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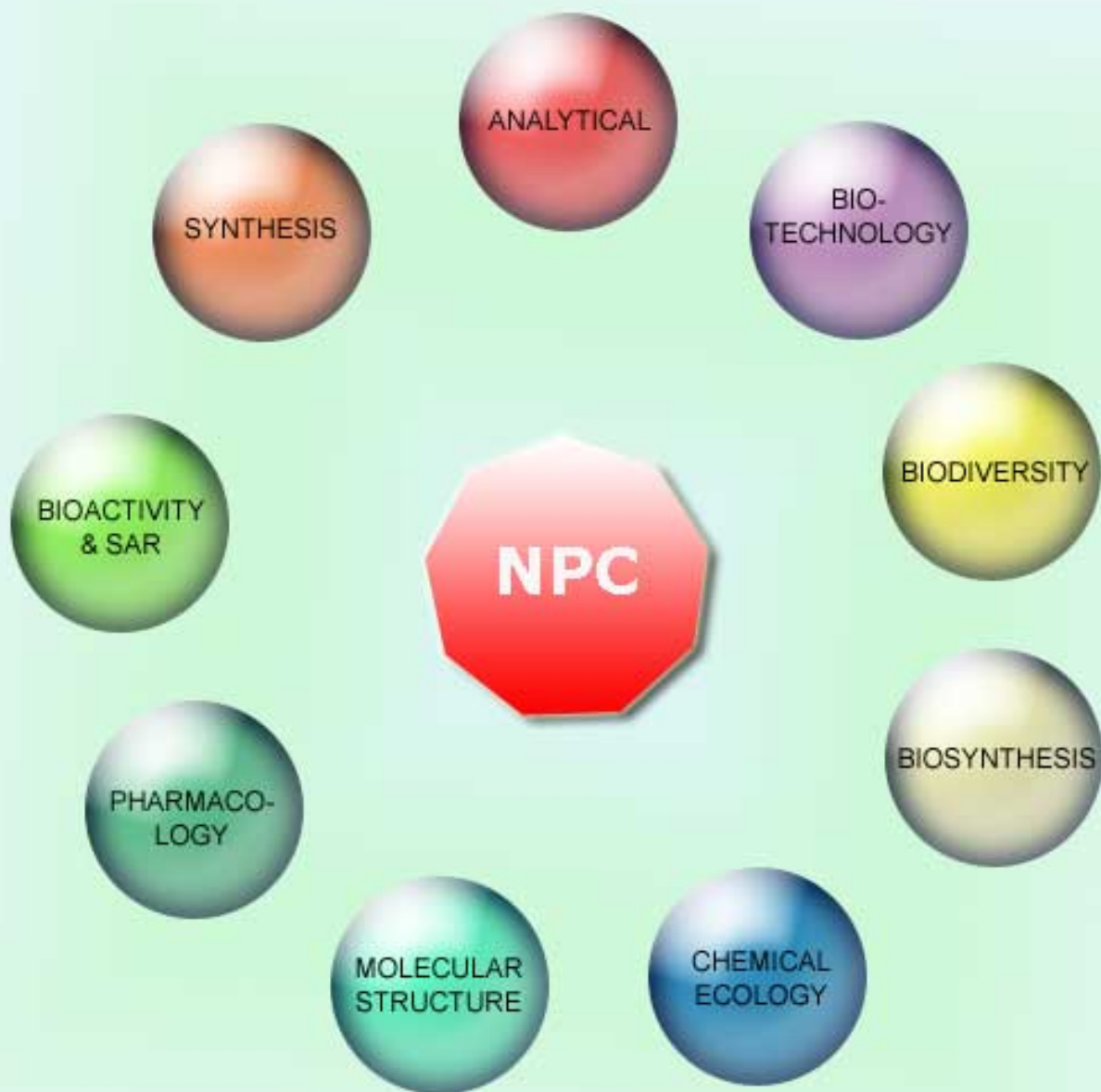
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# NATURAL PRODUCT COMMUNICATIONS

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on the Occasion of his 70<sup>th</sup> Birthday**

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## Characterization of By-products of Saffron (*Crocus sativus* L.) Production

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The stigma, stamens and sepals of *Crocus sativus* L., from two different geographical origins, were analyzed for their crocin and flavonol contents. Identification of crocins, safranal, picrocrocin, and flavonols was carried out by HPLC/DAD and HPLC/MS analysis. Both stigma samples, grown under natural conditions, exhibited high crocin contents (between 342 and 231 mg/g), while the stamens and sepals were rich in flavonols (between 6 and 10 mg/g). The stamens contain mainly kaempferol- 3-*O*-sophoroside, whereas the sepals contain mainly quercetin and methyl-quercetin glycosides. These data may be useful in order to find a possible exploitation of the by-products of saffron production, in which large quantities of *C. sativus* flowers are available.

