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.

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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
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 Safranchou 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 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, pentoses, 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 (mono-pentene 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-Fernandez, 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 to 16% 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 digentibioside, 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; Papandreu 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 α-glucose and safranal, the latter being the volatile oil in saffron. The six major volatile compounds in saffron are safranal, isosaffanal, 2,2,6-trimethyl-1,4-cyclohexanediol, 4-ketoisoporphone, 2-hydroxy-4,6,8-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; Sugiuera, Shoyama, Saito, & Abe, 1994; Zareena, Varyar, 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 (Sugiuera 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
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, Sadroeslami, 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.

<table>
<thead>
<tr>
<th>Saffron and its constituents tested</th>
<th>In vitro/in vivo model or human/animal subjects</th>
<th>Observations</th>
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<tr>
<td><strong>Beneficial health effects</strong></td>
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<td><strong>Gastric disorder prevention</strong></td>
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<td>Ulcer prevention</td>
<td>90 mg saffron in crude drug N-095</td>
<td>100 mg/kg or greater N-095 prevented gastric ulcers in rats under restraint and water immersion stress conditions 25, 100, or 250 mg/kg saffron extract prevented gastric lesions in diabetic rats</td>
<td>Inoue et al. (2005)</td>
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<td>Ethanolic saffron extract</td>
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<td>Male adult Wistar rats</td>
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<td></td>
<td>Crocin</td>
<td>12, 25, and 50 mg/kg prevented damage to the stomach mucosa</td>
<td>Kianbakht and Mozaffari (2009)</td>
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<td>Male Kunming and Sprague-Dawley rats</td>
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<td></td>
<td>Aqueous saffron extract</td>
<td>100 mg/kg caused higher outputs of gastric acid and pepsin</td>
<td>Nabavizadeh et al. (2009)</td>
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<td>Digestion enhancement</td>
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<td>Anti-cancer functions</td>
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<td>Ethanol extract, crocin, safranal, and picrocrocin</td>
<td>Human cervical carcinoma</td>
<td>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.</td>
<td>Tavakkol-Afshari et al. (2008)</td>
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<td></td>
<td>Human cervical carcinoma HeLa and hepatocellular carcinoma HepG2 cells</td>
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<td>Ethanol extract, crocin, safranal, and picrocrocin</td>
<td>Human cervical carcinoma HeLa cells</td>
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<td>Escrivan et al. (1996)</td>
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<td>Crocin</td>
<td>Human colon adenocarcinoma HT-29 cells and rat DHD/K12-PROb cells</td>
<td>The IC50 of crocin against HT-29 and DHD/K12-PROb cells are 0.4 mM and 1.0 mM, respectively</td>
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<td>Saffron extract, safranal, and picrocrocin</td>
<td>Human cervical carcinoma HeLa cells</td>
<td>IC 50 of saffron extract, safranal, and picrocrocin were 2.3 mg/ml, 0.8 mM and 3 mM, respectively</td>
<td>Escrivan et al. (1999), Abdulaleev (2002)</td>
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<td>Saffron extract</td>
<td>Human breast cancer MCF-7 cells</td>
<td>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</td>
<td>Mousavi et al. (2009)</td>
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<td>Crocin, trans-crocin-4, and safranal</td>
<td>Human breast cancer MCF-7 and MDA-MB-231 cells</td>
<td>Crocin, 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.</td>
<td>Chryssanthi et al. (2007)</td>
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<td>Crocin</td>
<td>Human rhabdomyosarcoma RD cells and African green monkey kidney Vero cells.</td>
<td>10, 15 and 20 μg/mL crocin demonstrated a cytotoxic selectivity towards malignant RD cells, with no effect on normal Vero cells</td>
<td>Jagadeeswaran et al. (2000)</td>
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<td>Crocin and diglucosylcrocetin</td>
<td>Mouse lymphoma cells</td>
<td>Crocin and diglucosylcrocetin inhibited early tumor antigen expression in adenosine infected cells</td>
<td>Molnar et al. (2000)</td>
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<td>Tumor inhibition</td>
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<td>Crocin</td>
<td>Colon tumour implanted female and male BD-4X rats</td>
<td>Significant effects on female rats at concentrations of 400 μg/kg body weight; no effect on male rats</td>
<td>Garcia-Olimo et al. (1999), Saloni et al. (1991)</td>
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<td>Saffron</td>
<td>MCA-induced soft tissue sarcomas in albino mice</td>
<td>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)</td>
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<td>Crocin</td>
<td>Mouse fibroblast NIH/3T3 cells</td>
<td>60 and 120 μM crocin inhibited TPA-induced protein kinase C (PKC) activity by 50% and 66%, respectively. (TPA :12-0 tetradecanoylphorbo1-13-acetate)</td>
<td>Wang et al. (1996)</td>
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<td>DNA destabilization</td>
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<td>Potential interaction with DNA was ordered crocin&lt;DMC&gt;crocin.</td>
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<td>Cardiovascular health promotion</td>
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<td>Crocin (from <em>Gardenia jasminoides</em> plant)</td>
<td>Calf thymus DNA (ctDNA) and oligonucleotides.</td>
<td>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.</td>
<td>He et al. (2005)</td>
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<tr>
<td>Crocin (from <em>Gardenia jasminoides</em> plant)</td>
<td>Quails</td>
<td>9-week treatment with crocin (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.</td>
<td>He et al. (2007)</td>
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<tr>
<td>Crocin</td>
<td>Rats</td>
<td>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.</td>
<td>Sheng et al. (2006)</td>
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<td>Insulin resistance prevention</td>
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<td>Crocin</td>
<td>Male Wistar rats</td>
<td>Crocin (40 mg/kg) prevented dexamethasone-induced insulin resistant.</td>
<td>Xi et al. (2005)</td>
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<tr>
<td>Crocin</td>
<td>Male Wistar rats</td>
<td>Crocin (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 leptin in epididymal white adipose tissue.</td>
<td>Xi et al. (2007)</td>
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</table>
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 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-Afsahi, 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, crocin 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, & Saktishekar, 2000). Recently Dhar's laboratory demonstrated that crocin 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-Afsahi 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-Afsahi, 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, García-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 et al. suggested 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 + crocin for a six-week period. The dexamethasone + crocin 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 crocin 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, hypertriglyceridermia, 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,
extracts were also investigated for antidepressant effects on human time (Hosseinzadeh et al., 2004). Immobility time in the forced swimming test indicates antidepressant and an increase in swimming time similar to Niapoor, 2004). Mice were administered crocin, safranal, aqueous, or in mice using the forced swimming test (Hosseinzadeh, Karimi, & 600 mg/kg). Safranal decreased immobility time, and increased 200, and 600 mg/kg dose), and increased climbing time (50, 200, and 600 mg/kg). The test measured immobility similar 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 (Hadjizadeh, 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.

3.4. 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 ethanol 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 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 (Hadjizadeh, 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 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 favored 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, Boulidakis, 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.

Hosseinazadeh 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 (Hosseinazadeh & 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 rotorod 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, hypnrotic, 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, bleeds 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 LD50 = 20 g/Kg which explains why toxicology researchers currently consider it safe to be used 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, Hosseinizadeh, & 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 active 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
