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Antioxidant Activity of Aqueous and Ethanolic Extracts of *Crocus sativus* L. Stigma and its Bioactive Constituents, Crocin and Safranal

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ABSTRACT

The antioxidant activity of aqueous and ethanol extracts of saffron (*Crocus sativus* L.) stigma and its constituents, crocin and safranal were evaluated using deoxyribose, erythrocyte membrane lipid peroxidation and liver microsomal non-enzymatic lipid peroxidation methods in vitro. The aqueous and ethanolic extracts in different concentrations (10, 100, 250, 500 and 1000 µg/ml), crocin and safranal at 0.1, 0.5, 1 and 2 mM were tested. Results showed that test compounds could diminish the extent of MDA generation in three methods (P<0.01 VS control). The effect of crocin at 1,2 mM and ethanolic extract at 500 and 1000 µg/ml in liver microsomal lipid peroxidation were similar to BHT 100 µM in liver microsomal lipid peroxidation (P>0.05). Crocin the main carotenoid in saffron extracts probably and relatively safranal may have an important role in antioxidant properties of the extracts.

Keywords: Antioxidant, Crocin, *Crocus sativus*, Lipid peroxidation, Saffron, Safranal.

INTRODUCTION1

Free radicals are involved in the etiology of different human disease such as vasculitis, rheumatoid arthritis, stroke (1), congestive heart failure (2), Parkinson, Alzheimer (1, 3) and cancer (4). Antioxidants are substances that can delay or inhibit oxidative stress, therefore they can help in prevention and therapy of disease caused by oxidative damage(4).

Crocus sativus L. commonly known as saffron is a stemless herb of the Iridaceae family. Its constituents that are considered pharmacologically active and important are safranal, crocin, picrocrocin and crocetin (5).

Saffron and its constituents are widely evaluated for their pharmacological activities such as treatment of memory impairment(6), antidepressant(7), anticonvulsant (8), antitussive (9), aphrodisiac activity (10), antianxiety

(11) and hypnotic (11) and especially for their antitumor effect (12).

Saffron or its constituents inhibited lipid peroxidation in renal (13), hippocampal (14) and muscle skeletal (15) homogenates during ischemia-reperfusion-induced oxidative damage in rats. Radical scavenging activity of *C. sativus* L. extract and its bioactive constituents, safranal and crocin have been shown using DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging test (16).

In this study we evaluated the antioxidant activity of ethanolic and aqueous extracts of saffron and its active constituents, crocin and safranal, in a range of lipid peroxidation using three experimental approaches i.e. deoxyribose assay, erythrocyte membrane peroxidation and rat liver microsomal lipid peroxidation induced by Fe²⁺/ascorbate.

MATERIALS AND METHODS*Material*

Crocin, safranal, sodium azid and deoxyribose obtained from Fluka.

Animal

Male Wistar rats, 200–250 g were housed in colony rooms with 12/12 h light/dark cycle at $21 \pm 2^\circ\text{C}$ and had free access to food and water. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical committee Acts.

Preparation of extracts

In the maceration method, 0.5g of sigma powder was macerated in 60 ml solvent (ethanol (80 v/v) and water) for 48 h. The mixture of plant and solvent was subsequently centrifuged (5 min, 3000 rpm) and the supernatants were evaporated to dryness under reduced pressure at 40°C . The yield of the extracts was 55% (w/w) for ethanolic extracts and 20% (w/w) for aqueous extract.

Deoxyribose assay

In this method all solutions were prepared freshly. 1.0 ml of the reaction mixture contained 100 μl of 28 mM 2-deoxy-2-ribose 500 μL solution of various concentrations of the material test (aqueous and ethanolic extracts of saffron, crocin and safranal in buffer), 200 μL of 200 μM FeCl_3 and 1.04 mM EDTA (1:1 v/v), 100 μL H_2O_2 (1 mM) and 100 μl ascorbic acid (1 mM). After an incubation period of 1 h at 37°C , the extent of deoxyribose degradation was measured by the TBA reaction. 1 ml of TBA (1% in 50 mM NaOH) and 1 ml of TCA were added to the reaction mixture and the tubes were heated at 100°C for 20 min. Absorbance was read at 532 nm (17). The percentage of inhibition of deoxyribose degradation was calculated using the following equation

$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

A_0 : Absorbance of the control in the absence of samples

A_1 : The absorbance in the presence of samples.

