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Review

Chemical and biological properties of the world's most expensive spice: Saffron

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ABSTRACT

Saffron (*Crocus sativus*, L.) is traditionally used as a coloring or flavoring agent, but recent research has shown its potential to promote health. The constituents of interest include crocin, crocetin, picrocrocin, and safranal which have all demonstrated health promoting properties. Previous studies have found that biological activity of saffron constituents alleviate or prevent such health problems as gastric disorders, cardiovascular disease, insulin resistance, depression, premenstrual syndrome, insomnia, and anxiety. Saffron also shows promise in the prevention and maintenance of cancer due to its antioxidant properties. The present review article highlights the constituents that are important in the treatment of each disorder as well as the mechanisms. Many of the studies were conducted using purified forms of the constituents or completed on animal subjects. The need for human subjects using saffron in its natural form is evident to determine the possible health benefits of dietary saffron.

Contents

1. Introduction

Saffron with its unique aroma, color, and flavor can by no means be considered a new introduction to 21st century cuisine and medicine. In fact, the history of saffron usage dates back nearly 3000 years, spanning many continents, civilizations, and cultures (Deo, 2003). Saffron, the highly desirable golden spice, is the dried elongated stigmas and styles of the blue-purple saffron flower (*Crocus sativus*, L.), a member of the Iridaceae (iris) family with origins in the Middle East. At nearly $40–50 per gram, it is the world's most expensive spice. It is estimated that it takes approximately 75,000 crocus blossoms or

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an astounding 225,000 stigmas to produce just one pound of this unique spice. The stigmas must be hand-picked from the delicate blossoms upon opening to preserve the desirable volatile components before evaporating in the heat of the day (Hill, 2004; Rau, 1969). With its unmatched signature bitter-like taste, slightly metallic sub-notes, and pungent hay-like aroma, saffron has found many precious uses ranging from fragrances to dyes to medicines, but it is especially favored as both a flavoring and coloring agent in food. While saffron is more tolerant to increasing temperatures, it easily degrades in the presence of light and oxidizing agents. As a result, the best saffron is usually sold whole (not powdered) in air-tight containers absent from light sources so as to preserve its integrity.

The limited production of saffron and its extremely high price have unfortunately made it the object of frequent adulteration at least as far back as the Middle Ages. In the 14th century, Nuremberg, Germany was the center of the European saffron trade with saffron grown in Crete, Austria, France, Greece, Sicily, and Spain passing through the hands of its enterprising merchants. But unfortunately much of the saffron was adulterated in ingenious and unsuspecting ways. To protect saffron's authenticity, the *Safranschou Code* was introduced and enforced, a code which contained specific standards for saffron as well as punishments for its adulteration. The penalties for fraud were quite severe as the code authorized officials to imprison or execute people found guilty of adulterating saffron. Strong measures are not only indicative of the lucrative profits to be made from the trade and sale of this golden spice, but is also indicative of the prevalence of its widespread fraud (Hagh-Nazari & Keifi, 2007). Today, a series of analytical methodologies have been developed to determine not only the quality but also to determine the adulteration type and level in the saffron. Natural and artificial additives such as coloring agents (*e.g.* ground paprika/turmeric common in ground saffron, water/moisture, and glycerin in intact saffron), organic compounds (*e.g.* honey and oil), and inorganic compounds (borates, sulfates, chlorides, and carbonates) have been used throughout the centuries (Hagh-Nazari & Keifi, 2007). Therefore, to ensure saffron's authenticity and quality, saffron is certified in the international trade market following the International Organisation for Standardization (ISO) 3632 Normative since 1993 (ISO 3632-1/2, Geneva, 2003).

Currently, the most popular usage of saffron continues to be as a food coloring and flavoring agent but there also appears to be adequate evidence to support the therapeutic benefits related to saffron consumption and also for its use in mediating various health disorders. These desirable benefits have been ascribed to the various chemical components found in varying amounts within the stigma themselves.

This review paper will outline the current understanding of the therapeutic properties of saffron and their relationship to the various phytochemicals commonly found in this golden spice.

2. Chemistry of saffron

2.1. Chemical composition of saffron

Chemical composition analyses have revealed a saffron composition of approximately 10% moisture, 12% protein, 5% fat, 5% minerals, 5% crude fibre, and 63% sugars including starch, reducing sugars, pentosans, gums, pectin, and dextrins (% w/w). Trace amounts of riboflavin and thiamine vitamins have also been identified in saffron (Rios, Recio, Giner, & Manez, 1996). Ranges of all chemical constituents can vary greatly due to growing conditions and country of origin.

Various analytical studies have been conducted to characterize the large number of potential biologically active compounds found within saffron. The four major bioactive compounds in saffron are crocin (mono-glycosyl or di-glycosyl polyene esters), crocetin (a natural carotenoid dicarboxylic acid precursor of crocin), picrocrocin (monoterpene glycoside precursor of safranal and product of zeaxanthin degradation), and safranal (Fig. 1), all contributing not only to the sensory profile of saffron (color, color, taste, and aroma, respectively), but also to the health-promoting properties which will be discussed in Section 3 (Rios et al., 1996).

Saffron's name is derived from the Arab word for yellow, a name reflecting the high concentration of carotenoid pigments present in the saffron flowers' stigmas which contribute most to the color profile of this spice. Both lipophilic carotenoids and hydrophilic carotenoids have been identified in saffron (Alonso, Salinas, Garijo, & Sanchez-Fernadez, 2001). The lipophilic carotenoids, lycopene, α-, and β-carotene, and zeaxanthin have been reported in trace amounts (Sampathu, Shivashankar, Lewis, & Wood, 1984). Of the carotenoids, the hydrophilic crocins constitutes approximately 6 to16% of saffron's total dry matter depending upon the variety, growing conditions, and processing methods (Gregory, Menary, & Davies, 2005). Crocin 1 (or α-crocin), a digentiobioside, is the most abundant crocin with a high solubility being attributed to these sugar moieties. Crocin, typically deep red in color, quickly dissolves in water to form an orange colored solution thereby making crocin widely used as a natural food colorant. In addition to being an excellent colorant, crocin also acts as an antioxidant by quenching free radicals, protecting cells and tissues against oxidation (Assimopoulou, Sinakos, & Papageorgiou, 2005; Papandreou et al., 2006; Soeda et al., 2007).

The actual taste of saffron is derived primarily from picrocrocin which is the second most abundant component (by weight), accounting for approximately 1% to 13% of saffron's dry matter (Alonso et al., 2001). Natural de-glycosylation of picrocrocin will yield another important chemical component, safranal, which is mainly responsible for the aroma of saffron.

Dehydration is not only important to the preservation of saffron but is actually critical in the release of safranal from picrocrocin via enzymatic activity, the reaction yielding D-glucose and safranal, the latter being the volatile oil in saffron. The six major volatile compounds in saffron are safranal, isophorone, 2,2,6-trimethyl-1,4-cyclohexanedione, 4-ketoisophorone, 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one as well as 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (Maggi et al., 2009) but more than 160 additional volatile components have been identified (Carmona, Zalacain, Salinas, & Alonso, 2007). Of these, safranal represents approximately 30 to 70% of essential oil and 0.001 to 0.006% of dry matter (Carmona et al., 2007; Maggi et al., 2009). Besides its typical spicy aromatic note, safranal has also been shown to have high antioxidant potential (Assimopoulou et al., 2005; Kanakis, Tarantilis, Tajmir-Riahi, & Polissiou, 2007) as well as cytotoxicity towards certain cancer cells *in vitro* (Escribano, Alonso, Coca-Prados, & Fernandez, 1996).

