
**ISSN Print:** 2394-7500
**ISSN Online:** 2394-5869
IJAR 2015; 1(5): 267-270
www.allresearchjournal.com
Received: 13-03-2015
Accepted: 23-04-2015

**Sajad H Wani**
Central Institute of Temperate Horticulture, Old Air Field Rangreth, Srinagar, Jammu & Kashmir, India

**Mir Faisal Mustafa**
Department of biochemistry, University of Kashmir, Jammu & Kashmir, India

**Shabir Ahmad Lone**
Department of biotechnology, Govt Degree College Boys, Ananthnag, Jammu & Kashmir, India

**Alima Shabir**
Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar, Jammu & Kashmir, India

**Correspondence**
**Sajad H Wani**
Central Institute of Temperate Horticulture, Old Air Field Rangreth, Srinagar, Jammu & Kashmir, India

# Biochemical evaluation of saffron *Crocus sativus* L. extracts from Jammu and Kashmir

**Sajad H Wani, Mir Faisal Mustafa, Shabir Ahmad Lone and Alima Shabir**

**Abstract**
Saffron selections of Jammu and Kashmir are heterogeneous for floral characteristics which are mainly attributed to the environmental factors, though genetic factors may have role with regard to its differential characteristics across various selections found in the area. Identification of high yielding selections using the existing gene pool of saffron may help in improving the productivity of this crop. Saffron, the most expensive spice in the world, is derived from the dry stigmata of the saffron flower and is having very high medicinal potential due to presence of some bioactive compounds like crocin and safranal. In present study quantitative analysis of different samples of *Crocus sativus* L. collected from different places of Jammu and Kashmir were done in order to estimate the levels of bioactive compounds. Reverse phase high pressure liquid chromatography (RP-HPLC) coupled with ultra violet diode array detector (UV-DAD) was carried out to quantitate safranal and crocin in the methanolic extracts of different samples of saffron and the content of these bioactive compounds varied significantly across different samples. Maximum crocin (38.6 mg/g of stigmas) and safranal (0.25mg/g of stigma) content was found in saffron samples collected from Kishtawar (K-1) and minimum crocin (17.64 mg/g of stigmas) and safranal (0.018mg/g of stigma) content was found in saffron samples collected from Budgam district (BD-1).

**Keywords:** *Crocus sativus*, safranal, crocin, RP-HPLC

## Introduction
*Crocus sativus* L. is an autumn-flowering geophyte extensively grown in the Mediterranean basin and Near East since the Late Bronze Age [1]. Saffron, the dried red stigmas of *C. sativus*, has been used as flavouring and colouring agent since then and is currently considered the world's most expensive spice. The major components of saffron are the apocarotenoids cis- and trans-crocins, picrocrocin (β-D-glucopyranoside of hydroxyl-β-cyclocitral), and its degradation product, the odour-active safranal [2]. Saffron contains more than 150 volatile and aroma compounds. It also has many non-volatile active components many of which are carotenoids, including zeaxanthin, lycopene, and various α- and β-carotenes. Saffron is rich source of phenolic compounds with antioxidant activity [3]. The most important compounds in saffron are crocin, picocrocin and safranal responsible for saffron color, flavor and aroma respectively [4]. The amounts of these main compounds are used to express the quality of saffron. Higher the amounts of these compounds in saffron, means its better quality. Saffron's golden yellow-orange colour is primarily because of α-crocin which is trans-crocetin di-(β-D-gentiobiosyl) ester (Systematic (IUPAC) name being 8, 8-diapo-8, 8-carotenoic acid. The bitter glycoside picrocrocin is responsible for saffron's flavour. Picrocrocin (Molecular formula: $C_{16}H_{26}O_7$; systematic IUPAC name: 4-(β-D-glucopyranosyloxy)-2, 6, 6- trimethylcyclohex-1-ene-1-carboxaldehyde) is derived from aldehyde sub-element known as safranal (Systematic IUPAC name: 2, 6, 6-trimethylcyclohexa-1, 3-diene-1- carboxaldehyde) and a carbohydrate. It has insecticidal and pesticidal properties, and may comprise up to 4% of dry saffron. Significantly, picrocrocin is a truncated version (Produced via oxidative cleavage) of the carotenoid zeaxanthin and is the glycoside of the terpene aldehyde safranal. The reddish-coloured zeaxanthin is, incidentally, one of the carotenoids naturally present within the retina of the human eye. When saffron is dried after its harvest, the heat, combined with enzymatic action, splits picrocrocin to yield D-glucose and a free safranal molecule. Safranal, a volatile oil, gives saffron much of its distinctive aroma.