DMSO was used as a positive control. (18)

Assay for site-specific reactions

This assay was done in the following three ways.

- 1- The assay was done as mentioned before except that 100 ml of FeCl_3 instead of 200 ml of Fe^{3+} -EDTA solution was added.
- 2- The assessment was performed without ascorbic acid as a starter for oxidation.
- 3- Deoxyribose itself was omitted from the assay (17).

Erythrocyte membrane peroxidation

The protective effects of saffron extracts, crocin and safranal were determined according to the method that was described before (18, 19) with modification. Wistar rats (200–250 g) were anesthized with chloroform, whole blood was drawn via a cardiac puncture and collected in to heparinized tubes. The RBC was separated from plasma by centrifugation at 1500 g for 15 min. Packed RBC was washed twice with NaCl 0.15 M, and preincubated with phosphate buffer (pH 7.4) containing sodium azid (1 mM) to inhibit catalase. Peroxidation was initiated by adding H_2O_2 (10 mM). 100 μl solution of various concentrations of the material test was added and the mixture was incubated at 37°C for 60 min. The reaction was terminated by addition of 28% (w/v) trichloroacetic acid. Lipid peroxidation was measured using thiobarbitoric method. The quantity of MDA was determined by measuring the absorbance at 532 nm (18, 19). BHT was used as a positive control (20).

Liver microsomal preparation

Liver microsomes were prepared according to the method that was described previously (18, 21) with minor changes. Briefly, male Wistar rats (200–250) were sacrificed. The liver was perfused with ice-cold saline through the portal vein until uniformly pale, immediately removed. After being minced, the pieces of liver were homogenized with 4 volume of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) containing 1.15% (w/v) KCl. The homogenate was centrifuged at 10000 g for 60 min. The supernatant was used for the study.

Microsomal lipid peroxidation induced by Fe^{2+} /ascorbat

To measure the antioxidant activity of the various material test, lipid peroxidation of rat liver microsomes was carried out in vitro according to the method that was described previously (21) with minor changes. Briefly the reaction mixture containing 0.2 ml of rat liver microsome (2mg/ml) 0.1 ml of FeSO_4 (final concentration 26% mM), 0.1 ml of ascorbate (final concentration 0.13 mM), 0.1 ml of the sample in 150 mM KCl/Tris-HCl buffer solution (pH 7.4). The mixture was incubated at 37°C for 60 min in a water bath; the reaction was stopped by addition of 0.75 ml of 2M trichloroacetic acid /1.7 MHCl.

After centrifugation (4000 rpm, 10 min) 0.5 ml of the supernatant was mixed with 0.15 ml TBA and the mixture was heated at 95°C for 10 min.

After cooling the quantity of MDA was determined by measuring the absorbance at 532 nm. Inhibition (I) of lipid peroxidation in percent was calculated by following equation. $\%I = (A_0 - A_1) / A_0 \times 100$

Table 1. Antioxidant activity of aqueous and ethanolic extracts of saffron in deoxyribose assay.

Concentration (µg/ml)	% Inhibition	
	Aqueous extract	Ethanolic extract
Control	—	—
10	15.51***	9.22***
100	26.03***	22.13***
250	40.48***	41.45***
500	58.26***	58.91***
1000	71.15***	72.26***
DMSO (20 mM) positive control	91.16***	91.16***

$$\% \text{ Inhibition} = \frac{A_{\text{control}}(532) - A_{\text{sample}}(532)}{A_{\text{control}}(532)} \times 100,$$

***P<0.001, as compared to control.

A₀: Absorbance of the control reaction

A₁: The absorbance in the presence of the agents.

BHT was used as a positive control (18).

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer post hoc test for multiple comparisons. The P value less than 5% 0.05 were considered to be statistically significant. PCS software was used to calculate IC₅₀ value.

RESULTS

Deoxyribose degradation assay

Absorption spectra of crocin aqueous and ethanolic extracts of saffron showed absorbance at 532 nm, therefore we prepared blank for each sample.