**Keywords:** Crocins, flavonols, HPLC/DAD/MS, sepals, stamens, stigma.

The dried, red stigmas of *Crocus sativus* L. are a very expensive spice known as saffron, which is used as a food flavoring and coloring agent and as a traditional herbal medicine [1a]. *Crocus* is cultivated in India, Iran, Spain, Greece and Italy. The production process involves a large amount of manual work and cannot be completely mechanized. In Italy, from a 1000 m<sup>2</sup> area, about 120,000-150,000 flowers can be obtained (4000-5000 kg), which give rise to 5-7 kg of fresh stigma, i.e. 1.0-1.3 kg of dried product.

Many papers deal with methods for the separation and determination of the biologically active [1b-1f] and aroma components [2a-2c]. The quality control of commercial saffron is checked using spectrophotometric [3a,3b], TLC [3c], GC [3d], HPLC [3e], and CE [3f] methods.

The purpose of this paper is the analysis of stigmas from *C. sativus* cultivated in Italy (Perugia and Fiesole) in order to characterize this commercial

saffron from a quality point of view. In these areas, cultivation is effected under natural conditions and without the use of any chemical product in the drying and conservation phases.

However, the most important part deals with the characterization of the biologically active components of the stamens and sepals in order to find a possible use for this material, which forms the major part of *C. sativus* flowers. The exploitation of stamens and sepals, notwithstanding their availability as by-products in the production of saffron, has not been taken into account, with the exception of one paper dealing with the isolation of flavonoids from crocus petals to study their tyrosinase inhibition action [4a]. Notwithstanding the lack of information on the polyphenol content of these tissues, petal extracts were used to control rat blood pressure [4b] and to test their antitussive effect in guinea pigs [1b]. The major biologically active components of saffron are crocin analogues, which are all glycosides of

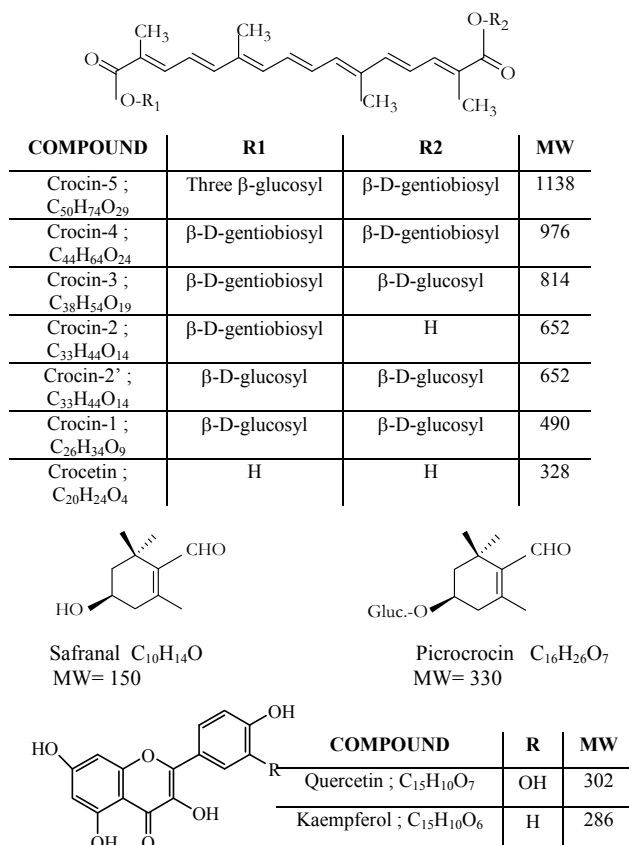


Figure 1: Chemical structures of saffron components

*trans*-crocetin, a carotenoid derivative, and which are responsible for the color. Safranal (2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde), which is responsible for the characteristic aroma of saffron, is formed during storage by dehydration of picrocrocin, which is responsible for its bitter taste. Flavonoids are found in stigma, sepals, and stamens (Figure 1).