2.2. Extraction and purification of saffron's bioactive constituents

Aqueous methanol, ethanol, or water is commonly used for the extraction of many bioactive constituents. The extraction and purification of crocins is well described in the literature (Pfister, Meyer, Steck, & Pfander, 1996; Sugiura, Shoyama, Saito, & Abe, 1994; Zareena, Variyar, Gholap, & Bongirwar, 2001). After defatting with diethyl ether, stigmas are commonly extracted 2 or 3 times using 70–90% methanol or ethanol (~10 mL solvent/g saffron). Solvents containing extract are pooled, evaporated to dryness, and then purified using silica gel column chromatography with ethanol–ethyl acetate–water (~6:3:1) as the mobile phase. Crocin and crocetin esters are eluted separately (Sugiura et al., 1994).

Picrocrocin can be effectively isolated from saffron stigma by successive, exhaustive Soxhlet extraction using light petroleum, diethyl ether, and methanol to obtain three fractions (Tarantilis, Polissiou, & Manfait, 1994). The diethyl ether phase containing picrocrocin and lipids is evaporated to dryness, defatted using Soxhlet for picrocrocin purification, and then dissolved in methanol for filtration. The filtrate is then analyzed using HPLC. Tarantilis and colleagues effectively analyzed picrocrocin using an HPLC LiChroCART

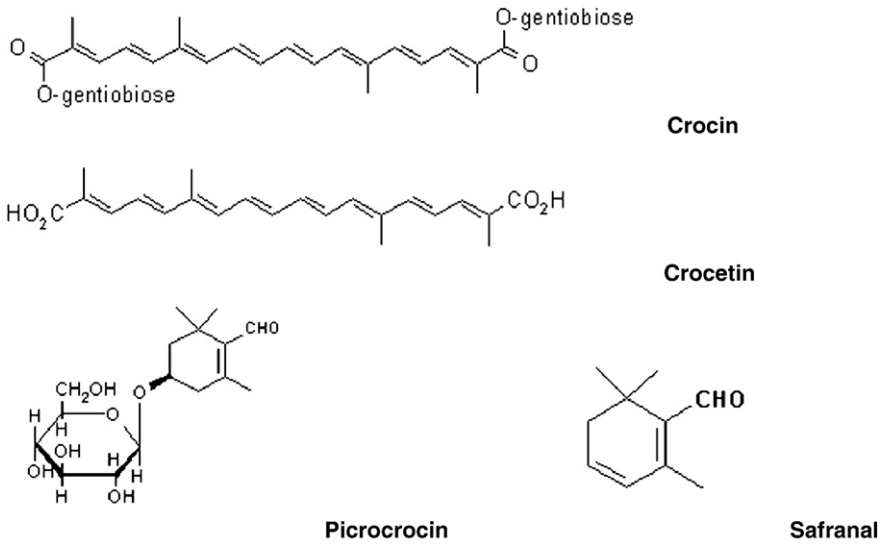

Fig. 1. Chemical structures of crocin, crocetin, picrocrocin, and safranal.

125-4 Superspher 100 RP-18 column and 20–100% ACN in water linear gradient mobile phase (Tarantilis et al., 1994).

Isolation of the volatile components in saffron stigma can be achieved by steam distillation or supercritical fluid extraction. Tarantilis and Polissiou (1997) tested three distillation methods for efficiency to extract representative volatile profiles of saffron. The steam distillation method is carried out for 4 h using 100 g of stigma. Micro-simultaneous steam distillation–solvent extraction (MSDE) is used to extract volatiles from 10 g of sample using 50 mL of deionized water and 3 mL of diethyl ether over 2 h. Vacuum head space distillation is achieved under vacuum with 100 g of stigma and water at 30 °C for 6 h. Condensed extract on dioxide snow-acetone at −70 °C is collected with 15 mL dichloromethane. Gas chromatography-mass spectroscopy reveals differences in the volatile components found with each method. MSDE was determined to extract a profile similar to saffron aroma, with 70% of the total extract being safranal. Isophorone, the second most abundant volatile, comprised 14% of the extract (Tarantilis & Polissiou, 1997). The supercritical fluid extraction (SFE) method described by Lozano and colleagues extracted safranal from 200 mg powdered saffron by holding supercritical fluid in the extraction chamber under pressure with sample for 5 min before circulating supercritical fluid through the extraction unit. Safranal was collected in 3 mL methanol for HPLC analysis, which revealed mainly safranal and a small amount of 4–hydroxy-2,6,6-trimethyl-1-carboxaldehyde-1-cyclohexane, a safranal precursor. The SFE method was developed as a non-destructive method for isolating safranal as the major volatile component in the extract (Lozano, Delgado, Gomez, Rubio, & Iborra, 2000).

3. Biological activity of saffron

With the development of *in vivo* and *in vitro* assays, various health-promoting properties of saffron have been reported. Some major reported biological functions attributable to saffron, as well as experimental conditions, dosage, and conclusions are summarized in Table 1.

3.1. The study of saffron on gastric disorders

In traditional Eastern medicine, saffron is commonly used for the treatment of gastric disorders. A 2009 study highlighted the effects of saffron as a potential anti-ulcer agent in mice (Kianbakht & Mozaffari, 2009). The study investigated the effectiveness of three different treatments (ethanolic saffron extract, commercial crocin, and commercial safranal) and determined that all three components demonstrated anti-ulcer activity similar to that of omeprazole, a proton pump inhibitor used to treat peptic ulcer disease. Saffron, crocin, and safranal displayed antioxidant properties that reduced ulcer formation by preventing indomethacin-induced gastric mucosa damage by increasing glutathione levels and preventing lipid oxidation (Kianbakht & Mozaffari, 2009). A similar study by Xu and colleagues investigated the ability of crocin to prevent stomach lesions in rats treated with indomethacin, an anti-inflammatory drug known to cause peptic ulcers (Xu et al., 2009). Results showed that crocin prevented damage to the stomach mucosa while damage was observed in the indomethacin-treated control rats (Xu et al., 2009). The study also highlighted the safety of crocin since the rats did not exhibit gastric ulceration at the highest crocin dose (50 mg/kg), the results being similar to a study by Kianbakht and colleagues who used a maximum dose of 10 mg/kg, also with no significant demonstrable safety concerns.

A similar study investigated the anti-ulcer properties of N-095, a nutrient drug containing 90 mg of saffron per daily dose (Inoue et al., 2005). N-095 has been attributed to many health benefits in rats including improved spatial cognition and increased blood flow in the hippocampus. After treating rats with N-095 for three days, the rats were subjected to stress or treated with ulcer-inducing histamine-2 HCl. Results showed that stress ulcers and histamine-induced ulcers were prevented due to treatment with N-095 (Inoue et al., 2005). However ulcer inhibition may not be solely attributed to the presence of saffron in the drug since it contains other potentially bioactive compounds that are used in traditional Chinese medicine, such as red ginseng, polygala root, antelope horn and aloe wood.

Saffron may also have health benefits by improving digestion. A recent study by Nabavizadeh and colleagues demonstrated the benefits of aqueous saffron extract on digestion (Nabavizadeh, Salimi, Sadroleslami, Karimian, & Vahedian, 2009). The study investigated gastric acid and pepsin outputs in mice fed saline or 100 mg/kg saffron and found that mice treated with saffron had a significantly higher output of acid and pepsin. Saffron may activate nitric oxide synthetase, which enhances histamine release from cells which increases gastric and pepsin secretions (Nabavizadeh et al., 2009). The results show the potential of saffron to improve digestion of proteins with the benefit of lower pH conditions aiding in digestion, however future studies are needed to determine the effect of increased gastric acid output on ulceration.