Table 2. Antioxidant activity of crocin and safranal in deoxyribose assay

Concentration (mM)	% Inhibition	
	Crocin	Safranal
Control	—	—
0.1	22.3***	30.6***
0.25	38.4***	40.91***
0.5	48.75***	64.06***
1	70.16***	73.1***
2	72.16***	78.61***
DMSO (20 mM) positive control	91.45***	91.45***

$$\% \text{ Inhibition} = \frac{A_{\text{control}}(532) - A_{\text{sample}}(532)}{A_{\text{control}}(532)} \times 100,$$

***P<0.001, as compared to control.

In deoxyribose degradation method, saffron aqueous and ethanolic extracts in various concentrations (10, 100, 250, 500, 1000 µg/ml) and its constituents crocin and safranal at (0.1, 0.25, 0.5, 1, 2 mM) were evaluated.

All of tested compound showed OH radical scavenging effect (P<0.001 VS control) and antioxidant activity increased with increasing concentration. The IC₅₀ values for aqueous, ethanolic extract, crocin and safranal were 324 µg/ml, 338 µg/ml, 0.47 mM and 0.31 mM respectively. The results are shown in tables 1 and 2.

Assay for site specific actions

To assay site specific reaction when EDTA omitted from the system test Fe³⁺ instead of Fe³⁺ EDTA bound to deoxyribose and stimulated MDA generation. All of tested agents inhibit deoxyribose degradation in this condition and showed metal chelating effect.

Table 3. Results of the assay for site-specific reactions of the ethanol and aqueous extracts of saffron and its constituents, crocin and safranal

Sample	Concentration	Omit EDTA	Omit Vit C	Omit deoxyribose
control	—	0.511	0.132	0.07
Ethanolic extract	10 (µg/ml)	0.473	0.113	0.0052
	100	0.481	0.113	0.016
	250	0.379	0.082	0.015
	500	0.228	0.148	0.041
	1000	0.13	0.183	0.055
Aqueous extract	10	0.399	0.108	0.012
	100	0.320	0.100	0.037
	250	0.285	0.082	0.02
	500	0.200	0.145	0.031
	1000	0.113	0.160	0.049
Crocin	0.1 (mM)	0.249	0.056	0.006
	0.25	0.225	0.049	0.0033
	0.5	0.183	0.049	0.0025
	1	0.150	0.103	0.008
	2	0.144	0.121	0.026
Safranal	0.1	0.4	0.022	0.0179
	0.25	0.365	0.020	0.0110
	0.5	0.31	0.030	0.059
	1	0.293	0.017	0.013
	2	0.263	0.022	0.0183

Values are absorbance of sample at 532 nm at test conditions

Saffron aqueous and ethanolic extracts in concentration 500, 100 µg/ml, crocin at 1.2 mM stimulated peroxidation under test condition.

In the absence of deoxyribose, non of the tested compounds were able to react with TBA to produce colored products. Results are shown in table 3.

RBC Lipid Peroxidation

Both extracts in concentration 250, 500 and 1000 µg/ml inhibited RBC lipid peroxidation and MDA generation as compared with control (P<0.001). Crocin and safranal in concentration 0.5, 1 and 2 mM showed antioxidant effect in this method (P<0.001 vs control). IC₅₀ value

for crocin was 1.14 mM. Because extracts and safranal showed antioxidant effect less than 50%, IC₅₀ for these agents were not calculated. Results are shown in tables 4-5.

Liver microsomal lipid peroxidation

Treatment of liver microsomal with saffron extracts are (10, 100, 250, 500 and 1000 µg/ml) produced a significant decrease of MDA generation as compared with control treatment (P<0.001).

Crocin and safranal in the tested concentration showed antioxidant activity. There was not distinct significant between BHT and ethanolic extract (500, 1000 µg/

Table 4. Effects of ethanolic and aqueous extracts of saffron in RBC lipid peroxidation assay.

Concentration	Aqueous extract			Ethanolic extract		
	MDA	P	%I	MDA	P	I%
Control	403.38 ± 3.84	–	–	365.51 ± 5.95	–	–
10	391.34 ± 3.48	Ns	3	346.99 ± 8.03	ns	5.3
100	374.00 ± 8.41	Ns	7.15	325.26 ± 14.86	ns	7.6
250	350.78 ± 5.28	0.05	13	302.8 ± 13.83	0.01	18
500	339.02 ± 2.87	0.01	16	292.25 ± 8.2	0.01	21
1000	275.14 ± 13.91	0.001	33	263.67 ± 6.22	0.001	39
BHT (0.04 mM) positive control	66.83 ± 2.31	0.001	87	70.77 ± 2.93	0.001	84

Values are mean ± SEM (n=6)