Safranal is less bitter than picrocrocin and may constitute up to 70% of dry saffron's volatile fraction in some samples. Saffron is somewhat more resistant to heat. The colouring strength and bitter taste of dehydrated saffron are five times more concentrated than those of fresh saffron [5].

Due to presence of crocin, safranal. picrocrocin and their properties there is growing interest to decipher the chemotherapeutic potential of this crop. The uses of saffron dates back in to ancient Egypt and Rome, wherein it was used for perfumery, spice and dying purposes. But the uses of saffron in traditional medicine for treatment of illnesses, including cough, colic, insomnia, chronic uterine hemorrhage, cardiovascular disorders and tumors have been reported [6]. Potential uses of saffron extract as anticancer and antitumor properties and cytotoxic effect has been studied in the breast cancer cell lines, MCF-7 [7]. There are also some reports on antiproliferation of lung cancer cell lines by saffron extracts [8]. However, there is no evidence on the therapeutic effects of saffron extracts from Jammu and Kashmir region on cancer cell line. Therefore, the aim of the present study was to assess the potential cytotoxic and antiproliferative effects of saffron (*C. sativus* L.) in human lung and colon cancer cell lines.

## Materials and Methods

### Collection of samples

The samples were collected from different ecogeographical zones of Jammu and Kashmir covering high altitude regions like Kishtwar, Pulwama, Budgam and Anantnag (Table 1) and voucher specimens were deposited in the Herbarium of the institute under voucher specimens (No.I302/2012-I105/2013).

**Table 1:** Summary for the tested samples of *Saffron*

| No. | Code | Samples | Sources | Altitude (ft) | Collection date |
|---|---|---|---|---|---|
| 1 | SF-6 | *Crocus sativus* | Sambora, Pampore | 5,350 | Oct-nov. 2013 |
| 2 | CF-10 | *Crocus sativus* | Charisharief, Budgam | 10,479 | Oct-nov. 2013 |
| 3 | NP-2 | *Crocus sativus* | Nehama, Pulwama | 5,350 | Oct-nov. 2013 |
| 4 | BD-1 | *Crocus sativus* | Nagam,Budgam | 10,479 | Oct-nov. 2013 |
| 5 | K-1 | *Crocus sativus* | Puchel,Kishtwar | | Oct-nov. 2013 |
| 6 | K-2 | *Crocus sativus* | Berwar, Kishtwar | | Oct-nov. 2013 |
| 7 | SF-11 | *Crocus sativus* | Duru,Anantnag | 5,350 | Oct-nov. 2013 |
| 8 | NP-1 | *Crocus sativus* | Patlibal, Pampore | 5,350 | Oct-nov. 2013 |
| 9 | SF-5 | *Crocus sativus* | Wolarhama Anantnag | 5,350 | Oct-nov. 2013 |
| 10 | SF-1 | *Crocus sativus* | Parigam,Pulwama | 5,253 | Oct-nov. 2013 |

### Extraction of apocarotenoids for HPLC analysis

For the analysis of apocarotenoids, saffron stigma were extracted with methanol (100 mL) in a microcentrifuge tube for 5 min on ice. Tris-HCl (50 mM, pH 7.5; containing 1 M NaCl) was then added (100 mL) and a incubated for 10 min on ice. The precipitate was collected by centrifugation at 3,000g for 5 min at 4ºC. The pellet was then reground in acetone (400 mL) and incubated on ice for 10min. The mixture was centrifuged at 3,000rpm for 5 min at 4ºC. This step was repeated until no color was detected in the pellet. The supernatants were pooled and evaporated and the dried residues were stored at -80ºC until HPLC analysis.

### Sample preparation for HPLC

Extracted samples were dissolved in HPLC grade methanol at final concentration of 100 mg/ml. Samples were filtered through 0.2 µm syringe filters. A 20 µl filtered sample was then injected into an HPLC coupled to a PDA detector.

### HPLC analysis

The analysis was carried out in a Shimadzu HPLC (Kyoto, Japan) equipped with quaternary pumps, degasser coupled to a photo-diode-array detector and injection valve with a 20 µL loop. Separation was carried out with an injection volume of 20 µL, a flow rate of 1 ml min−1 with 35-40 minutes of run time. The analyses were triplicated for each sample. Safranal was detected at 310 nm, picrocrocin at 257nm and crocin at 440 nm, whereas the standards for respective apocarotenoids were detected at the above mentioned wavelengths [9]. Standards of crocin, safranal and picrocrocin were obtained from Sigma Aldrich. Chromatographic separations were performed on C18 (250 mm × 4.6 mm), 5 µm column using a solvent system consisting of 75% acetonitrile and 25% methanol in an isocratic mode. The mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA) before analysis. Class WP software (version 6.1) from Shimadzu was used for instrument control, data acquisition and data processing. Quantitative determinations were made by taking into account the respective peak areas of standards at particular retention time versus concentration and expressed in milligrams per gram of saffron stigmas.