3.2. The study of saffron's anti-carcinogenic properties

Among the many reported biological properties of saffron, the anti-carcinogenic properties are of great interest and are intensively

Table 1
Major reported biological functions attributed to saffron.

	Saffron and its constituents tested	*In vitro/in vivo* model or human/animal subjects	Observations	Reference
Beneficial health effects				
Gastric disorder prevention				
Ulcer prevention	90 mg saffron in crude drug N-095	Wistar/ST rats	100 mg/kg or greater N-095 prevented gastric ulcers in rats under restraint and water immersion stress conditions	Inoue et al. (2005)
	Ethanolic saffron extract	Male adult Wistar rats	25, 100, or 250 mg/kg saffron extract prevented gastric lesions in diabetic rats	Kianbakht and Mozaffari (2009)
	Crocin	Male Kunming and Sprague–Dawley rats	12, 25, and 50 mg/kg prevented damage to the stomach mucosa	Xu et al. (2009)
Digestion enhancement	Aqueous saffron extract	Wistar rats	100 mg/kg caused higher outputs of gastric acid and pepsin	Nabavizadeh et al. (2009)
Anti-cancer functions				
Antiproliferation or cytotoxicity on tumor cells	Ethanolic saffron extract	Human cervical carcinoma HeLa and hepatocellular carcinoma HepG2 cells	The 50% cell growth inhibition (IC50) values of ethanolic saffron extract against HeLa and HepG2 were 800 and 950 μg/ml after 48 h, respectively.	Tavakkol-Afshari et al. (2008)
	Ethanolic saffron extract, crocin, crocetin, safranal, and picrocrocin	Human cervical carcinoma HeLa cells	The IC50 values against HeLa were 2.3 mg/ml for an ethanolic saffron extract, 3 mM for crocin, 0.8 mM for safranal and 3 mM for picrocrocin. Crocetin did not show cytotoxic effect.	Escribano et al. (1996)
	Crocin	Human colon adenocarcinoma HT-29 cells and rat DHD/K12-PROb cells	The IC50 of crocin against HT-29 and DHD/K12-PROb cells are 0.4 mM and 1.0 mM, respectively	Garcia-Olmo et al. (1999)
	Saffron extract, safranal, and picrocrocin	Human cervical carcinoma HeLa cells	IC 50 of saffron extract, safranal, and picrocrocin were 2.3 mg/ml, 0.8 mM and 3 mM, respectively	Escribano et al. (1996), Abdullaev (2002)
	Saffron extract	Human breast cancer MCF-7 cells	200–2000 μg/ml saffron extract inhibited proliferation of MCF-7 cells in a dose-time dependent manner. IC50 = 400 ± 18.5 μg/ml after 48 h	Mousavi et al. (2009)
	Crocetin, trans-crocin-4, and safranal	Human breast cancer MCF-7 and MDA-MB-231 cells	Crocetin, trans-crocin-4, and safranal inhibited the growth of both cancer cells. The antiproliferative effect is attributed to the constituent crocins irrespective of the degree of glycosylation.	Chryssanthi et al. (2007)
	Crocetin	Human rhabdomyosarcoma RD cells and African green monkey kidney Vero cells.	10, 15 and 20 μg/mL crocetin demonstrated a cytotoxic selectivity towards malignant RD cells, with no effect on normal Vero cells	Jagadeeswaran et al. (2000)
	Crocin and diglucosylcrocetin	Mouse lymphoma cells	Crocin and diglucosylcrocetin inhibited early tumor antigen expression in adenovirus infected cells	Molnar et al. (2000)
Tumor inhibition	Crocin	Colon tumour implanted female and male BD-IX rats	Significant effects on female rats at concentrations of 400 mg/kg body weight; no effect on male rats	Garcia-Olmo et al. (1999)
	Saffron	MCA-induced soft tissue sarcomas in albino mice	Oral administration of saffron (100 mg/kg body wt) for 30 days restricted 10% MCA-induced tumor incidence compared with 100% in MCA-treated controls. (MCA: 20-methyl-cholanthrene)	Salomi et al. (1991)
	Crocetin	Mouse fibroblast NIH/3T3 cells	60 and 120 pM crocetin inhibited TPA-induced protein kinase C (PKC) activity by 50% and 66%, respectively. (TPA :12-0 tetradecanoy1phorbo1-13-acetate)	Wang et al. (1996)
DNA destabilization	Crocin, crocetin, and dimethylcrocetin (DMC)	Calf thymus DNA (ctDNA) and oligonucleotides.	Potential interaction with DNA was ordered crocetin>DMC>>crocin.	Bathaie et al. (2007)
Cardiovascular health promotion				
Anti-atherosclerosis	Crocin (from *Gardenia jasminoids* plant)	Bovine aortic endothelial cells (EC), bovine aortic smooth muscle cells (SMC), and quails	Crocin decreased Ox-LDL induced EC apoptosis as well as SMC proliferation. Crocin decreased Ox-LDL and thus inhibited the formation of atherosclerosis in quails.	He et al. (2005)
	Crocetin (from *Gardenia jasminoids* plant)	Quails	9-week treatment with crocetin (25, 50, 100 mg/kg/day) reduced serum, total cholesterol, triglycerides, LDL cholesterol levels, and inhibited the formation of aortic plaque, reduced malonaldehyde, and prevented decreased nitric oxide in serum.	He et al. (2007)
	Crocin	Rats	10-day treatment with crocin (25 to 100 mg/kg/day) significantly reduced serum triglyceride, total cholesterol, LDL cholesterol and VLDL cholesterol levels. The hyperlipidemic effect of crocin is attributed to its pancreatic lipase inhibition.	Sheng et al. (2006)
	Crocetin	Atherosclerotic rabbits	Crocetin suppressed vascular cell adhesion molecule-1 (VCAM-1) expression and ameliorated atherosclerosis.	Zheng et al. (2005)
Insulin resistance prevention	Crocetin	Male Wistar rats	Crocetin (40 mg/kg) prevented dexanethasone-induced insulin resistant.	Xi et al. (2005)
	Crocetin	Male Wistar rats	Crocetin (40 mg/kg) improved insulin sensitivity in fructose-fed rats via normalizing the expression of both protein and mRNA of adiponectin (an insulin-sensitizing adipocytokine), TNF-α, and leptinin epididymal white adipose tissue.	Xi et al. (2007)

Table 1 (*continued*)

