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REVIEW

Evaluation of antioxidant activities of bioactive compounds and various extracts obtained from saffron (*Crocus sativus* L.): a review

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Abstract Saffron (*Crocus sativus* L. stigma), the most valuable medicinal food product, belongs to the *Iridaceae* family which has been widely used as a coloring and flavoring agent. These properties are basically related to its crocins, picrocrocin and safranal contents which have all demonstrated health promoting properties. The present review article highlights the phytochemical constituents (phenolic and flavonoid compounds, degraded carotenoid compounds crocins and crocetin) that are important in antioxidant activity of saffron extracts. However, the synergistic effect of all the bioactive components presence in saffron gave a significant antioxidant activity similar to vegetables rich in carotenoids. Our study provides an updated overview focused on the antioxidant activity of saffron related to its bioactive compounds to design the different functional products in food, medicine and cosmetic industries.

Keywords Saffron · Antioxidant activity · Bioactive compounds · Saffron extracts

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Introduction

A wide range of factors such as psychological stress, toxins, industrial lifestyle, infections, drugs, smoking, and obesity can cause many people to develop abnormally high levels oxidative stress (Makhlouf et al. 2011). Oxidative stress is considered to play an important role in a variety of neurodegenerative disorders such as Alzhemer's disease, Parkinson's disease and etc. (Soeda et al. 2007). Reactive oxygen species (ROS) and free radical (FR) have been implicated in the etiology of various human diseases including inflammation, cardiovascular disease, cancer, diabetes through induction of lipid peroxidation in cellular membranes (Choil et al. 2006). In addition to the body's natural antioxidant defense for removing FR and ROS, there would be need to provide the body with a constant supply of antioxidants through dietary supplementation. Natural products have long been used to prevent and treat many diseases including cancer and thus they are good candidates for the development of anti cancer drugs (Papandreou et al. 2011).

Saffron (*Crocus Sativus* Linnaeus) is a perennial herb and belongs to the *Iridaceae* family (Fig. 1; ISO 3632 2011) which has been cultivated in Iran, Greece, Morocco, India, Spain and Italy (Ebrahim-Habibi et al. 2010; Pintado et al. 2011; Modaghegh et al. 2008). Although the source of saffron is obscure, it is apparently originated from Asia Minor and Iran (Ahmad et al. 2011). It is estimated that saffron world production is about 205 t per year. Annual production of Iranian saffron is 80 % of the total which 85 % was obtained from Khorasan province, where has the best quality saffron of worldwide (Ahmad et al. 2011).

Saffron is used as one of the most commonly spices with flavoring and coloring properties in bakery products and beverages at a level of 260±1 ppm (Selim et al. 2000). Many studies have demonstrated various pharmacological effects of saffron and or its important compounds including antioxidant activity, anti tumor, memory and learning enhancing, treatment of hepatic disorders, anti inflammatory, anti depressant and insulin resistant reducing (Sanchez-Vioque et al. 2012; Makhlouf et al. 2011; Umigai et al. 2011; Ghadrdoost et al. 2011; Amin et al. 2011; Dhar et al. 2009; Nkhaei et al. 2008; Ochiai et al. 2007; Soeda et al. 2007; Abdullaev and Espinosa-Aguirre 2004). All these properties have been attributed to the stigmas, the sole commercially valuable part of the plant whereas other parts of the plant have been much less studied. It should be noted that several in vivo studies in animals have been indicated a very low or even non-existent toxicity of both saffron and its extracts. Other reports showed the toxic effect of 5 g and more amounts, with a lethal dose of approximately 20 g/kg which explains why toxicology researches currently consider it to be safe for human consumption (Bathaie and Mousavi 2010; Melnyk et al. 2010). The saffron use in food applications has been recently increased, despite its high price due to changes in consumer preference toward natural products (Amin et al. 2011).

Carotenoids are the red, orange and yellow tetraterpene pigments found in plants, algae and microorganisms, and they are thought to function as important antioxidants and functional ingredients in foods (Gharibzahedi et al. 2013). Interest in the impact of saffron carotenoids on human health is due to their high antioxidant capacity which was investigated by many researchers (Makhlouf et al. 2011; Asdaq and Inamdar 2010; Karimi et al. 2010; Bathaie and Mousavi 2010; Tavakkol-Afshari et al. 2008; Kanakis et al. 2007; Soeda et al. 2007; Magesh et al. 2006; Ochiai et al. 2004; Selim et al. 2000; Verma and Bordia 1998; Nair et al. 1995). One of hypothesis for the modes of anti carcinogenic and anti tumor actions of saffron has been proposed the inhibitory effect on free radical chain reactions (Makhlouf et al. 2011). Many studies have been shown the antioxidant and free- radical scavenging activities of saffron. Figure 2 demonstrates the percent of done studies on the antioxidant activity of saffron in different years.

