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Comparison of phytochemical constituents and antioxidant activities of aqueous and alcoholic extracts of saffron

S. Rahaiee[1], M. Hashemi[2*], S. Moini[1*], S. Abbas Shojaosadati[3] and S.H. Razavi[1]

[1]*Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, P.O. Box 4111, Karaj 31587-77871, Iran;* [2]*Department of Microbial Biotechnology and Biosafety, Agricultural Biotechnology Research Institute of Iran (ABRII), Shahid Fahmideh street, P.O. Box 31535-1897, Karaj, Iran;* [3]*Biotechnology Group, Department of Chemical Engineering, Tarbiat Modarres University, P.O. Box 14115-143, Tehran 82883803, Iran; dr_smoini@yahoo.com; hashemim@abrii.ac.ir*

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RESEARCH ARTICLE

Abstract

In the present work, phytochemical compounds and antioxidant activities of three aqueous, ethanolic (50 and 80%, v/v) and methanolic (50 and 80%, v/v) extracts of saffron during 1 and 24 h of shaking time were compared. Results showed that the ethanolic solution (80%, v/v) extracted for 24 h had the highest amounts of phenolic compounds (29.2 g gallic acid (Gal)/g dry weight (DW)), followed by 1 h aqueous extract (17.53 mg Gal/g DW) and 1 h methanol extract (50%, v/v, 14.5 mg Gal/g DW). The total flavonoid contents for all extracts were varied from 0.20 to 0.56 mg quercetin/g DW. Although all the extracts were capable to scavenge the 2,2'-diphenyl-1-picrylhydrazyl free-radical, ethanolic extract was found to be the best antioxidant activity (84.22%). Solvent type and extraction time had significant effects on the antioxidant capacities and phytochemical contents of the saffron extracts. A significant positive correlation was found between the antioxidant activity, total phenolic (r^2=0.991) and flavonoid (r^2=0.996) contents (P<0.05) for 24 h the extracted extracts. High performance liquid chromatography analysis also demonstrated that the components amounts of crocins, picrocrocin and safranal were varied in the saffron samples extracted from the different solvents.

Keywords: saffron, extraction, phytochemical compounds, antioxidant behaviour

1. Introduction

Antioxidants are important groups which possess the ability of protecting organisms from damage caused by free radical-induced oxidative stress. Antioxidants such as vitamins, carotenoids and phenolic compounds are introduced into the human body in form of food components (Mozafari *et al.*, 2006). Carotenoids as high-potential functional ingredients belong to the tetraterpenes family are shown biological activities such as antioxidant activity and anti-carcinogenesis (Gharibzahedi *et al.*, 2013). Phytochemicals such as phenolic and flavonoid compounds can act as free radical scavenger, strong antioxidant agents as well as anti-carcinogens in food and human body (Zheng and Wang, 2001). Nowadays, search for new and safe natural antioxidant, especially with plant origin, has increased due to the significance of such bioactive molecules in areas like pharmaceutical, food and cosmetic industries as well as considering the side effects of synthetic antioxidants when taken *in vivo*.

Saffron (*Crocus sativus* L.) is an autumn flowering herb and belongs to the subfamily *Crocoideae* which is well-known for its very expensive dried dark red stigmas in the world. By far the most important producer country is Iran, followed by Greece, Morocco, India, Spain and Italy (Sanchez-Vioque *et al.*, 2014). Several recent studies have highlighted the ability of saffron extracts to act on nervous and cardiovascular system, as anticancer, anti-inflammatory, antioxidant, antigenotoxic, insulin resistance reducing in the treatment of hepatic disorders and depression (Bathaie and Mousavi, 2010; Melnyk *et al.*, 2010). Saffron is able to

lend a unique mixture of colour, bitterness taste and aroma to food and beverages. The molecules responsible for the colour, taste and the particular aroma are water-soluble carotenoids crocins, picrocrocin and safranal, respectively (Lozano *et al.*, 1999).