	Saffron and its constituents tested	*In vitro*/*in vivo* model or human/animal subjects	Observations	Reference
Depression treatment				
	Capsulated ethanolic saffron extract	Thirty adult outpatients	In the 6-week randomized and double-blind clinical trial, saffron (30 mg capsules of saffron per day) was found to be effective similar to imipramine (100 mg capsule of imipramine per day) in the treatment of mild to moderate depression.	Akhondzadeh et al. (2004)
	Capsulated ethanolic saffron extract	Forty adult outpatients	In a 6-week double-blind, placebo-controlled and randomized trial, supplementation of 30 mg capsules of saffron per day was effective in the treatment of mild to moderate depression.	Akhondzadeh et al. (2005)
	Saffron petal extract	Forty adult outpatients	In the 8-week pilot, double-blind randomized trial, petal extract (30 mg/day) was effective similar to fluoxetine (20 mg/day) in the treatment of mild to moderate depression.	Akhondzadeh Basti et al. (2007)
	Saffron petal extract	Forty adult outpatients	In the 6-week double-blind, placebo-controlled and randomized trial, 30 mg/day petal extract was effective in the treatment of mild to moderate depression.	Moshiri et al. (2006)
	Capsulated ethanolic saffron extract	Forty adult outpatients	In the 6-week randomized and double-blind clinical trial, saffron (30 mg capsule per day) was found to be effective similar to fluoxetine (20 mg capsule per day) in the treatment of mild to moderate depression.	Noorbala et al. (2005)
	Aqueous and ethanolic saffron extract, crocin, and safranal	Male BALB/c mice	The antidepressant activity was evaluated via forced swimming test. The aqueous and ethanolic extracts of stigma (0.2–0.8 g/kg), safranal (0.15–0.5 ml/kg) and crocin (50–600 mg/kg) reduced immobility time. Both extracts, safranal, and crocin increased swimming time.	Hosseinzadeh et al. (2004)
Premenstrual syndrome (PMS) treatment				
	Capsulated ethanolic saffron extract	Women aged 20–45 years with regular menstrual cycles and experience of PMS symptoms for at least 6 months	In the double-blind, randomised and placebo-controlled trial, supplementations of 30 mg/day saffron capsules (15 mg twice a day; morning and evening) was effective in the treatment of PMS in women.	Agha-Hosseini et al. (2008)
Anxiolytic and hypnotic effects				
	Crocin	Male Wistar rats	50 mg/kg crocin had anxiolytic effects; 15 and 30 mg/kg crocin did not influence animals' behaviour.	Pitsikas et al. (2008)
	Aqueous saffron extract, and safranal	Razi male mice	Saffron aqueous extract (0.56 g/kg) and safranal (0.15 and 0.35 mL/kg) had anxiolytic and hypnotic effects. Crocin had no effect.	Hosseinzadeh and Noraei (2009)
Detrimental health effects				
Nausea, vomiting, uterus bleeding, abortion	Saffron	Human and pregnant women	1.2 g to 2 g provokes symptoms.	Schmidt et al. (2007)
Allergic reactions	Saffron	Human	An anaphylactic reaction due to saffron is rare.	Lucas et al. (2001)

investigated using both *in vitro* and *in vivo* assays and are summarized in Table 1.

3.2.1. In vitro observations of anticancer properties of saffron

In vitro studies have shown that crocin is regarded as the most promising anticancer compound in saffron as it has been reported to have inhibitory effects against a wide range of cancer cells (Abdullaev, 2002). Spanish researchers extracted four major compounds from their saffron samples (*i.e.* crocin, crocetin, picrocrocin, and safranal) and evaluated their inhibitory effects on the proliferation of human cervical carcinoma Hela cells (Escribano et al., 1996). Their results indicated that of the four compounds investigated, crocin produced the best inhibitory effects. Garcia-Olmo and colleagues also reported that crocin had a potent cytotoxic effect on human and animal adenocarcinoma cells (HT-29 and DHD/K12-PROb cells, 50% LD = 0.4 and 1.0 mM, respectively) (Garcia-Olmo et al., 1999). Cells with the crocin treatment exhibited a remarkable loss of cytoplasm and large cytoplasmic vacuole-like areas. Chryssanthi and colleagues have also demonstrated the anti-proliferation effect of crocins on breast cancer cells, MCF-7 and MDA-MB-231, which was later confirmed by Mousavi using saffron extract (Chryssanthi et al., 2007; Mousavi, Tavakkol-Afshari, Brook, & Jafari-Anarkooli, 2009). An investigation by Molnar and colleagues reported that both crocin and diglucosyl-crocetin possessed inhibitory effects on the early tumor antigen expression of adenovirus-infected cells (Molnar et al., 2000). On the other hand, crocetin at concentration of 5–20 μg/mL has been shown to have selective cytotoxicity toward human rhabdomyosarcoma (RD) cells with less effect on the normal (Vero) cells compared with the anticancer drug cisplatin treatments. Cisplatin is a commonly used drug for human RD treatment (Jagadeeswaran, Thirunavukkarasu, Gunasekaran, Ramamurty, & Sakthisekaran, 2000). Recently Dhar's laboratory demonstrated that crocetin in both *in vitro* and *in vivo* experiments demonstrated significant anti-tumorigenic effects on pancreatic cancer (Dhar et al., 2009). Most of these *in vivo* studies were interested in the isolated bioactive compounds of saffron. Little research has been done to examine anticancer properties of saffron in its natural form. Tavakkol-Afshari and colleagues reported that 96% ethanol saffron extract is selectively cytotoxic against epithelial-like human hepatocellular carcinoma cells (HepG2) as well as human cervical carcinoma cells (HeLa) but nontoxic towards normal mouse fibroblast cells (L929) (Tavakkol-Afshari, Brook, & Mousavi, 2008). The additive and synergistic effects among the different phytochemicals in saffron however, may enhance its anti-carcinogenic properties (Liu, 2004). Based on these current results, more studies are warranted to determine the beneficial effects of saffron in its natural form using human subjects.

3.2.2. In vivo observations of anticancer properties of saffron

Many *in vivo* experiments have also demonstrated the ability of saffron extracts to inhibit the growth of soft tissue sarcomas (Salomi, Nair, & Panikkar, 1991). Salomi, Nair, & Panikkar designed a 12-week trial to test saffron extract's ability to modulate chemically induced cancer in albino mice. They found that 90% of mice developed papillomas (2–7 papillomas per mouse) without saffron treatment while saffron treated (100 mg/kg) mice only developed 0.26–1.0 papilloma (Salomi et al., 1991). Previously, Wang and colleagues demonstrated that crocetin was a potent inhibitor of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion in mouse skin. They were able to successfully demonstrate that 60 and 120 μM crocetin can inhibit enzymatic activity of TPA-induced protein kinase C (PKC) in the particulate fraction of mouse fibroblast NIH/3T3 cells by 50% and 66%, respectively, without changing enzyme levels (Wang et al., 1996). Interestingly, Garcia-Olmo's laboratory also reported that supplementations of crocin slowed tumor growth but only in female rats, with no significant anti-tumour effect in male rats (Garcia-Olmo et al., 1999) suggesting a possible protective contribution or mitigating effect by at least one of the hormones unique to females.

3.2.3. Potential mechanisms of saffron's anti-carcinogenic properties

Despite accumulating studies demonstrating that saffron may be a promising cancer therapy agent, mechanisms of saffron anticancer actions are still largely unknown. Many proposed mechanisms have been reported. Researchers have suggested that saffron may directly target DNA sequences and modulate gene expression. Bathaie found that saffron carotenoids (crocin, crocetin, and dimethylcrocetin) directly bind to DNA minor grooves and induce conformational changes of targeted DNA (Bathaie et al., 2007). It has also been suggested that saffron may be a good apoptotic inducers of tumor cells. The induction of apoptosis of saffron has been identified to play an important role in the death of human hepatocellular carcinoma cells (HepG2) and human cervical carcinoma cells (HeLa) (Tavakkol-Afshari et al., 2008). Although saffron demonstrates potential as an anticancer drug, little data is available from clinical trials. More studies are needed to determine the effective dose and the mechanisms behind saffron's anti-carcinogenic properties.