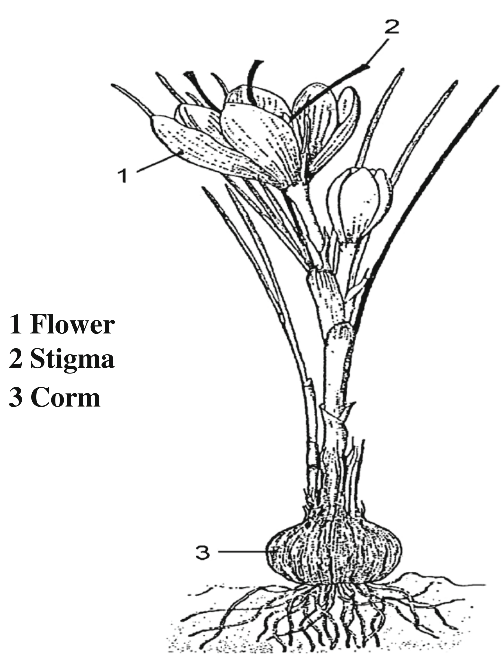

Fig. 1 Diagram of the organography of *Crocus sativus* L. (ISO 3632; 2011)

Therefore, this review paper will outline the current understanding of the antioxidant and free-radical scavenging activities of saffron capacities and various bioactive compounds commonly found in this golden spice.

Saffron chemistry

Crocus sativus L. stigma

The stigmas of the saffron flower contain many chemical substances. Ranges of all chemical constituents can vary greatly due to growing conditions and country of origin. Among the estimated more than 150 volatile and several non volatile compounds of saffron, approximately 40–50 constituents have already been identified (Bathaie and Mousavi 2010; Melnyk et al. 2010; Abdullaev and Espinosa-Aguirre 2004). Table 1 illustrates the chemical composition analyses of saffron stigma.

The obtained data showed that red stigmatic lobs of the *C. sativus* flower contain three main metabolites crocins, picrocrocin and safranal. The crocins include crocin 1–4 which are a group of water soluble and red colored carotenoids formed by esterification of crocetin (a dicarboxylic 20-carbon carotenoid, $C_{20}H_{24}O_4$) with different glycosids. Unlike most carotenoids, crocins are soluble in water due to a saccharide link with glucose, gentibiose or neapolitanose. Among the four crocins, crocin 1 or α-crocin is the most abundant in saffron and has been extensively studied for its antioxidant activity by quenching free radicals and protecting cells and tissues against oxidation. Besides, other minor carotenoids are also present in saffron such as carotenes, lycopene, zeaxanthin, mangicrocin and xanthone-carotenoid glycosidic conjugate (Licon et al. 2012; Maggi et al. 2011; Bathaie and Mousavi 2010; Hadizedeh et al. 2010; Asai et al. 2005; Li et al. 1999). picrocrocin ($C_{16}H_{26}O_7$) which is a colorless glycoside and product of zeaxanthin degradation and is the main substance responsible for the bitter taste of saffron. Picrocrocin is the second most abundant component (by weight) accounting for approximately 1 % to 13 % of saffron's dry matter. Safranal ($C_{10}H_{14}O$) is a product of natural deglycosylation of picrocrocin and responsible for the individual odor of saffron. Safranal represents about 30–70 % of volatile components and 0.001–0.006 % of dry matter of saffron. safranal is present in low amount in fresh stigmas and after drying of the stigmas or the passage of time, it is produced in high amount from its precursor (Maggi et al. 2011; Melnyk et al. 2010; Jalali-Heravi et al. 2009; Abdullaev and Espinosa-Aguirre 2004).