As regards stigma, the composition of the extract was similar to that found by other authors regarding crocins, picrocrocin, and safranal. Three kaempferol derivatives (two triglycosides and one diglycoside) were identified, according to previous findings [1f,5a]. In the case of stamens, a lesser number of crocins was found and quercetin, as well as kaempferol derivatives were detected. Also, methyl-quercetin derivatives in quite large amounts were recorded. There were no differences, from a qualitative point of view, between the two sampling zones; in fact only a quantitative variation was found in the samples from the different geographic regions [5a].

Table 1 reports the quantitative data for the dried stigma. It should be noted that the two samples differ are present in largest amount in the two samples. These compounds, together with *cis*-crocin 4, were

Table 1: Quantitative data for dried stigma. Average value ± SD of three samples. Data are expressed as mg/g fresh sample.

COMPOUNDS (Rt)	Stigma (FI)	Stigma (PG)
<i>trans</i> crocin-5 (10.30)	2.4±0.09	2.0±0.07
crocine derivative (11.14)	2.1±0.08	0.8±0.04
crocine derivative (11.46)	0.3±0.01	0.3±0.01
crocine derivative (11.87)	0.3±0.009	0.1±0.007
<i>trans</i> crocin-4 (12.84)	238.9±2.86	148.5±2.66
crocine derivative (13.88)	1.3±0.06	0.5±0.02
<i>trans</i> crocin-3 (14.39)	65.6±1.84	46.2±1.38
crocine derivative (14.99)	0.2±0.01	0.2±0.009
crocine derivative (15.90)	0.6±0.03	0.5±0.02
<i>trans</i> crocin-2' (16.17)	2.1±0.07	1.5±0.06
crocine derivative (17.37)	0.3±0.01	0.3±0.009
<i>cis</i> crocin-4 (17.79)	9.5±0.33	14.1±0.49
<i>trans</i> crocin-2 (19.33)	16.9±0.51	14.8±0.50
crocine derivative (20.40)	0.2±0.009	traces
crocine derivative (21.11)	0.3±0.01	traces
<i>cis</i> crocin-1 (22.02)	1.0±0.05	0.8±0.04
crocine derivative (22.81)	0.2±0.01	traces
crocine derivative (23.17)	traces	0.5±0.02
<b>TOTAL</b>	<b>342.02</b>	<b>231.1</b>
<b>Picrocrocin (6.34)</b>	111.1±2.33	68.9±1.79
<b>Safranal (24.87)</b>	2.2±0.09	2.6±0.09
K-3-sophoroside -7- glucoside (3.78)	4.7±0.2	3.3±0.14
K -3,7,4'-triglucoside (5.90)	1.2±0.05	0.9±0.04
K-3-sophoroside (8.49)	6.2±0.22	5.4±0.17
<b>TOTAL</b>	<b>12.1</b>	<b>9.64</b>

mainly in *trans*-crocin 4, *trans*-crocin 3 and picrocrocin contents, i.e. the three compounds which also the main compounds found by Caballero-Ortega *et al.* [5b] in a study of 11 saffron samples from different origins. The crocins content of the two samples is quite high giving evidence for the very good quality of the two samples. Among flavonols, kaempferol-3-*O*-sophoroside was the main compound reported for a Spanish sample analyzed by Carmona *et al.* [5a].

Table 2 reports the crocin contents of sepals and stamens. The amount of crocins is low, while that of flavonols (Table 3) ranged from 10.1 to 6.1 mg/g. Stamens and sepals differ mainly in their kaempferol-3-*O*-sophoroside content, which is the most abundant flavonol in the sepals.

The flavonols composition of the two tissues is different: in sepals, kaempferol derivatives ranged between 91 -93 %, whereas in stamens, quercetin and methyl-quercetin derivatives ranged between 52-71%. From all these data the possible exploitation of alternative tissues like stamens and sepals as phytochemical resources can be pointed out. For each kg of stigma, about 1000 kg of flowers are processed; therefore, sepals and stamens are important by-products of saffron production and their use could increase the economic value of *C. sativus* flowers.