3.3. The study of saffron on the cardiovascular system

3.3.1. Cardiovascular related disorders

Cardiovascular disease is the greatest cause of death globally, claiming approximately 17 million lives each year (World Health Organization, 2009). The high death rate highlights the need for effective methods to treat the many disorders classified as cardiovascular diseases. Recent studies, summarized in Table 1, have shown the potential of saffron constituents in the treatment of atherosclerosis (He et al., 2005, 2007; Zheng, Qian, Tang, & Sheng, 2005). In the 2005 study, Zheng and colleagues administered crocetin, the natural carotenoid antioxidant, to rabbits to determine its effect on the development of atherosclerosis. New Zealand white rabbits were randomly assigned to three different diets for eight weeks — a standard diet, a high lipid diet (HLD), or a high lipid + crocetin diet. The HLD group developed hypercholesterolemia and atherosclerosis, while the crocetin-supplemented group decreased the negative health effects of a high lipid diet (Zheng et al., 2005). Results did not show a significant difference in plasma lipid levels (total-, low density lipoprotein-, and high density lipoprotein cholesterol) between the HLD and crocetin groups, but did show a significant decrease in aorta cholesterol deposits, atheroma, foam cells, and atherosclerotic lesions in the crocetin-fed group. Zheng suggested that nuclear factor kappa B (NF-κB) activation in aortas is suppressed by antioxidants such as crocetin which in turn decreases the vascular cell adhesion molecule-1 (VCAM-1) expression. Since VCAM-1 expression is associated with increased foam cells and lipids within arterial walls, a reduction in VCAM-1 may help reduce lipid deposition in arterial walls. The study shows the potential of antioxidants such as crocetin to ameliorate atherosclerosis and aortic lesions in rabbits (Zheng et al., 2005). Results of this study were confirmed by He's research team in quails using crocetin and crocin extracted from *Gardenia jasminoides* plants (He et al., 2005, 2007). In the 2007 study, control group and hyperlipidemic diet group were compared to crocetin groups fed 25, 50, and 100 mg/kg/day, with results showing inhibition of increased total serum cholesterol, LDL, and very low density lipoproteins (VLDL) that were significant compared to the hyperlipidemic diet fed group (He et al., 2007). Aortic intima thickening and accumulation of foam cells within the aortic intima were also inhibited in both crocin and crocetin fed groups (He et al., 2005, 2007). The studies also demonstrated the potential of crocin and crocetin to prevent increased levels of serum malonaldehyde and decreases in serum nitric oxide levels compared to the hyperlipidemic fed group. These results show the potential of crocin and crocetin to lower serum and aortic lipid accumulation, prevent lipid peroxidation, and prevent atherosclerotic lesions in quails (He et al., 2005, 2007). The mechanisms by which crocin inhibited atherosclerosis in quails is attributed to the decreased uptake of oxidized-LDL, inhibiting the formation of foam cells as well as mitigating atherosclerotic symptoms (He et al., 2005). A 2006 study by Sheng and colleagues looked at an alternative mechanism for crocin's atherosclerotic properties (Sheng, Qian, Zheng, & Xi, 2006). Crocin inhibited an increase in serum triglycerides, total-, LDL-, and VLDL cholesterol compared to the control group as seen before, however results also showed a significant increase in fecal excretion of fat and cholesterol in the crocin group (100 mg/kg/day). Further studies determined that crocin inhibited pancreatic and gastric lipase activity, although a potential mechanism was not offered. Since pancreatic lipase is responsible for fat absorption by hydrolyzing fat, inhibition of pancreatic lipase activity resulted in low lipid absorption. With a lack of potential pancreatic lipase inhibitors available, crocin shows promise as a drug for treating hyperlipidemia (Sheng et al., 2006).

3.3.2. Insulin resistance

Recent studies have also demonstrated the potential of crocetin to reduce insulin resistance in rats (Xi, Qian, Shen, Wen, & Zhang, 2005; Xi et al., 2007). In a 2005 study, rats were administered dexamethasone (a glucocorticoid that contributes to insulin resistance) and dexamethasone + crocetin for a six-week period. The dexamethasone + crocetin fed group had significantly lower levels of serum insulin, free fatty acids (FFA), triglycerides, and tumor necrosis factor-α (TNF-α) compared to the pure dexamethasone group. TNF-α, over-expressed in insulin resistant states, leads to reduced activity of glucose transporter 4 which leads to a deficiency in insulin-stimulated glucose absorption (Xi et al., 2005). Reduced TNF-α and serum insulin levels in the crocetin fed groups provides evidence of crocetin as a treatment against insulin resistance. No possible mechanisms were reported (Xi et al., 2005). The 2007 study by the same group compared the effect of crocetin extracted from saffron to rats fed a high fructose diet, with results showing significantly lower blood pressure as well as lower levels of epididymal adipose tissue, serum triglycerides, FFA, LDL-C, HDL-C, and serum insulin in the high-dose crocetin group (Xi et al., 2007). The fructose fed group exhibited increases in all, compared to the crocetin group, highlighting the potential of crocetin to treat insulin resistance.

The cardiovascular related studies have determined that crocetin (and crocin) can ameliorate the effects of atherosclerosis and related diseases; hypercholesterolemia, hyperlipidemia, hyperinsulinemia, hypertriglyceridemia, hypertension, and insulin resistance (He et al., 2005, 2007; Sheng et al., 2006; Xi et al., 2005, 2007; Zheng et al., 2005). It is important to note, however, that most of the studies have utilized crocetin extracted from a source other than saffron (*C. sativus*,

L.); namely from the *G. jasminoides* plant (He et al., 2005, 2007; Sheng et al., 2006; Xi et al., 2005). More studies comparing crocetin from saffron and other sources are necessary to confirm the effects of crocetin from the ingestion of saffron. Large scale double-blind placebo controlled trials would be beneficial to determine if the positive health benefits can be translated to humans.

3.4. The study of saffron on depression

Depression is a serious mental disorder that is experienced by many individuals spanning age, gender, and cultural boundaries. Many synthetic drugs have been developed to treat depression but can have undesirable side effects. As a result, people have turned to natural herbal treatments such as saffron in the hopes of alleviating their depression. Recent studies on saffron's antidepressant effects are compared in Table 1.

Saffron is considered an excellent therapeutic plant for depression treatment in traditional medicine (Akhondzadeh et al., 2005). In the first clinical trials ever conducted, Akhondzadeh and colleagues investigated the effectiveness of saffron stigma ethanolic extract to treat mild to moderate depression (Akhondzadeh, Fallah-Pour, Afkham, Jamshidi, & Khalighi-Cigaroudi, 2004; Akhondzadeh et al., 2005). In the 2005 study, they found that saffron statistically improved the moods of people compared to placebo group after receiving 30 mg/day of saffron for six weeks based on the Hamilton Depression Rating Scale (HAM-D). One year earlier, the same research group in Iran conducted a similar study comparing saffron stigma extract to the antidepressant drug imipramine and reported that both had a positive effect on depression based on the HAM-D with no significant difference between the two treatments (Akhondzadeh et al., 2004). A similar study by Noorbala and colleagues determined that saffron extracts were effective in treating mild to moderate depression similar to fluoxetine (the antidepressant, Prozac) after 30 mg/day intake for six weeks (Noorbala, Akhondzadeh, Tahmacebi-Pour, & Jamshidi, 2005). The study administered saffron capsules or fluoxetine to patients and measured their response to treatment using the HAM-D. The study found no significant differences between saffron and fluoxetine (Noorbala et al., 2005) but highlighted saffron's potential to be used as natural antidepressant, a replacement for the synthetically produced drugs fluoxetine and imipramine.

In addition to the recent studies using human subjects to highlight the antidepressant activity of saffron, experiments have been conducted using mice and rats to further support this claim. Hosseinzadeh, Karimi, and Niapoor investigated four saffron treatments, fluoxetine, and imipramine for their ability to reduce depression symptoms in mice using the forced swimming test (Hosseinzadeh, Karimi, & Niapoor, 2004). Mice were administered crocin, safranal, aqueous, or ethanol stigma extracts intraperitoneally. The test measured immobility time, swimming time, and climbing time as an indicator for antidepressant activity in mice. Crocin decreased immobility time (50, 200, and 600 mg/kg dose), and increased climbing time (50, 200, and 600 mg/kg). Safranal decreased immobility time, and increased swimming and climbing time at 0.5 mL/kg. The aqueous and ethanol extract generally showed a significant decrease in immobility time, and an increase in swimming time similar to fluoxetine. A decrease in immobility time in the forced swimming test indicates antidepressant activity. All four treatments resulted in a decrease in mouse immobility time (Hosseinzadeh et al., 2004).