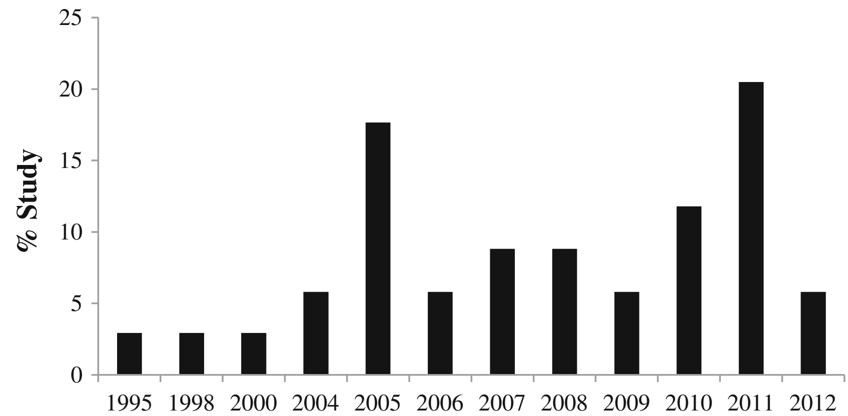

Fig. 2 Percent of literatures on the antioxidant activity of saffron in different years (obtained from Scopus database)

Saffron extracts

Aqueous methanol, ethanol or water is commonly used for the extraction of many bioactive constituents. Crocin as a water-soluble carotenoid can be effectively isolated from saffron stigma using the solvent-assisted extraction methods (Fig. 3). Effect of the use of water and different organic solvents such as ethanol or methanol at various concentrations on the total polyphenol content and antioxidant activity was studied for saffron (Makhlouf et al. 2011; Karimi et al. 2010). Makhlouf et al. (2011) have been studied the different conditions for extraction of saffron components. The following extracts were obtained from saffron soaked and boiled and extracted with water and methanol 50 % (v/v) using maceration method. Karimi et al. 2010 prepared saffron extracts using three different solvents of ethanol 80 % (v/v), methanol 80 % (v/v) and boiling water by shaking conventional method. In another study, the extraction yield of saffron components with water and 80 % (v/v) ethanol using a following maceration step (5 days at 4 °C) was respectively increased to 71.0 % and 58.4 % (Amin et al. 2011).

The results showed that solvent with different polarity had significant effect on polyphenol content and antioxidant activity. A high correlation between polyphenol content and antioxidant activity of saffron extracts was observed (Makhlouf et al. 2011; Amin et al. 2011; Karimi et al. 2010; Chen et al. 2008). Change in solvent polarity alters its ability to dissolve a selected group of antioxidant compounds and influences the antioxidant activity estimation.

Table 1 Analytical results of main components of saffron (Hosseinpour-Chermahini et al. 2010)

Type of component	Size (w/w %)
Proteins	12
Fats	5
Minerals	5
Crude fibre	5
Carbohydrate (starch, reducing sugars, gums, pectin and dextrins)	63
moisture	10

Antioxidant activity of bioactive compounds of saffron

Phenolic and flavonoid compounds

Several researches have shown that spices containing phenolic and flavonoid compounds indicated antioxidant activities, so they are frequently used as antioxidant food supplements (Rahaiee et al. 2012; Karimi et al. 2010; Martinez-Tome et al. 2001). The antioxidant property of *C. sativus* stigma could be credited to its phenolic content as well as to its active ingredients such as safranal, crocin, crocetin and carotene, all of which have been reported to have antioxidant properties (Karimi et al. 2010). Table 2 shows the antioxidative properties and scavenging activity of bioactive compounds of saffron.

Makhlouf et al. (2011) found that the total dosage of polyphenols in saffron (16 mg gallic acid/l) is higher than that in white grape juice (6.285 mg gallic acid/l). Their study showed that saffron extract obtained from the C. sativus flower had high amounts of polyphenol components which can effectively reduce activity of free radicals and can provide a strong protection for the different organs (liver, kidneys, lung and heart) against some oxidative damages under a dose-dependent behaviour. It was demonstrated that saffron as an excellent antioxidant source has more antioxidant capacity compared to white grape (Makhlouf et al. 2011).

The high dose of saffron was found to be more in counteracting the manifestation of hyperlipidemia than high dose of crocin. These propose that apart from crocin of saffron, there are other components responsible for synergistic antihyperlipidemic and antioxidant potential of saffron. The powerful hyperlipidemic activities can be directly linked with the presence of flavonoids in saffron as it is known that flavonoids have powerful hypolipidemic properties (Asdaq and Inamdar 2010).

