetic and natural safranal. Moreover, it was found that SFE allowed various volatile compounds to be selectively extracted from saffron under optical conditions. It is a quicker and safer way to extract volatile saffron compounds.⁴⁰ G. L. Alonso *et al.*, (1998) used the Thermal-desorption Gas Chromatography-Mass Spectrometry technique for analyzing the authenticity of Spanish saffron and found that major components of the aromatic composition are safranal. The fingerprint of the chromatograph was obtained for both genuine and fake saffron, which were different. In adulterated saffron, a chromatograph peak appeared, which was not produced by pure saffron fingerprint. Such fingerprint was similar to compound 2,6,6-trimethyl cyclohexane-

carboxaldehyde, and it could be concluded that if beta-cyclocitral peak appears in chromatograph fingerprint, the saffron was adulterated.⁴¹ Zalacian *et al.*, (2005) researched a testing tool for various colorant identification. This method was based on removing crocins from the sample by precipitation before the adsorption of colorants on a Polyamide Solid-phase Extraction (SPE) cartridge. Elution with methanol-ammonia solution was performed and after washing identification process was carried out with a spectrometer.⁴² Anna Torelli *et al.*, (2004) used a system based on Sequence-Characterized Amplified Regions (SCARs) to detect adulteration with specific agents using various food products containing saffron. The use of SCAR markers proved to be an effective, quick, and low-cost screening method for authenticating saffron-containing food products.⁴³ The HPLC process for identifying, detecting and quantifying saffron metabolites was studied by Haghghi *et al.*, (2007) to detect adulterants like colored styles of *Crocus sativa*, safflower red beet, etc. The chromatograms were obtained at desired wavelengths, and then applying ANOVA to the chromatographic data obtained, the presence of adulterants in saffron were detected significantly.⁴⁴ In 2007, Gonzalo *et al.*, published a method for HPLC-DAD to simultaneously classify crocins and picrocrocin in aqueous saffron spice extracts. This method was able not only to determine crocins and picrocrocin but also to detect adulteration of saffron with water-soluble colorants.⁴⁵ Application of Proton Transfer Reaction-Mass Spectrometry (PTR-MS) in saffron quality control was studied by Nikolaos *et al* (2014). A minute quantity sample was used to capture volatile fingerprints. IPTR-MS/chemometry was examined to detect fresh addition of low-quality saffron to fine quality⁴⁶. Kobra Heidarbeigi *et al.*, (2015) developed an electronic nose system combined with principal component analysis and an artificial neural network to detect adulteration in saffron. This was the first approach towards the detection of adulteration using sensors. The electronic nose could detect complex odors via an array of sensors. The specimen odors are drawn into the electronic nose chamber and then passed through the sensor array process, resulting in a reversible physical change or chemical alteration in the sensing material associated with electrical

properties like conductivity.⁴⁷ Ordoudi *et al.*, (2014) researched a multi-step method for detecting saffron fraud. HPLC, UV-Vis, FT-IR, and NMR were used to examine the saffron fraud. UV-Vis and FT-IR data were used to reveal the artificial colors used. NMR was used to identify others. The identification of authentic saffron by using a molecular genetic approach was investigated by Ma XQ *et al.* (2000). After analysis, they found that the nucleotide sequence of all the samples was distinct and different and served as a marker for authentic identification of pure saffron from counterfeit saffron.⁴⁸ N Javanmardi (2011) studied the petals of the sunflower plant that are mostly used as an adulterant of saffron. In their study, they employed the application DNA analysis (RAPD) to detect adulteration in saffron. DNA from safflower petals and saffron was extracted and after analysis, it was found two monomorphic bands (500 and 700 bp) present in safflower were found to be absent in saffron.⁴⁹ Luana magi *et al.*, (2011) studied the spectroscopic method to determine the safranal content in the saffron. To check the quality of saffron spice, this quantitative safranal study was based on non-polar solvent extraction followed by spectrometric analysis. Ultrasound-assisted safranal extraction was performed and UV-Vis spectrophotometric analysis showed satisfactory results in the performance of repeatability, linearity, and recovery.⁵⁰ Karimi *et al.*, (2016) used FTIR spectroscopy with pattern recognition to differentiate between true saffron and those samples adulterated with various food adulterants.

The FTIR spectrum was obtained for all the samples and it was found that three region bands corresponding 1800-1830, 2600-2900 and 3700-3850 were responsible for recognizing true saffron from adulterated ones.⁴ Soffritti G *et al.*, (2016) in their study, isolated DNA from the saffron sample, adulterated sample, and possible adulterants, then from isolated DNA new markers were developed to recognize genuine saffron from fake or adulterated saffron. This method could recognize saffron from a mixture with a low percentage of adulterants.⁵¹

CONCLUSION

Plants are tremendously rich in phytochemicals with a significant role in biological functions. Saffron has a substantial amount of crocin and safranal as phytochemical compounds that can treat diseases like diabetes, inflammation, neuro degenerative disorders besides helping in the fight against human pathogens. Although the bioactivity of saffron and its compounds is plenty, the mode of action is confined to *in-vitro* studies. As a result, the molecular mechanism of its action remains obscure and needs further study with regard to its therapeutic potential. Due to high demand and price, saffron is prone to adulteration. Lack of quality management is a threat to the saffron industry since a significant portion of the market share of saffron is exported through the selling of counterfeit saffron, necessitating the creation of a system for detecting adulterants on the spot. **IJFMP**

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