TBARS were expressed with pmol MDA produced in the presence of different concentrations of extracts.

ns: not significant

Table 5. Effects of crocin and safranal in RBC lipid peroxidation

Concentration (mM)	Crocine			Safranal		
	MDA	P	%I	MDA	P	I%
Control	368.076±6.16	–	–	357.02±9.37	–	–
0.1	316.11±5.34	ns	15	343.11±4.38	ns	4.2
0.5	273.40±8.66	0.001	27	300.35±8.31	0.001	16.5
1	224.8±15.10	0.001	41	275.01±5.86	0.001	24
2	122.95±4.52	0.001	69.5	205.14±7.69	0.001	44.5
BHT(0.04 mM) positive control	65.32±1.77	0.001	83	72.26±2.78	0.001	81

Values are mean ± SEM (n=6)

TBARS were expressed with pmol MDA produced in the presence of different concentrations of crocin and safranal.

ns: not significant

Table 6. Effects of ethanolic and aqueous extracts of saffron in microsomal lipid peroxidation

Concentration (mM)	Ethanolic extract			Aqueous extract		
	MDA	P	%I	MDA	P	I%
Control	1.98 ± 0.15	–	–	1.98 ± 0.15	–	–
10	1.22 ± 0.064	0.001	39	1.69 ± 0.058	ns	14
100	0.866 ± 0.033	0.001	55	1.26 ± 0.051	0.001	35
250	0.778 ± 0.028	0.001	62	1.05 ± 0.033	0.001	47
500	0.61 ± 0.027	0.001	69	0.96 ± 0.029	0.001	51.5
1000	0.389 ± 0.034	0.001	80	0.74 ± 0.043	0.001	62.3
BHT(100 µM) positive control	0.540 ± 0.035	0.001	73.5	0.54 ± 0.035	0.001	72.7

Values are mean ± SEM (n=6)

TBARS were expressed with nmol MDA produced in the presence of different concentrations of extracts.

ns: not significant

Table 7. Effects of crocin and safranal in microsomal lipid peroxidation assay

Concentration (mM)	crocin			safranal		
	MDA	P	%I	MDA	P	%I
Control	1.877 ± 0.076	–	–	1.879 ± .075	–	–
0.1	1.344 ± 0.031	0.001	27	1.65 ± 0.045	0.05	12
0.5	0.815 ± 0.031	0.001	55	1.41 ± 0.042	0.001	24.5
1	0.465 ± 0.026	0.001	74	1.35 ± 0.033	0.001	28
2	0.343 ± 0.022	0.001	81.5	1.17 ± 0.039	0.001	37
BHT(0.04 mM) positive control	0.489 ± 0.036	0.001	74	0.489 ± 0.036	0.001	74

Values are mean ± SEM (n=6)

TBARS were expressed with nmol MDA produced in the presence of different concentrations of crocin and safranal.

ml) or crocin (1, 2 mM) in inhibition lipid peroxidation ($P > 0.05$).

IC₅₀ values for aqueous, ethanolic extract and crocin were 372.58 µg/ml, 44.8 µg/ml and 0.34 mM respectively. Results are shown in Tables 6–7

DISCUSSION

In this study we evaluated the antioxidant activity of ethanolic and aqueous extracts of saffron and its major bioactive constituents, crocin and safranal, under in vitro methods.

In deoxyribose assay all agents showed hydroxyl radical scavenging activity in a dose-dependent manner. In site specific assay, in the absence of EDTA, crocin, safranal and extracts exhibited ferrous ion chelating activity. In the absence of ascorbic acid crocin (1.2 mM) and ethanolic extract (500 and 100 µg/mL) stimulated deoxyribose degradation and showed oxidant activity.

Metal ion chelation activity of an antioxidant compound prevents oxyradical generation and the consequent oxidative damage (22). The inhibition of deoxyribose degradation in the absence of EDTA indicates the ability of agent to trap OH radicals and also to chelating iron (23). The presence of carotenoids in saffron extracts may be responsible for the antioxidant properties (5, 24, 25) but carotenoids do not necessarily act as antioxidant and it was demonstrated that carotenoids could increase the total yield of free radicals depending on the oxidation potential of the carotenoids and the nature of the radicals (24).

Membrane lipids are particularly susceptible to oxidative damage. This is not only because of their high polyunsaturated fatty acid content, but also because of their association in the cell membrane with enzymatic and non-enzymatic system able to generate free radical species (1). Lipid peroxidation is important process in many pathological events and is one of the reactions that induced by oxidative stress (2–4).