**Table 2:** Crocins content of sepals and stamens. Average value  $\pm$  SD of three samples.

COMPOUNDS (Rt)	Sepals (FI)	Sepals (PG)	Stamens (FI)	Stamens (PG)
<i>trans</i> crocin-4 (12.84) crocin der. (13.88)	3.1 $\pm$ 0.17	traces	112.2 $\pm$ 5.65	4.0 $\pm$ 0.19
<i>trans</i> crocin-3 (14.39) crocin der. (14.99) crocin der. (15.99)	0.8 $\pm$ 0.04	traces	33.4 $\pm$ 1.74	traces
<i>trans</i> crocin-2' (16.17) cis crocin-4 (17.79)			traces	traces
<i>trans</i> crocin-2 (19.33) cis crocin-1 (22.02) crocin der. (22.81) crocin der. (23.17)	traces	traces	22.0 $\pm$ 1.14	0.1 $\pm$ 0.006
cis crocin-2 (24.82)	0.3 $\pm$ 0.02		20.7 $\pm$ 1.07	1.3 $\pm$ 0.08
			7.0 $\pm$ 0.38	traces
			0.3 $\pm$ 0.02	traces
			0.1 $\pm$ 0.008	traces
<b>TOTAL</b>	<b>4.2</b>	<b>traces</b>	<b>196.3</b>	<b>5.4</b>

## Experimental

**Sample preparation:** Sepals, stamens and dried stigma samples were obtained from plants harvested in 2005 from Fiesole (FI, Italy) and Perugia (PG, Italy). Sepals and stamens (500 mg) were suspended in 50 mL of 70% ethanol, adjusted to pH 2.0 with formic acid, and left overnight. After extraction, the

samples were filtered to eliminate plant residues, and the filtrate evaporated to dryness under vacuum at room temperature. The residue was redissolved in EtOH/H<sub>2</sub>O (70:30) and adjusted to pH 2.0 with formic acid to a final volume of 3 mL.

Saffron stigmas (50 mg) were extracted with 10 mL of 70% ethanol, adjusted to pH 2.0 with formic acid, left overnight and then filtered to eliminate plant residues. The extracts were analysed by HPLC/DAD/MS for the determination of saffron components.

Authentic standards of crocin were purchased from Fluka (St. Louis, USA), safranal from Sigma-Aldrich (St. Louis, USA), and *p*-hydroxybenzoic acid, kaempferol 3-*O*-glucoside, rutin and curcumin from Extrasynthèse S.A. (Lyon, France). All solvents were of HPLC grade purity (BDH Laboratory Supplies, United Kingdom).

**HPLC/DAD analysis:** Analysis for flavonols and crocins was carried out using a HP 1100L liquid chromatograph equipped with a DAD detector and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Flavonols and crocins were separated by using a 150  $\times$  3.9 mm i.d. 4  $\mu$ m Nova-Pak C18 column (Waters) operating at 27°C. UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 250, 308, 350 and 440 nm. The mobile phase was a

**Table 3:** Flavonols content of sepals and stamens. Average value  $\pm$  SD of three samples. Data are expressed as  $\mu$ g/g fresh sample.