In addition to stigma extract and crocin studies, saffron petal extracts were also investigated for antidepressant effects on human subjects (Akhondzadeh Basti et al., 2007; Moshiri et al., 2006). Ethanolic petal extract (30 mg/day) or placebo capsules were administered for eight weeks, after which patients in the saffron group showed significantly lower readings on the HAM-D (Moshiri et al., 2006). One year later, the same group compared the effect of petal extract (30 mg/day) to fluoxetine (20 mg/day) with results showing a significant decrease in HAM-D score, similar in both treatment groups (Akhondzadeh Basti et al., 2007). In the same year, these results were confirmed in both mice and rats in a forced swimming test in which both rodents received kaempferol, saffron petal's active constituent, in doses of 100 and 200 mg/kg in mice and 50 mg/kg in rats. The results showed a significant decrease in immobility comparable to fluoxetine in both the mice and the rats. Although petal extract does not contain crocin and other active constituents of the stigma discussed in this review, it does contain kaempferol, a natural flavonoid shown to have antidepressant effects (Hadizadeh, Khalili, Hosseinzadeh, & Khair-Aldine, 2003). These studies highlight the potential to utilize the remaining, less expensive flower components not used in saffron stigma extraction that can be obtained in large quantities for the use as natural drugs.

3.5. The study of saffron on premenstrual syndrome

Premenstrual syndrome (PMS) is a diagnosable disorder affecting up to 40% of women of reproductive age. It is characterized as a group of physical and emotional disorders experienced during the second half of the menstrual cycle (Agha-Hosseini et al., 2008). With recent studies highlighting the potential of saffron to treat mild depression, saffron has been suggested as an effective means to alleviate the emotional symptoms associated with PMS (Akhondzadeh et al., 2005; Moshiri et al., 2006; Noorbala et al., 2005). A recent study by Agha-Hosseini and colleagues demonstrated that saffron petal extract reduced the symptoms of PMS in women (Agha-Hosseini et al., 2008). Two groups of women receiving either 30 mg/day saffron or placebo were evaluated for PMS symptoms using a Premenstrual Daily Symptoms (PDS) questionnaire and Hamilton Depression Rating Scale (HAM-D). Results showed a significant improvement in both tests (PDS and HAM-D) for the women in the saffron group compared to their pre-treatment symptoms. They also showed a significant improvement in symptoms compared to the control group (Agha-Hosseini et al., 2008). Further studies in this area may help to confirm saffron constituents as an effective treatment for alleviating PMS symptoms in women.

3.6. The study of saffron on anxiety and insomnia

Insomnia is a challenging health problem to treat and is characterized by having difficulty falling asleep or staying asleep, leading to tiredness, anxiety, or disruption of normal habits for period of one month or more (Morin, 2000). Approximately 58% of American adults suffer insomnia at least several nights per week. Using natural substances to treat insomnia and anxiety is favoured by the public, because of their relatively mild side effects. Saffron has demonstrated such treatment potential in recent research.

A study by Pitsikas and others in 2008 demonstrated the anti-anxiety activity of crocin compared to diazepam (anti-anxiety drug) on rats using a light/dark test (Pitsikas, Boultadakis, Georgiadou, Tarantilis, & Sakellaridis, 2008). The test places rats in a two-chambered box, one light and one dark, and measures time spent in each as an indicator of anxiety based on the idea that rats will desire the dark area if experiencing high anxiety. Results showed that crocin administered intraperitoneally at the highest dose (50 mg/kg) reduced anxiety similar to diazepam as demonstrated by increased latency to initially enter the dark chamber and increased overall time spent in the light chamber.

Hosseinzadeh and Noraei used a pentobarbital sleeping time test, an elevated plus maze test, an open field test, and a rotarod test to evaluate hypnotic activity, anxiolytic activity, locomotor activity as well as motor coordination and balance respective of saffron, safranal, and crocin in mice (Hosseinzadeh & Noraei, 2009). With doses of 56 and 80 mg/kg saffron, mice showed a significant increase in the percentage of time spent in the open areas of the maze which was considered to reflect a good anxiolytic effect. A dose of 560 mg/kg

saffron significantly increased the sleeping time in mice compared with the controls which indicated high hypnotic activity. The saffron extracts also decreased motion balance and function in the rotarod system at 30 and 60 min after injection as well as decreased the open field parameters at most doses. In terms of saffron's two major bioactive compounds, only safranal has anxiolytic effect at concentrations of 0.15 and 0.35 mL/kg while crocin has no demonstrable anxiolytic, hypnotic, or myorelaxation effects. These findings indicate a different effect resulting from consumption of whole saffron compared to its individual components. Again the synergistic effects of different phytochemicals may contribute to saffron's more powerful treatment of insomnia and anxiety. The mechanisms are still unknown.

4. Detrimental effects of saffron

The lack of saffron safety information has resulted in research focusing precisely on this important issue. The few studies that have been conducted have produced contradictory results. In some cases, injections of 1.2 to 2 g/per average body weight of saffron may cause nausea, vomiting, diarrhea, and bleeding whereas in other cases, no adverse affects accompanied the ingestion of 4 g of saffron per day for several days, even in pregnant women. However it is not clear whether these German studies used *C. sativus* or if the studies were conducted using meadow saffron (*Colchicum autumnale*), which is abundant in Germany (Schmidt, Betti, & Hensel, 2007). According to another study, doses of more than 10 g of saffron may induce abortion with reported side effects including decreased appetite, drowsiness, nausea, vomiting, uterine bleeding, haematuria, bleeding of the gastrointestinal mucosa, vertigo, and dizziness (Schmidt et al., 2007). In only very rare cases does saffron extract cause allergic reactions (Lucas, Hallagan, & Taylor, 2001). Saffron has a high $LD_{50} = 20$ g/Kg which explains why toxicology researchers currently consider it to be safe for human consumption (Bisset & Wichtl, 1994). The actual amount used in daily food consumption is much lower than the dose causing any of the reported side effects. The dose required for positive health benefits reported in this review are in accordance with the amount of saffron used in various cuisines.

Compared to human trials, *in vivo* studies in animals indicate a very low or even non-existent toxicity of both saffron and its extracts (Karimi, Hosseinzadeh, & Khaleghpanah, 2001; Nair, Kurumboor, & Hasegawa, 1995; Nair, Pannikar, & Panikkar, 1991). It should be noted that many of the positive effects of saffron have been established using *in vitro* or *in vivo* animal studies, but whether these positive effects are identical in humans still remains unclear. Further clinical research to elucidate the potential benefits or detrimental effects in humans is warranted.

5. Conclusion

The use of saffron for food coloring and flavoring by the general public is widely accepted throughout the world and by many cultural groups. However, scientists worldwide are more attracted to saffron's potential for biological or pharmacological function, which may be attributed to the large number of phytochemicals found in saffron. Among these phytochemicals, crocins, crocetin, picrocrocin, and safranal are considered the most medicinally bioactive and the most frequently examined in many *in vitro* and *in vivo* studies. These experimental studies clearly indicate that consumption of saffron positively correlates with a lower risk of diseases including metabolic disorders (gastric disorder), premenstrual syndrome, depression, insomnia and anxiety, cardiovascular disease, as well as many types of cancers. The most positive observations, however, were observed in animal models which raises many questions: How do those potential benefits translate to human models? Are there differences between animal tolerance of saffron and that of humans? How safe is saffron for humans? It should also be noted that different effects due to saffron crude extracts (containing all phytochemicals) and its bioactive compounds (purified phytochemicals) could be possible. Further studies are needed to understand the difference in health benefits due to crude extracts and purified forms. In terms of medical usage, further large-scale epidemiological investigations, clinical trials, and laboratory research are needed to elucidate the mechanisms and effects of saffron on human health.