## Results and Discussion

### Relative apocarotenoid quantification through HPLC analysis

Apocarotenoid content of 10 different saffron samples collected from different sites of Jammu and Kashmir varying in altitude was determined using reverse phase C18 HPLC. The chromatographic conditions employed allowed quantification of crocin and safranal in each sample. Each component was identified by comparison of its retention time as previously described in the literature [9-12]. Table 2 shows the concentration of each detected compound in the 10 different saffron samples. The results indicate that there is some significant variation among selected samples with respect to apocarotenoid contents. Saffron selection from Kishtawar K-1 had highest total crocin content (38.6 mg/g of stigmas) whereas Budgam selection BD-1 had lowest crocin concentration (17.64 mg/g of stigma). Highest concentration of safranal (0.25mg/g of stigma) was observed again in selection from Kishtawar (Puchel) K-1 while the selection BD-1 showed the lowest concentration (0.018 mg/g of stigma). The average values for crocin and safranal in all the 10 saffron selections were 35.4 mg/g and 0.178 mg/g of dried stigma respectively. Therefore it is clear that selection K-1 is superior to other selections with respect to crocin and safranal content and selection BD-1 is inferior to all other selections. Thus promotion of such selection on large scale will yield the saffron of high quality and promise. Total content of crocin and safranal depends upon storage of stigma and extraction method of apocarotenoids [12] hence the amount of these compounds reported by different workers varies. Alonso *et al*.

2001 reported that crocins vary from 0.85% to 32.4% dry weight. Other values reported vary between 2.9 mg% (29 mg/g) (Li *et al*., 1999) and 4.6 mg% (45.99 mg/g) [12] for Iranian saffron and 6.7 mg% (67.3 mg/g) [14] for Indian saffron. Safranal levels reported by some researcher are around 0.80 mg% (8 mg/g) [14]. Safranal vary between a 0.06 mg/g and 0.29 mg/g [23]. Lage *et al* 2009 reported that crocin, safranal and picrocrocin content vary between 17-37%, 0.1-0.48% and 0.4-2.8% respectively under Moroccan conditions. It is clear from our results that selected 10 saffron selections showed significant variation in apocarotenoid content, therefore offering an opportunity for exploiting such finding for saffron crop improvement.

**Table 2:** Apocarotenoid quantification though HPLC in ten *Crocus Sativus* L. Selections

| S. No | Sample code | Crocin content (mg/g) | Safranal content (mg/g) |
|---|---|---|---|
| 1. | SF6 | $37.33937^{C}$±0.007079 | $0.005667^{B}$±0.002348 |
| 2. | CF10 | $27.35407^{H}$±0.001671 | $0.158333^{AB}$±0.005727 |
| 3. | NP1 | $37.12297^{D}$±0.001035 | $0.1203^{AB}$±0.000751 |
| 4. | BD1 | $17.6458^{I}$±0.171906 | $0.011533^{B}$±0.00082 |
| 5. | K2 | $37.75047^{B}$±0.010717 | $0.201567^{AB}$±0.000234 |
| S6. | K1 | $38.67473^{A}$±0.011433 | $0.251333^{AB}$±0.000233 |
| 7. | SF5 | $37.25803^{CD}$±0.001301 | $0.057167^{B}$±0.000579 |
| 8. | SF1 | $32.62607^{E}$±0.003601 | $0.1552^{AB}$±0.000322 |
| 9. | NP2 | $28.1327^{G}$±0.001533 | $0.126333^{AB}$±0.000883 |
| 10. | SF11 | $28.71113^{F}$±0.005463 | $0.078133^{A}$±0.000145 |

Means followed by the same letter within the columns are not significantly different (*P*= 0.05) using DMRT

**Acknowledgement**
Authors are greatly thankful to Director, ICAR-Central Institute of Temperate Horticulture for providing the facilities to conduct the research activities. Authors are thankful to Department of Chemistry and Department of Biotechnology, University of Kashmir for providing facilities and support during the course of research activities.