COMPOUNDS (Rt)	Sepals (FI)	Sepals (PG)	Stamen(FI)	Stamen(PG)
Kaempferol derivative (3.71)	76 $\pm$ 4.10			
Kaempferol-3-sophoroside-7-glucoside (3.78)			511 $\pm$ 22.84	923 $\pm$ 41.53
Kaempferol derivative (5.81)			24 $\pm$ 1.18	77 $\pm$ 4.15
Kaempferol diglucoside (5.89)	97 $\pm$ 4.85	113 $\pm$ 5.6		
Kaempferol derivative (6.49)	15 $\pm$ 1.03	34 $\pm$ 1.83		
Kaempferol diglucoside (7.30)			416 $\pm$ 21.16	755 $\pm$ 33.75
Quercetin diglucoside (7.30)	480 $\pm$ 22.08	738 $\pm$ 32.16	1037 $\pm$ 37.32	1227 $\pm$ 47.81
Methyl quercetin diglucoside (7.82)	82 $\pm$ 4.16	84 $\pm$ 4.21	628 $\pm$ 28.88	2091 $\pm$ 61.74
Quercetin derivative (8.15)			27 $\pm$ 1.15	39 $\pm$ 2.14
Methyl quercetin di glucoside (8.42)			209 $\pm$ 10.03	249 $\pm$ 11.73
Kaempferol-3-sophoroside (8.49)	6415 $\pm$ 192.45	8304 $\pm$ 215.9	1702 $\pm$ 64.7	377 $\pm$ 17.72
Kaempferol glucosyl rhamnoside (9.29)	41 $\pm$ 2.13	66 $\pm$ 3.20		
Methyl quercetin derivative (9.34)			691 $\pm$ 31.09	1188 $\pm$ 46.32
Quercetin derivative (9.44)			239 $\pm$ 11.47	303 $\pm$ 14.54
Quercetin diglucoside (9.58)	24 $\pm$ 1.27	60 $\pm$ 3.18		
Kaempferol sinapoyl glucoside (10.59)	306 $\pm$ 14.38	309 $\pm$ 14.25	140 $\pm$ 5.81	
Kaempferol derivative (10.86)				39 $\pm$ 2.25
Kaempferol glucoside (10.98)	421 $\pm$ 19.78	399 $\pm$ 18.75	93 $\pm$ 4.65	
Methyl quercetin glucoside (11.13)			52 $\pm$ 2.75	176 $\pm$ 8.62
Quercetin derivatives (11.55-12.21)			26 $\pm$ 1.19	66 $\pm$ 3.43
Kaempferol derivative (12.99)	21 $\pm$ 1.15	17 $\pm$ 0.078		
Quercetin <i>p</i> -cumaroyl glucoside (13.76)			199 $\pm$ 9.75	237 $\pm$ 110.61
Quercetin derivative (14.09)			4 $\pm$ 0.22	26 $\pm$ 1.20
Kaempferol <i>p</i> -cumaroyl glucoside (15.42)			35 $\pm$ 2.05	52 $\pm$ 2.65
Methyl quercetin <i>p</i> -cumaroyl glucoside (15.61)			26 $\pm$ 1.21	40 $\pm$ 2.12
Kaempferol (18.43)	20 $\pm$ 1.16	14 $\pm$ 0.74		8 $\pm$ 0.44
<b>TOTAL</b>	<b>7998</b>	<b>10138</b>	<b>6059</b>	<b>7873</b>

one-step linear solvent gradient system, starting from 90% H<sub>2</sub>O (adjusted to pH 3.2 with HCOOH) up to 100% CH<sub>3</sub>CN during a 60-min period; flow rate 0.8 mL min<sup>-1</sup>.

**HPLC/MS analysis:** HPLC/MS analysis was performed using a HP 1100L liquid chromatograph linked to a HP 1100 MSD mass spectrometer with an API/electrospray interface (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer operating conditions were: gas temperature, 350°C; nitrogen flow rate, 10.5 L/min, nebulizer pressure, 40 psi; quadrupole temperature, 30°C; and capillary voltage, 3500 V. The mass spectrometer was operated in positive mode at 120 eV.

**Identification and quantification of individual polyphenols:** Quantification of individual compounds was directly performed by HPLC/DAD using a five-point regression curve ( $r^2 \geq 0.998$ ) in the range

0-30 µg on the basis of authentic standards. In particular, crocin derivatives were determined at 440 nm using curcumin as reference compound; safranal was determined at 308 nm using safranal as reference compound and picrocrocin was determined at 250 nm using *p*-hydroxybenzoic acid as reference compound. Flavonols, like kaempferol and quercetin derivatives, were determined at 350 nm using kaempferol-3-*O*-glucoside and rutin, respectively, as reference compounds. In all cases, actual concentrations of the derivatives were calculated after applying corrections for differences in molecular weight.