In this study all agents decrease MDA generation in RBC lipid peroxidation induced by H₂O₂ and liver microsomal non-enzymatic lipid peroxidation.

The antioxidant activity of saffron and its bioactive constituents may be responsible for its various pharmacological effects (26) such as inhibition of human cancer cells proliferation (27–30), protection of hepatocytes (5) and prevention muscle skeletal (15), renal and cerebral ischemia-reperfusion induced oxidative injury(13, 14). Crocin a major carotenoid pigment of saffron inhibited PC12 lipid peroxidation and restored SOD activity (31) and also protected RBC against AAPH induced hemolysis (26).

Safranal is monoterpene aldehyde which is the major constituent of the essential oil of saffron and is obtained by picrocrocin degradation (16, 30) showed radical scavenging activity (16) and ameliorated ischemia-reperfusion injury induced oxidative damage in rat hippocampus (14). In this study safranal also exhibited antioxidant activity in three methods, thus the significant antioxidant activity of the saffron extracts should probably be attributed to an action of the main bioactive constituent mainly crocin but also safranal. The role of crocin in antioxidant activity of saffron extracts is more important because safranal is usually present in less than 1% in saffron extracts (32).

CONCLUSION

The present study showed that ethanolic and aqueous extracts of saffron have antioxidant activity in different in vitro methods. Crocin, a major carotenoid of saffron may be have more role for antioxidant properties of saffron. Safranal a monoterpene aldehyde which is the major constituent essential oil of saffron also showed antioxidant activity.