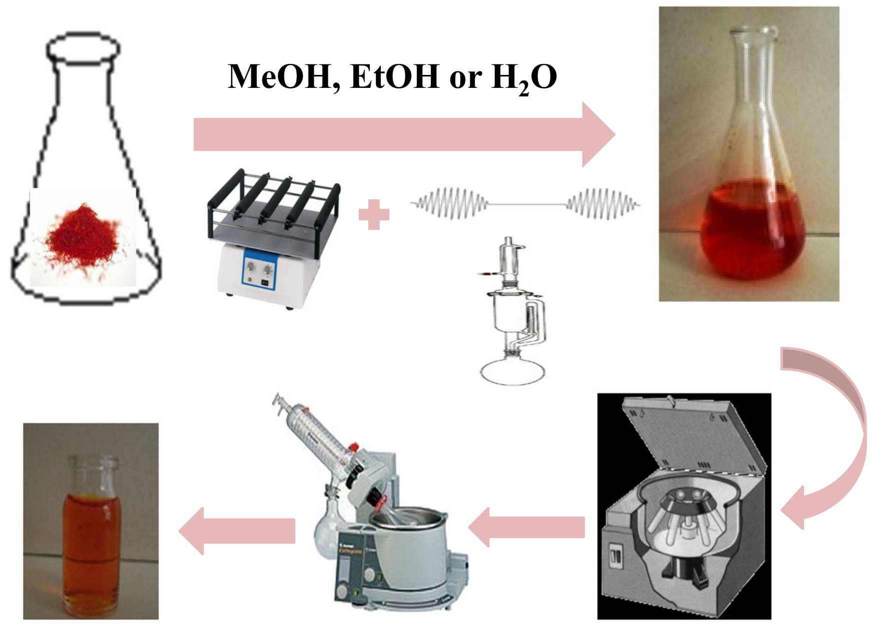

Fig. 3 Preparation stages of saffron extracts

Crocin and crocetin

Crocin bleaching assay was suggested as a suitable method for screening radical scavenging activity and assessment of total antioxidant activity of plasma. This method designed according to important property of crocin carotenoid as a basic element for the antioxidant activity of saffron (Bathaie et al. 2011; Bathaie and Mousavi 2010). Several studies suggested that sugars attached to the crocetin moiety probably play a key role in biological effects of crocins (Papandreou et al. 2006; Abe et al. 1998; Escribano et al. 1996). To the best of our

Table 2 Biological functions attributed to *Crocus sativus* L. (saffron)

Bioactive compounds	Type of function	Ref.
Phenolic content		
Safranal	ABTS radical scavenging activities	
Crocin	DPPH radical scavenging activities	Amin et al. 2011
Crocetin	Exhibition of significant reducing power	
Carotene		
Crocetin	Having potent antioxidant	Papandreou et al. 2011
	Showing protective effects against hepatotoxicity Genotoxicity	
Crocin	Potential of hypolipidemic	Asdaq and Inamdar 2010
	Antioxidant activity	
Crocin	Antioxidant activity	Karimi et al. 2010
Safranal		
Crocin	Prevention of the formation of peroxidized lipids Partly restored superoxide dismutase (SOD) activity	Bathaie and Mousavi 2010
Crocin	Antioxidant	Tavakkol-Afshari et al. 2008
Crocetin	Free radical scavenging activity	
Crocin	Treating neurodegenerative damage	Soeda et al. 2007
Crocetin	Antioxidative properties	Kanakis et al. 2007
Safranal		
Crocetin	Inhibition of lipid peroxidation	Magesh et al. 2006
	Increasing antioxidant status	
Crocin	Antioxidant effect	Ochiai et al. 2004
Crocetin	Antioxidant activity	Selim et al. 2000

knowledge, little work has been done to investigate the structure- antioxidant relationships of crocins. Thus, the protective effects of crocetin, crocin and saffron extract related to their structures require further in vivo comparative examination. Also, it is suggested that some of the variations in antioxidant capacity might be attributed to genotypic and environmental differences within species, the part of the plant studied, the time of year, the samples were taken and the analytical methods used (Shan et al. 2005).

Asdaq and Inamdar (2010) evaluated the hypolipidemic and antioxidant potential of saffron and its carotenoid pigment, crocin, in heperlipidemic rats. This study suggested the possible role of other constituents apart from crocin for potent antioxidant action of saffron. As discussed earlier, crocin, dimethyl crocetin, safranal and flavonoids quench free radicals and have antioxidant effects (Asdaq and Inamdar 2010). Although the importance of such characteristics for the activity of saffron water soluble carotenoids is not yet clear, it is accepted that the mechanistic paths for scavenging of free radicals are similar to those of the most known non polar carotenoids (Pham et al. 2000). It is postulated when bioactive molecules crocin and crocetin absorbed into blood plasma, they may modulate intracellular oxidative stress by activation of body's natural antioxidant enzymes (Ordoudi et al. 2009).