**References**

1. Negbi M. Saffron Cultivation: Past, Present and Future Prospects, In: M. Negbi, Ed., *Saffron Crocus sativus* L. Harwood academic publishers, Amsterdam, 1999, 1- 19.
2. Kanakis CD, Daferera DJ, Tarantilis PA, Polissiou MG. Qualitative determination of volatile compounds and quantitative evaluation of safranal and 4-hydroxy-2, 6, 6 trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) in Greek saffron. J Agr. Food Chem. 2004; 52:4515-4521.
3. Goli SAH, Mokhtari F, Mehdi R. Phenolic Compounds and Antioxidant Activity from Saffron (*Crocus sativus* L.) Petal. Journal of Agricultural Science. 2012; 4:2010-2012.
4. Fernandez JA. Biology, biotechnology and biomedicine of saffron. Recent Res. Dev. Plant Sci. 2004; 2:127-159.
5. Alonso GL, Sánchez MA, Salinas MR, Esteban-Infantes FJ. Kinetics of the Loss of Color Power in Saffron Extract at Different Temperatures. 9th Congreso Nacional de Química Sevilla, España, 1993.
6. Joukar S, Najafipour H, Khaksari M, Sepehri G, Shahrokhi N, Dabiri S. The effect of saffron consumption on biochemical and histopathological heart indices of rats with myocardial infarction. Cardiovasc Toxicol. 2010; 10:66-71.
7. Tavakkol-Afshari J, Brook A, Mousavi SH. Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. Food Chem Toxicol. 2008; 46:3443-7.
8. Saeed S, Mohammad Hossein B, Saideh D. Use of *in vitro* assays to assess the potential antiproliferative and cytotoxic effects of saffron (*Crocus sativus* L.) in human lung cancer cell line. Pharmacopgnocy Magazine. 2010; 24:309-314.
9. Lonzano P, Castellar MR, Simancas MJ, Ibora JL. A Quantitative High-Performance Liquid Chromatographic Method to Analyze Commercial Saffron (*Crocus sativus* L.) Products. Journal of Chromatography. 1999; 830:477-483.
10. Tarantilis PA, Soupras G, Polissiou M. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV/Visible photodiode-array detection-mass spectrometry. J Chromatogr. 1995, 699:107-118.
11. Li N, Lin G, Kwan Y, Min Z. Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. J Chromatography. 1999; 849:349-355.
12. Caballero-Ortega H, Pereda-Miranda R, Abdullaev FI. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. Food Chemistry. 2007; 100:1126-1131.
13. Alonso GL, Salinas MR, Garijo J, Sànchez MA. Composition of Crocins and Picrocrocins from Spanish Saffron (*Crocus sativus* L.). Food Quality. 2001; 24:219-233.
14. Sujata V, Ravishankar A, Venkataraman LV. Methods for the analysis of the saffron metabolites crocin, crocetins, picrocrocin and safranal for the determination of the quality of the spice using thin-layer chromatography, high performance liquid chromatography and gas chromatography. J Chromatogr. 1992; 624:497-502.
15. Pinelo M, Rubilar M, Sineiro J, Nunez MJ. Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). Food Chemistry. 2004; 85:267-273.
16. Kosar M, Goger F, Baser KHC. *In vitro* antioxidant properties and phenolic composition of *Salvia halophila* hedge from turkey. Food Chemistry. 2011; 129:374-379.
17. Zhu KX, Lian CX, Guo XN, Peng W, Zhou HM. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. Food Chemistry. 2011; 126:1122-1126.
18. Angelo G, Mariagiovanna S, Lorena C, Antonella C. Biochemical, Antioxidant and Antineoplastic Properties of Italian Saffron (*Crocus sativus* L.). American Journal of Plant Sciences. 2012; 3:1573-1580.
19. Karimi E, Oskoueian E, Hendra R, Jaafar HZ. Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules. 2010; 15:6244-6256.

20. Tseng TH, Chu CY, Huang JM, Shiow SJ, Wang CJ. Crocetin protects against oxidative damage in rat primary hepatocytes. Cancer Lett. 1995; 97:61-7.
21. Giaccio M. Crocetin from saffron: An active component of an ancient spice. Crit Rev Food Sci Nutr. 2004; 44:155-72.
22. Khuloud B, Jan S, Stefanie D, Amr A, Regine SS. The anticancer effect of saffron in two p53 isogenic colorectal cancer cell lines. BMC Complementary and Alternative Medicine. 2012; 12:69.
23. Hadizadeh F, Mahdaci M, Emami SA, Khashayarmanesh Z, Hassanzadeh M, Asili J *et al*. Evaluation of ISO method in saffron quantification. In: Koosheki, Nassiri, Ghorbani, (Eds.), Proceedings of the second international Symposium on Saffron Biology and Technology, Acta Hort. (ISHS). 2007; 739:405-410.