References

Abdullaev, F. I. (2002). Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). *Experimental Biology and Medicine*, *227*, 20—25.

Agha-Hosseini, M., Kashani, L., Aleyaseen, A., Ghoreishi, A., Rahmanpour, H., Zarrinara, A. R., et al. (2008). *Crocus sativus* L. (saffron) in the treatment of premenstrual syndrome: A double-blind, randomised and placebo-controlled trial. *BJOG — An International Journal of Obstetrics and Gynaecology*, *115*(4), 515—519.

Akhondzadeh Basti, A., Moshiri, E., Noorbala, A., Jamshidi, A., Abbasi, S. H., & Akhondzadeh, S. (2007). Comparison of petal of *Crocus sativus* L. and fluoxetine in the treatment of depressed outpatients: A pilot double-blind randomized trial. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *31*(2), 439—442.

Akhondzadeh, S., Fallah-Pour, H., Afkham, K., Jamshidi, A., & Khalighi-Cigaroudi, F. (2004). Comparison of *Crocus sativus* L. and imipramine in the treatment of mild to moderate depression: A pilot double-blind randomized trial. *BMC Complementary Alternative Medicine*, *4*, 12.

Akhondzadeh, S., Tahmacebi-Pour, N., Noorbala, A., Amini, H., Fallah-Pour, H., Jamshidi, A., et al. (2005). *Crocus sativus* L. in the treatment of mild to moderate depression: A double-blind, randomized and placebo controlled trial. *Phytotherapy Research*, *19*, 148—151.

Alonso, G. L., Salinas, M. R., Garijo, J., & Sanchez-Fernadez, M. A. (2001). Composition of crocins and picrocrocin from Spanish saffron (*Crocus sativus* L.). *Journal of Food Quality*, *24*(3), 219—233.

Assimopoulou, A. N., Sinakos, Z., & Papageorgiou, V. P. (2005). Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytotherapy Research*, *19*, 997—1000.

Bathaie, S. Z., Bolhasani, A., Hoshyar, R., Ranjbar, B., Sabouni, F., & Moosavi-Movahedi, A. (2007). Interaction of saffron carotenoids as anticancer compounds with ctDNA, Oligo (dG.dC)15, and Oligo (dA.dT)15. *DNA and Cell Biology*, *26*(8), 533—540.

Bisset, N. G., & Wichtl, M. (1994). *Herbal drugs and phytopharmaceuticals*, 3rd ed. Stuttgart: Medpharm GmbH Scientific Publishers.

Carmona, M., Zalacain, A., Salinas, M. R., & Alonso, G. L. (2007). A new approach to saffron aroma. *Critical Reviews in Food Science and Nutrition*, *47*, 145—159.

Chryssanthi, D. G., Lamari, F. N., Iatrou, G., Pylara, A., Karamanos, N. K., & Cordopatis, P. (2007). Inhibition of breast cancer cell proliferation by style constituents of different *Crocus* species. *Anticancer Research*, *27*(1A), 357—362.

Deo, B. (2003). Growing saffron — The world's most expensive spice. *Crop & Food Research*, *20*, 1—4.

Dhar, A., Mehta, S., Dhar, G., Dhar, K., Banerjee, S., Van Veldhuizen, P., et al. (2009). Crocetin inhibits pancreatic cancer cell proliferation and tumor progression in a xenograft mouse model. *Molecular Cancer Therapy*, *8*(2), 315—323.

Escribano, J., Alonso, G. L., Coca-Prados, M., & Fernandez, J. A. (1996). Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. *Cancer Letters*, *100*, 23—30.

Garcia-Olmo, D. C., Riese, H. H., Escribano, J., Ontanon, J., Fernandez, J. A., Atienzar, M., et al. (1999). Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (*Crocus sativus* L.): An experimental study in the rat. *Nutrition and Cancer*, *35*(2), 120—126.

Gregory, M. J., Menary, R. C., & Davies, N. W. (2005). Effect of drying temperature and air flow on the production and retention of secondary metabolites in saffron. *Journal of Agricultural and Food Chemistry*, *53*(15), 5969—5975.

Hadizadeh, F., Khalili, N., Hosseinzadeh, H., & Khair-Aldine, R. (2003). Kaempferol from saffron petals. *Iranian Journal of Pharmaceutical Research*, *2*, 251—252.

Hagh-Nazari, S., & Keifi, N. (2007). Saffron and various fraud manners in its production and trades. *Acta Horticulturae*, *739*, 411—416.

He, S. Y., Qian, Z. Y., Tang, F. T., Wen, N., Xu, G. L., & Sheng, L. (2005). Effect of crocin on experimental atherosclerosis in quails and its mechanisms. *Life Sciences*, *77*(8), 907—921.

He, S. Y., Qian, Z. Y., Wen, N., Tang, F. T., Xu, G. L., & Zhou, C. H. (2007). Influence of crocetin on experimental atherosclerosis in hyperlipidemic-diet quails. *European Journal of Pharmacology*, *554*(2–3), 191—195.

Hill, T. (2004). *The contemporary encyclopedia of herbs and spices: Seasonings for the global kitchen*. Hoboken: John Wiley & Sons, Inc.

Hosseinzadeh, H., Karimi, G., & Niapoor, M. (2004). Antidepressant effect of *Crocus sativus* L. stigma extracts and their constituents, crocin and safranal, in mice. *Proceedings of the 1st International Symposium on Saffron Biology and Biotechnolgy*, *650*, 435—445.

Hosseinzadeh, H., & Noraei, N. B. (2009). Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents, crocin and safranal, in mice. *Phytotherapy Research*, *23*(6), 768—774.

Inoue, E., Shimizu, Y., Shoji, M., Tsuchida, H., Sano, Y., & Ito, C. (2005). Pharmacological properties of N-095, a drug containing red ginseng, polygala root, saffron, antelope horn and aloe wood. *American Journal of Chinese Medicine*, *33*(1), 49—60.

ISO Technical Specifications 3632-1/2. (2003). *Saffron (Crocus sativus L.). Part 1: Specifications, and Part 2: Test methods.*

Jagadeeswaran, R., Thirunavukkarasu, C., Gunasekaran, P., Ramamurty, N., & Sakthisekaran, D. (2000). *In vitro* studies on the selective cytotoxic effect of crocetin and quercetin. *Fitoterapia*, *71*(4), 395−399.

Kanakis, C. D., Tarantilis, P. A., Tajmir-Riahi, H. A., & Polissiou, M. G. (2007). Crocetin, dimethylcrocetin, and safranal bind human serum albumin: Stability and antoxidative properties. *Journal of Agricultural and Food Chemistry*, *55*(3), 970−977.

Karimi, G., Hosseinzadeh, H., & Khaleghpanah, P. (2001). Study of antidepressant effect of aqueous and ethanolic extracts of *Crocus sativus* in mice. *Iranian Journal of Basic Medical Science*, *4*(3–11), 11−15.

Kianbakht, S., & Mozaffari, K. (2009). Effects of saffron and its active constituents, crocin and safranal on prevention of indomethacin induced gastric ulcers in diabetic and nondiabetic rats. *Journal of Medicinal Plants*, *8*(5), 30−38.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *Journal of Nutrition*, *134*(12), 3479S−3485S.