Ochiai et al. (2007) assessed the antioxidant activity of each crocin by monitoring the inhibition of coupled auto-oxidation of ß-carotene and linolenic acid emulsion in cell lysates. They showed that crocetin, a polyene di carboxylic acid consisting of seven conjugated double bonds and four side-chain methyl groups, had a key role in the antioxidant activity of carotenoids while, picrocrocin had no activity.

Depriving the Pc-12 cells (pheochtomocytoma cells) of serum/glucose caused peroxidation of their cell membrane lipids and decrease intercellular superoxide dismutase (SOD) activity. Ochiai et al. (2004) investigated crocin's neuroprotective effect by focusing on its initial action on the cell membrane of serum/glucose–deprived Pc-12 cells. The results suggested that crocin's antioxidant effect is stronger than that of α-tocopherol.

In a study by Soeda et al. (2007), crocin, as the unique water-soluble carotenoids in the nature, was found to become active in maintaining normal serum/glucose-deprived cells morphology than α-tocopherol. They concluded that crocin may have potential for treating neurodegenerative damage induced by oxidative stress. If antioxidant activity varies directly as the number of double bonds, therefore crocin which has seven double bonds in a molecule should be as more effective antioxidant than α-tocopherol (Bathaie and Mousavi 2010; Soeda et al. 2007). Assimopoulou et al. (2005) believed that the antioxidant activity of *C. sativus* stigma could be attributed to two bioactive compounds, crocin and safranal. Both contents were affected by nature of the solvent used. Crocin and safranal showed high radical scavenging activity, 50 % and 34 % for 500 ppm solution in methanol, respectively. Zheng et al. (2005) showed that administration of crocetin caused an increase the resistance of LDL to in vitro oxidation and decreased plasma levels of oxidized LDL, indicating that crocetin can exert an antioxidant effect in vivo.

Cancer continues to represent the largest cause of mortality in the world and needs to anticancer drugs with high efficacy and low toxicity. Since the relationship between saffron compounds and cancer is an important problem, comprehensive studies need to be conducted further along the following issues such as determination of the mechanism(s) involved in the therapeutic properties of saffron, study the mechanism(s) involved in saffron cancer chemoprevention, determine the biologically active components of saffron and human studies to define efficacy of saffron in cancer treatment and prevention (Hosseinpour-Chermahini et al. 2010). Among the different hypothesis underlying the modes of anticarcinogenic and anti tumor actions of saffron and its bioactive components, it is suggested that the inhibitory effect on free radical chain reactions may be related to antioxidant properties of saffron especially crocin compound which is regarded as the most promising anticancer compound in saffron (Melnyk et al. 2010; Hosseinpour-Chermahini et al. 2010). Thus, it is proposed that saffron and constituents, alone or in combination with other chemical substances, could attain particular relevance in future treatment of some cancers.

Antioxidant activity of aqueous and alcoholic extracts of saffron

Aqueous extract

The saffron was found to be superior to crocin, safranal etc. indicating the involvement of other potential constituents of saffron apart from crocin for its synergistic behavior of quenching the free radicals (Asdaq and Inamdar 2010). The antioxidant activity of saffron extract is dose dependent (Botsoglou et al. 2005). The antioxidant activity of different extracts of saffron is reported in Table 3.

Martinez-Tome et al. (2001) reported that saffron extract had significant hydrogen peroxide scavenging

Table 3 Antioxidant activity of different extracts of saffron

Type of extract	Observation	Ref.
Ethanolic extract Methanolic extract	Having radical scavenging activity	Montoro et al. 2012
Ethanolic extract Aqueous extract	The highest phenolic content in ehtanolic E. The best antioxidant activity in ethanolic E.	Amin et al. 2011
Aqueous extract	Reduction of free radicals	Makhlouf et al. 2011
Aqueous extract	Having antioxidant effects in chronic stress	Ghadrdoost et al. 2011
Boiling water Ethanolic extract Methanolic extract	Higher reductive potential (FRAP assay) in Methanolic extract Showing strong free radical scavenging activity	Karimi et al. 2010
Methanolic extract Water–methanol extract	Hydrogen peroxide scavenging activity	Bathaie and Mousavi 2010
Methanolic extract	Remarkable intracellular antioxidant activity	Ordoudi et al. 2009
Ethanolic extract	Showing good antioxidant activity by in vitro antioxidant models (Anti-hemolysis, DPPH radical scavenging Lipid peroxidation assay and phosphor molybdenum)	Chen et al. 2008
Aqueous extract	Reducing lipid peroxidation products Increasing antioxidant power	Hosseinzadeh et al. 2005

activity and lipid peroxidation inhibitory effect. Ghadrdoost et al. (2011) demonstrated the protective effects of saffron aqueous extract and crocin against chronic stress induced impairment of learning and memory. This finding suggested antioxidant effects of these substances in chronic stress.