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REFERENCES

- Halliwell B., Gutteridge J.M.C., and Lester N.G. Packer and Alexander. [1] Role of free radicals and catalytic metal ions in human disease: An overview, *Method Enzymol.* **186**: 1–85 (1990).
- Polidori M.C., Savino K., Alunni G., Freddio M., Senin U., Sies H., Stahl W., and Mecocci P. Plasma lipophilic antioxidants and malondialdehyde in congestive heart failure patients: relationship to disease severity. *Free Radic Biol Med.* **32**(2): 148–52 (2002).
- Smith D.G., Cappai R., and Barnham K.J. Theredox chemistry of the Alzheimer's disease amyloid [beta] peptide. *Biochimica et Biophysica Acta (BBA) - Biomembranes.* **1768**(8): 1976–1990 (2007).
- Tucker J.M. and Townsend D.M. Alpha-tocopherol: roles in prevention and therapy of human disease. *Biomed Pharmacother.* **59**(7): 380–7 (2005).
- Rios J.L., Recio M.C., Giner R.M., and Menez S. An update review of saffron and its active constituents. *Phytother Res.* **10**(3): 189–193 (1996).
- Hossein-zadeh H. and Ziaei T. Effects of *Crocus sativus* stigma extract and its constituents, crocin and safranal, on intact memory and scopolamine-induced learning deficits in rats performing the Morris water maze task. *J Med Plants.* **5**(19): 40–50 (2006).
- Hossein-zadeh H., Karimi G., and Niapoor M. Antidepressant effects of *Crocus sativus* stigma extract and its constituents, crocin and safranal, in mice. *J Med Plants.* **3**(11): 48–58 (2004).
- Hossein-zadeh H. and Talebzadeh F. Anticonvulsant evaluation of safranal and crocin from *Crocus sativus* in mice. *Fitoterapia.* **76**(7–8): 722–4 (2005).
- Hossein-zadeh H. and Ghenaati J. Evaluation of the antitussive effect of stigma and petals of saffron (*Crocus sativus*) and its components, safranal and crocin in guinea pigs. *Fitoterapia.* **77**(6): 446–8 (2006).
- Hossein-zadeh H., Ziaee T., and Sadeghi A. The effect of saffron, *Crocus sativus* stigma, extract and its constituents, safranal and crocin on sexual behaviors in normal male rats. *Phytomedicine.* **15**(6–7): 491–495 (2008).
- Hossein-zadeh H. and Noraei N.B. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents, crocin and safranal, in mice. *Phytother Res.* **23**(6): 768–774 (2009).
- Abdullaev F.I. and Espinosa-Aguirre J.J. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. *Cancer Detec Prev.* **28**(6): 426–432 (2004).
- Hossein-zadeh H., Sadeghnia H.R., Ziaee T., and Danaee A., Protective effect of aqueous saffron extract (*Crocus sativus* L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. *J Pharm Pharm Sci.* **8**(3): 387–93 (2005).
- Hossein-zadeh H. and Sadeghnia H.R., Safranal, a constituent of *Crocus sativus* (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus. *J Pharm Pharm Sci.* **8**(3): 394–9 (2005).
- Hossein-zadeh H., Modagheh M.H., and Saffari Z., *Crocus sativus* L. (saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle. *eCAM.* 1–8 (2007).
- Assimopoulou A.N., Sinakos Z., and Papageorgiou V.P. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytother Res.* **19**(11): 997–1000 (2005).
- Burits M. and Bucar F. Antioxidant activity of Nigella sativa essential oil. *Phytother Res.* **14**(5): 323–8 (2000).
- Schinella G.R., Tournier H.A., Prieto J.M., de Buschiazzo P.M., and Rios J.L. Antioxidant activity of anti-inflammatory plant extracts. *Life Sci.* **70**(9): 1023–1033 (2002).
- Liu M., Wallin R., Wallmon A., and Saldeen T. Mixed tocopherols have a stronger inhibitory effect on lipid peroxidation than α -tocopherol alone. *J Cardiovasc Pharmacol.* **39**(5): 714–721 (2002).
- Dwight J.F.S.J. and Hendry B.M. The effects of tert-butyl hydroperoxide on human erythrocyte membrane ion transport and the protective actions of antioxidants. *Clinica Chimica Acta.* **249**(1–2): 167–181 (1996).
- Kim S.W., Park S.S., Min T.J., and Yu K.H. Antioxidant activity of ergosterol peroxide (5,8-epidioxy-5 α ,8 α -ergosta-6,22e-dien-3 β -ol) in *Armillariella mellea*. *Bull Korean Chem Soc.* **20**(7): 819–823 (1999).
- Duh P.D., Tu Y.Y., and Yen G.C. Antioxidant Activity of Water Extract of *Huang Jyur* (*Chrysanthemum morifolium* Ramat). *LWT - Food Sci Technol.* **32**(5): 269–277 (1999).
- Halliwell B., Gutteridge J.M.C., and Aruoma O.I. The deoxyribose method: A simple “test-tube” assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem.* **165**(1): 215–219 (1987).
- Polyakov N.E., Leshina T.V., Konovalova T.A., and Kispert L.D. Carotenoids as scavengers of free radicals in a fenton reaction: antioxidants or pro-oxidants. *Free Radic Biol Med.* **31**(3): 398–404 (2001).
- Zhang P. and Omaye S.T. [beta]-Carotene and protein oxidation: effects of ascorbic acid and [alpha]-tocopherol. *Toxicology.* **146**: 37–47 (2000).
- Chen Y., Zhang H., Tian X., Zhao C., Cai L., Liu Y., Jia L., Yin H.-X., and Chen C. Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents. *Food Chem.* **109**(3): 484–492 (2008).
- Aung H.H., Wang C.Z., Ni M., Fishbein A., Mehendale S.R., Xie J.T., Shoyama C.Y., and Yuan C.S. Crocin from *Crocus sativus* possesses significant anti-proliferation effects on human colorectal cancer cells. *Exp Oncol.* **29**(3): 175–80 (2007).
- Nair S.C., Kurumboor S.K., and Hasegawa J.H. Saffron chemoprevention in biology and medicine: A review. *Cancer Biothe.* **10**(4): 257–264 (1995).
- Escribano J., Alonso G.L., Coca-Prados M., and Fernandez J.A., Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. *Cancer Lett.* **100**(1–2): 23–30 (1996).
- Fernandez J.-A. and Mahmud T.H.K.a.A.A. Anticancer properties of saffron, *Crocus sativus* Linn. *Advan Phytomed.* **2**: 313–316 (2006).
- Ochiai T., Ohno S., Soeda S., Tanaka H., Shoyama Y., and Shimeno H. Crocin prevents the death of rat pheochromocytoma (PC-12) cells by its antioxidant effects stronger than those of [alpha]-tocopherol. *Neurosci Lett.* **362**(1): 61–64 (2004).
- Caballero-Ortega H., Pereda-Miranda R., and Abdullaev F.I. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. *Food Chem.* **100**(3): 1126–1131 (2007).