Lozano, P., Delgado, D., Gomez, D., Rubio, M., & Iborra, J. L. (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*(1–3), 367−378.

Lucas, C. D., Hallagan, J. B., & Taylor, S. L. (2001). The role of natural color additives in food allergy. *Advances in Food and Nutrition Research*, *43*, 195−216.

Maggi, L., Carmona, M., del Campo, C. P., Kanakis, C. D., Anastasaki, E., Tarantilis, P. A., et al. (2009). Worldwide market screening of saffron volatile composition. *Journal of the Science of Food and Agriculture*, *89*(11), 1950−1954.

Molnar, J., Szabo, D., Pusztai, R., Mucsi, I., Berek, L., Ocsovszki, I., et al. (2000). Membrane associated antitumor effects of crocine-, ginsenoside- and cannabinoid derivates. *Anticancer Research*, *20*(2A), 861−867.

Morin, C. M. (2000). The nature of insomnia and the need to refine our diagnostic criteria. *Psychosomatic Medicine*, *62*(4), 483−485.

Moshiri, E., Akhondzadeh Basti, A., Noorbala, A. A., Jamshidi, A. H., Abbasi, S. H., & Akhondzadeh, S. (2006). *Crocus sativus* L. (petal) in the treatment of mild-to-moderate depression: A double-blind, randomized and placebo-controlled trial. *Phytomedicine*, *13*(9–10), 607−611.

Mousavi, S. H., Tavakkol-Afshari, J., Brook, A., & Jafari-Anarkooli, I. (2009). Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells. *Food and Chemical Toxicology*, *47*(8), 1909−1913.

Nabavizadeh, F., Salimi, E., Sadroleslami, Z., Karimian, S. M., & Vahedian, J. (2009). Saffron (*Crocus sativus*) increases gastric acid and pepsin secretions in rats: Role of nitric oxide (NO). *African Journal of Pharmacy and Pharmacology*, *3*(5), 181−184.

Nair, S. C., Kurumboor, S. K., & Hasegawa, J. H. (1995). Saffron chemoprevention in biology and medicine: A review. *Cancer Biotherapy*, *10*(4), 257−264.

Nair, S. C., Pannikar, B., & Panikkar, K. R. (1991). Antitumour activity of saffron (*Crocus sativus*). *Cancer Letters*, *57*(2), 109−114.

Noorbala, A. A., Akhondzadeh, S., Tahmacebi-Pour, N., & Jamshidi, A. H. (2005). Hydro-alcoholic extract of *Crocus sativus* L. versus fluoxetine in the treatment of mild to moderate depression: A double-blind, randomized pilot trial. *Journal of Ethnopharmacology*, *97*(2), 281−284.

Papandreou, M. A., Kanakis, C. D., Polissiou, M. G., Efthimiopoulos, S., Cordopatis, P., Margarity, M., et al. (2006). Inhibitory activity on amyloid-β aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its crocin constituents. *Journal of Agricultural and Food Chemistry*, *54*(23), 8762−8768.

Pfister, S., Meyer, P., Steck, A., & Pfander, H. (1996). Isolation and structure elucidation of carotenoid-glycosol esters in gardenia fruits (*Gardenia jasminoides* Ellis) and saffron (*Crocus sativus* Linne). *Journal of Agricultural and Food Chemistry*, *44*(9), 2612−2615.

Pitsikas, N., Boultadakis, A., Georgiadou, G., Tarantilis, P. A., & Sakellaridis, N. (2008). Effects of the active constituents of *Crocus sativus* L., crocins, in animal model of anxiety. *Phytomedicine*, *15*(12), 1135−1139.

Rau, S. R. (1969). *The cooking of India (foods of the world)*. USA: Time Life Education.

Rios, J. L., Recio, M. C., Giner, R. M., & Manez, S. (1996). A update review of saffron and its active constituents. *Phytotherapy Research*, *10*, 189−193.

Salomi, M. J., Nair, S. C., & Panikkar, K. R. (1991). Inhibitory effects of *Nigella sativa* and saffron (*Crocus sativus*) on chemical carcinogenesis in mice. *Nutrition and Cancer*, *16*(1), 67−72.

Sampathu, S. R., Shivashankar, S., Lewis, Y. S., & Wood, A. B. (1984). Saffron (*Crocus sativus* Linn.) − Cultivation, processing, chemistry and standardization. *Critical Reviews in Food Science and Nutrition*, *20*(2), 123−157.

Schmidt, M., Betti, G., & Hensel, A. (2007). Saffron in phytotherapy: Pharmacology and clinical uses. *Wiener Medizinische Wochenschrift*, *157*(13–14), 315−319.

Sheng, L., Qian, Z., Zheng, S., & Xi, L. (2006). Mechanism of hypolipidemic effect of crocin in rats: Crocin inhibits pancreatic lipase. *European Journal of Pharmacology*, *543*(1–3), 116−122.

Soeda, S., Ochiai, T., Shimeno, H., Saito, H., Abe, K., Tanaka, H., et al. (2007). Pharmacological activities of crocin in saffron. *Journal of Natural Medicine*, *61*(2), 102−111.

Sugiura, M., Shoyama, Y., Saito, H., & Abe, K. (1994). Crocin (crocetin di-gentiobiose ester) prevents the inhibitory effect of ethanol on long-term potentiation in the dentate gyrus in vivo. *The Journal of Pharmacology and Experimental Therapeutics*, *271*(2), 703−707.

Tarantilis, P. A., & Polissiou, M. G. (1997). Isolation and identification of the aroma components from saffron (*Crocus sativus*). *Journal of Agricultural and Food Chemistry*, *45*(2), 456−462.

Tarantilis, P. A., 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*(1), 55−61.

Tavakkol-Afshari, J., Brook, A., & Mousavi, S. H. (2008). Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. *Food and Chemical Toxicology*, *46*(11), 3443−3447.

Wang, C. J., Cheng, T. C., Liu, J. Y., Chou, F. P., Kuo, M. L., & Lin, J. K. (1996). Inhibition of protein kinase C and proto-oncogene expression by crocetin in NIH/3T3 cells. *Molecular Carcinogenesis*, *17*(4), 235−240.

World Health Organization (2009). *Cardiovascular diseases fact sheet number 317*. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/index.html. Accessed February, 2010.

Xi, L., Qian, Z. Y., Shen, X. C., Wen, N., & Zhang, Y. B. (2005). Crocetin prevents dexamethasone-induced insulin resistance in rats. *Planta Medica*, *71*(10), 917−922.

Xi, L., Qian, Z., Xu, G., Zheng, S., Sun, S., Wen, N., et al. (2007). Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructose-fed rats. *Journal of Nutritional Biochemistry*, *18*(1), 64−72.

Xu, G. L., Li, G., Ma, H. P., Zhong, H., Liu, F., & Ao, G. Z. (2009). Preventive effect of crocin in inflamed animals and in LPS-challenged RAW 264.7 cells. *Journal of Agricultural and Food Chemistry*, *57*(18), 8325−8330.

Zareena, A. V., Variyar, P. S., Gholap, A. S., & Bongirwar, D. R. (2001). Chemical investigation of gamma-irradiated saffron (*Crocus sativus* L.). *Journal of Agricultural and Food Chemistry*, *49*(2), 687−691.

Zheng, S. G., Qian, Z. Y., Tang, F. T., & Sheng, L. (2005). Suppression of vascular cell adhesion molecule-1 expression by crocetin contributes to attenuation of atherosclerosis in hypercholesterolemic rabbits. *Biochemical Pharmacology*, *70*(8), 1192−1199.