Alcoholic extract

Phenolic and flavonoid content analyses of saffron extracts (boiling water, ehanolic and methanolic) by Karimi et al. (2010) revealed that different solvents had different contents of total phenolics and flavonoids. Phenolic and flavonoid contents were markedly higher in the methanolic extract; however, the antioxidant activity was affected by the nature of solvent used. Methanolic extract showed a higher reductive potential (by FRAP assay) and a strong free radical scavenging activity (by DPPH radical scavenging assay) than the boiling water and ehanolic extracts. This might be due to the presence of higher total Phenolics and flavonoids which play a major role in antioxidant capacities. Gallic acid as the major phenolic and pyrogallol as the major flavonoid compound in methanolic extract were also identified (Karimi et al. 2010). The antioxidant potential of ethanolic extract of *c. sativus* was also analyzed by Chen et al. (2008) who confirmed the antioxidant activities of this alcoholic extract using in vitro antioxidant modes including anti-hemolysis, DPPH radical scavenging, lipid peroxidation assay and phosphor molybdenum method. They evidenced that crocins in saffron extract have potent antioxidant effects and these properties are influenced by the sugars attached to the crocetin moiety. It was shown that the antioxidant activity of both methanol extract and water–methanol extract of saffron was higher than tomatos and carrots (Papandreou et al. 2006; Assimopoulou et al. 2005).

Referring to Ordoudi et al. (2009), the results reinforced the perception of saffron bioactivity through antioxidant mechanism of action. They pointed out that saffron methanolic extracts exhibit a remarkable intracellular antioxidant activity by using a single-cell model system (human monocyte) as effectively as the selected phenolic antioxidants such as rosmarinic acid, curcumin and Trolox.

Conclusion

Saffron is a useful medicine plant with a very low toxicity and shows the beneficial effect in many diseases. It acts as a multi potential drug and effects on various systems simultaneously. Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones.

Saffron extracts and its biological active compounds including crocin, crocetin, carotene and safranal have been shown both in vivo and in vitro antioxidant property. Many studies showed the Pharmacological and biological effects of saffron especially its alcoholic extracts due to its antioxidant activity. However, the synergistic effect of all the bioactive components gave saffron a significant antioxidant activity the same as vegetables rich in carotenoids. According to the results, it can be concluded that there are various causes for antioxidant activity of saffron including glucose sugars attached to crocetin, polyphenolic compounds and the presence of more double bonds. This increases its therapeutic importance and calls for more studies especially as a promising chemotherapeutic agent in cancer treatment in the future. This finding above makes the spice an excellent candidate for being a functional food.

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References

Abdullaev FI, Espinosa-Aguirre JJ (2004) Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detect Prev 28:426–432

Abe K, Sugiura M, Shoyama Y, Saito H (1998) Crocin antagonizes ethanol inhibition of NMDA recptor-mediated responses in rat hippocampal neurons. Brain Res 787:132–138

Ahmad M, Zaffar G, Mir SD, Razvi SM, Rather MA, Mir MR (2011) Saffron (*crocus sativus L.*) strategies for enhancing productivity. Res J Med Plant 5(6):630–649

Amin A, Hamza AA, Bajbouj K, Ashraf S, Daoud S (2011) Saffron: a potential candidate for a novel anticancer drug against hepatocellular carcinoma. Hepatology 54(3):857–867

Asai A, Nakano T, Takahashi M, Nagao A (2005) Orally administered crocetin and crocins are absorbed into blood plasma as crocetin and its glucuronide conjugates in mice. J Agric Food Chem 53:7302–7306

Asdaq SMB, Inamdar MN (2010) Potential of *Crocus sativus* (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. Appl Biochem Biotechnol 162:358–372

Assimopoulou AN, Sinakos Z, Papageorgiou VP (2005) Radical scavenging activity of *crocus sativus L.* extract and its bioactive constituents. Phytother Res 19:997–1000

Bathaie SZ, Mousavi SZ (2010) New applications and mechanisms of action of saffron and its important ingredients. Crit Rev Food Sci Nutr 50(8):761–768

Bathaie SZ, Moghadas Zadeh F, Shams A (2011) Crocin bleaching assay using purified Di-gentiobiosyl crocin (α-crocin) form Iranian saffron. Iran J Basic Med Sci 14(5):399–406