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## References

- [1] (a) Xi L, Qian Z. (2006) Pharmacological properties of crocetin and crocin (digentiobiosyl) ester of crocetin from saffron. *Natural Product Communications*, **1**, 65-75; (b) Alonso GL, Salinas M., Garijo J, Sanchez-Fernandez MA. (2001) Composition of crocins and picrocrocin from Spanish saffron (*Crocus sativus* L.), *Journal of Food Quality*, **24**, 219-233; (c) Li N, Lin G, Kwan YW, Min ZD. (1999) Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. *Journal of Chromatography A*, **849**, 349-355; (d) Pfander H, Rychener M. (1982) Separation of crocetin glycosyl esters by high-performance liquid chromatography. *Journal of Chromatography A*, **234**, 443-447; (e) Tarantilis PA, Polissiou M, Manfait M. (1994) Separation of picrocrocin, *cis-trans*-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. *Journal of Chromatography A*, **664**, 55-61; (f) Tarantilis PA, Tsoupras G, Polissiou M. (1995) Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. *Journal of Chromatography A*, **699**, 107-118
- [2] (a) Lozano P, Delgado D, Gomez D, Rubio M, Iborra JL. (2000) A non-destructive method to determine the safranal content of saffron (*Crocus sativus* L.) by supercritical carbon dioxide extraction combined with high performance liquid chromatography and gas chromatography. *Journal of Biochemical and Biophysical Methods*, **43**, 367-378; (b) Loskutov AV, Beninger CW, Hosfield GL, Sink KC. (2000) Development of an improved procedure for extraction and quantitation of safranal in stigmas of *Crocus sativus* L. using high performance liquid chromatography. *Food Chemistry*, **69**, 87-95; (c) Straubinger M, Bau B, Eckstein S, Fink M, Winterhalter P. (1998) Identification of novel glycosidic aroma precursors in saffron (*Crocus sativus* L.). *Journal of Agricultural and Food Chemistry*, **46**, 3238-3243.
- [3] (a) Carmona M, Carrion ME, Zalacain A, Alonso GL. (2004) Detection of adulterated saffron through UV-Vis spectral analysis. *Journal of Food Science & Technology*, **41**, 451-455; (b) Zalacain A, Ordoqui SA, Blazquez I, Diaz-Plaza EM, Carmona M, Tsimidou MZ, Alonso GL. (2005) Screening method for the detection of artificial colours in saffron using derivative UV-Vis spectrometry after precipitation of crocetin. *Food Additives and Contaminants*, **22**, 607-615; (c) Corti P, Mazzei E, Ferri S, Granchi GG, Dreassi E. (1996) High performance thin layer chromatographic quantitative analysis of picrocrocin and crocetin, active principles of saffron (*Crocus sativus* L.): a new method. *Phytochemical Analysis*, **7**, 201-203; (d) Alonso GL, Salinas MR, Garijo J. (1998) Method to determine the authenticity of aroma of saffron (*Crocus sativus* L.). *Journal of Food Protection*, **61**, 1525-1528; (e) Lozano P, Castellar MR, Simancas MJ, Iborra JL. (1999) Quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. *Journal of Chromatography A*, **830**, 477-483; (f) Zougagh M, Simonet BM, Rios A, Valcarcel M. (2005) Use of non-aqueous capillary electrophoresis for the quality control of commercial saffron samples. *Journal of Chromatography A*, **1085**, 293-298.
- [4] (a) Kubo I, Kinoshita H. (1999) Flavonols from saffron flower: tyrosinase inhibitory activity and inhibition mechanism. *Journal of Agricultural and Food Chemistry*, **47**, 4121-4125; (b) Fatehi M, Rashidabady T, Fatehi-Hassanabad Z. (2003) Effects of *Crocus sativus* petals extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea pig ileum. *Journal of Ethnopharmacology*, **84**, 199-203; (c) Hosseinzadeh H, Ghenaati J. (2006) Evaluation of the antitussive effect of stigma and petals of saffron (*Crocus sativus*) and its components safranal and crocin in guinea pig. *Fitoterapia*, **66**, 446-448.
- [5] (a) Carmona M, Sanchez AM, Ferreres F, Zalacain A, Tomas-Barberan F, Alonso GL. (2007) Identification of the flavonoids fraction in saffron spice by LC/DAD/MS/MS: comparative study of samples from different geographic origins. *Food Chemistry*, **100**, 445-450; (b) Caballero-Ortega H, Pereda-Miranda R., Abdullaev FI. (2007) HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. *Food Chemistry*, **100**, 1126-1131.

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