Botsoglou NA, Florou-Paneri P, Nikolakakis I, Giannenas I, Dotas V, Botsoglou EN, Aggelopoulos S (2005) Effect of dietary saffron (*Crocus sativus* L.) on the oxidative stability of egg yolk. Br Poult Sci 46:701–707

Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, Jia L, Yin HX, Chen HX (2008) Antioxidant potential of crocins and ethanol exracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: a relationahip investigation between antioxidant activity and crocin content. Food Chem 109:484–492

Choil WS, Kwak SS, Kim H (2006) Improvement of bioavailability of water insoluble drugs: potential of nano-sized grinding technique. Asian J pharm Sci 1(1):27–30

Dhar A, Mehta S, Dhar G, Dhar K, Banerjee S, Veldhuizen PV, Campbell DR, Banerjee SK (2009) Crocetin inhibits pancreatic cancer cell proliferation and tumor progression in a xenograft mouse model. Mol Cancer Ther 8(2):315–322

Ebrahim-Habibi MB, Amininasab M, Ebrahim-Habibi A, Sabbaghian M, Nemat-Gorgani M (2010) Fibrillation of α-lactalbumin: effect of crocin and safranal, two natural small molecules from *Crocus sativus.* Biopolymers 93:854–864

Escribano J, Alonso GL, Coca-prados M, Fernandez JA (1996) Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. Cancer Lett 100:23–30

Ghadrdoost B, Vafaei AA, Rashidy-Pour A, Bandegi AR, Motamedi F, Haghighi S, Samani HR, Pahlvan SH (2011) Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. Eur J Pharmacol 667(1–3):222–229

Gharibzahedi SMT, Razavi SH, Mousavi SM (2013) Comparison of antioxidant and free radical scavenging activities ofbiocolorant synthesized by *Dietzia natronolimnaea HS-1* cells grown inbatch, fed-batch and continuous cultures. Ind Crop Prod 49:10–16

Hadizedeh F, Mohajeri SA, Seifi M (2010) Extraction and purification of crocin from saffron stigma employing a simple and efficient crystallization method. Pak J Biol Sci 13(14):691–698

Hosseinpour-Chermahini S, Adibah-Ahd F, Roji-Sarmidi M, Taghizadeh E, Salehnezhad S (2010) Impact of saffron as an anti-cance and anti-tumor herb. Afr J Pharm Pharmacol 4(11):834–840

Hosseinzadeh H, Sadeghnia HR, Ziaee T, Danaee A (2005) 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 8:387–393

ISO 3632 (2011) Saffron (*Crocus sativus* L.). Part 1: Specification, Part 2: Test methods. International Organization for Standaization (ISO), Geneva

Jalali-Heravi M, Parastar H, Ebrahimi-Najafabadi H (2009) Characterization of volatile components of Iranian saffron using factorial-based response surface modeling of ultrasonic extraction combined with gas chromatography-mass spectrometry analysis. J Chrom A 1216: 6088–6097

Kanakis CD, Tarantilis PA, Taimir-Riahi H, Polissiou MG (2007) Crocetin, dimethylcrocetin, and safranal bind human serum albumin: stability and antioxidative properties. J Agric Food Chem 55: 970–977

Karimi E, Oskoueian E, Hendra R, Jaafar HZE (2010) Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules 15:6244–6256

Li N, Lin G, Kwan YW, Min ZD (1999) Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. J Chrom A 849:349–355

Licon CC, Rubio R, Molina A, Berruga MI (2012) Preliminary study of saffron (*Crocus sativus L.* stigma) color extraction in a dairy matrix. Dyes Pigm 92:1355–1360

Magesh V, Singh JPV, Selvendiran K, Ekambaram G, Sakthisekaran D (2006) Antitumor activity of crocetin in accordance to tumor incidence, antioxidant status, drug methabolizing enzymes and histopathological studies. Mol Cell Biochem 287:127–135

Maggi L, Carmona M, Kelly S, Marigheto N, Alonso GL (2011) Geographical origin differentiation of saffron spice (*Crocus sativus L.* stigmas) preliminary investigation using chemical and multi-element (H, C, N) stable isotope analysis. Food Chem 128:543–548

Makhlouf H, Saksouk M, Habib J, Chahine R (2011) Determination of antioxidant activity of saffron taken from the flower of *Crocus sativus* grown in Lebanon. Afr J Biotechnol 10(41):8093–8100

Martinez-Tome M, Jimenez AM, Ruggieri S, Frega N, Strabbioli R, Murcia MA (2001) Antioxidant properties of Mediterranean spices compared with common food additives. J Food Prot 64(1412):1419

Melnyk JP, Wang S, Marcone MF (2010) Chemical and biological properties of the world's most expensive spice: saffron. Food Res Int 43:1981–1989

Modaghegh MH, Shahabian M, Esmaeili H, Rajbai O, Hosseinzadeh H (2008) Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers. Phytomedicine 15:1032–1037

Montoro P, Maldini M, Luciani L, Tuberoso CIG, Congiu F, Pizza C (2012) Radical scavenging activity and LC-MS metabolic profiling of petals, stamens, and flowers of *Crocus sativus* L. J Food Sci 77(8): C893–C900

Nair SC, Kurumboor SK, Hasegawa JH (1995) Saffron chemoprevention in biology and medicine: a review. Cancer Biother 10(4):257–264

Nkhaei M, Khaje-Karamoddin M, Ramezani M (2008) Inhibition of *Helicobacter pylori* growth in vitro by saffron (*crocus sativus L.*). Iran J Basic Med Sci 11(2):91–96

Ochiai T, Ohno SH, Soedo SH, Tanaka H, Shoyama Y, Shimeno H (2004) Crocin prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effect stonger than those of α-tocopherol. Neurosci Lett 362:61–64

Ochiai T, Shimeno H, Mishima K, Iwasaki K, Fujiwara M (2007) Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. Biochim Biophys Acta 1770:578–584

Ordoudi SA, Befani CD, Nenadis N, Koliakos GG, Tsimidou MZ (2009) Further examination of antiradical properties of *crocus sativus* stigmas extract rich in crocins. J Agric Food Chem 57:3080–3086

Papandreou MA, Kanakis CD, Polissiou MG, Efthimiopoulos S, Cordopatis P (2006) Inhibitory activity on amyloid-ß-aggregation and antioxidant properties of *Crocus sativus* stigma extract and its crocin constituents. J Agric Food Chem 54:8762–8768

Papandreou MA, Tsachaki M, Efthimiopoulos S, Cordopatis P, Lamari FN, Margarity M (2011) Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. Behav Brain Res 219:197–204

Pham TQ, Cormier F, Farnworth E, Van Tong H, Van Calsteren M (2000) Antioxidant properties of crocin from *Gardenia jasminoides ellis* and study of the reactions of crocin with linoleic acid and crocin with oxygen. J Agric Food Chem 48:1455–1461

Pintado C, Miguel A, Acevedo O, Nozal L, Novella JL, Rotger R (2011) Bactericidal effect of saffron (*Crocus sativus L.*) on *salmonella enterica* during storage. Food Control 22:638–642

Rahaiee S, Gharibzahedi SMT, Razavi SH, Jafari SM (2012) Recent developments on new formulations based on nutrient-dense ingredients for the production of healthy-functional bread: a review. J Food Sci Technol. doi:10.1007/s13197-012-0833-6

Sanchez-Vioque R, Rodrigues-Conde MF, Reina-Urena JV, Escolano-Tercero MA, Herraiz-Penalver D, Santana-Meridas O (2012) In vitro antioxidant and metal chelating properties of corm, petal and leaf from saffron (*Crocus sativus* L.). Ind Crop Prod 39: 149–153

Selim K, Tsimidou M, Biliaderis CG (2000) Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices. Food Chem 71:199–206

Shan B, Cai YZ, Sun M, Croke H (2005) Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J Agric Food Chem 53:7749–7759

Soeda SH, Ochiai T, Shimeno H, Saito H, Abe K, Tanaka H, Shoyama Y (2007) Pharmacological activities of crocin in saffron. J Nat Med 61: 102–111

Tavakkol-Afshari J, Brook A, Mousavi SH (2008) Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. Food Chem Toxicol 46:3443–3447

Umigai N, Murakami K, Antonio LS, Shirotori M, Morikawa H, Nakano T (2011) The pharmacokinetic profile of crocetin in healthy adult human volunteers after a single oral administration. Phytomedicine 18:575–578

Verma SK, Bordia A (1998) Antioxidant property of saffron in man. Ind J Med Sci 52:205–207

Zheng SG, Qian ZY, Tang FT, Sheng L (2005) Suppression of vascular cell adhesion molecule-1 expression by crocetin contributes to attenuation of atherosclerosis in hypercholesterolemic rabbits. Biochem Pharmacol 70:1192–1199