During the last decade, researches have been focused on the antitumor and anticarcinogenic properties of saffron (Tavakkol-Afshari *et al.*, 2008) and more recently on its antioxidant and free radical scavenging activity (Sariri *et al.*, 2011; Serrano-Diaz *et al.*, 2012) and even on antioxidant activity of saffron byproducts (Montoro *et al.*, 2012; Sanchez-Vioque *et al.*, 2012). Solvent extraction is most commonly used technique for isolation of phytochemical compounds. However, the extraction yields and extracted components of the plant materials are strongly dependent on the nature of extracting solvent, due to the presence of different phytochemical compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix. The most appropriate of these solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Methanol and ethanol have been extensively used to extract antioxidant compounds from various plants and vegetables (Sultana *et al.*, 2009).

The best method for saffron quality characteristics determination recommended by the International Standardisation Organisation (ISO) is UV-vis spectrophotometry. This method permits the determination of the main characteristics of saffron related to picrocrocin, crocins and safranal. Higher amounts of above mentioned components mean higher quality of saffron (ISO, 2003). Various analytical methods have been developed (Caballero-Ortega *et al.*, 2007; Cullere *et al.*, 2011; Iborra *et al.*, 1992; Lage and Cantrell, 2009; Tarantilis *et al.*, 1994). High performance liquid chromatography (HPLC) is the most efficient analytical technique for the analysis of sensitive compounds in complex extracts of natural products such as spices and medicinal herbs (Alonso *et al.*, 2001). Therefore, this study sought to determine data on Iranian saffron characteristics as well as antioxidant activity related to phenolic and flavonoid content and free radical scavenging activity of saffron extracted successively with water, ethanol and methanol.

2. Materials and methods

Chemicals and reagents

Folin-ciocalteu reagent, potassium acetate, gallic acid, quercetin, 2,2'-diphenyl-2-picrylhydrasyl (DPPH), α-tocopherol and crocin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium carbonate and aluminium chloride were obtained from Fluka while methanol and acetonitrile from Romil-SPS (Cambridge, UK). Deionised water and ethanol were supplied by Bahre Zolal Tehran Co. (Teheran, Iran) and Bidestan Co. (Qazvin, Iran), respectively.

Preparation of saffron extracts

Qaen Negin saffron was supplied by Novin Zaferan Co. (Mashahd, Iran) and ground to powder using a pestle and mortar. After grinding, 500 mg powder was extracted with 100 ml ethanol (50 and 80%, v/v), methanol (50 and 80%, v/v) and deionised water in shaker for 1 and 24 h extraction times. The extract was filtered and concentrated in a vacuum evaporator at 40 °C. The extracts kept at 2-6 °C (refrigerator) for subsequent analysis.

Determination of plant extract yield

The yield of evaporated extracts based on dry weight was calculated using following equation:

$$\text{Yield} = \frac{W_1}{W_2} \times 100 \quad (1)$$

Where W_1 was the weight of the extract after the solvent evaporation and W_2 was the weight of the dry plant material.

Determination of main characteristics of saffron by UV-visible spectrometric method

2.5 g of saffron stigmas previously powdered by grinding were dried in an oven at 103±2 °C for 16 h at atmospheric pressure. Determination of colouring strength was carried out according to the technical specification ISO/TS 3632-2:2003 (ISO, 2003). Picrocrocin, safranal and crocins have been identified and quantified using an UV spectroscopy determination (Varian-Cary 300 spectrophotometer; Agilent, Santa Clara, CA, USA) of the absorbance maxima at 257, 330 and 440 nm, respectively. These main components of saffron are expressed as direct reading of the absorbance of 1% aqueous solution of dried saffron.

$$E^{1\%} = \frac{D \times 10{,}000}{m \times (100 - H)} \quad (2)$$

Where $E^{1\%}$ is the maximum absorbance of crocin, safranal and picrocrocin, D is the specific absorbance, m is the mass of the saffron sample and H is the moisture and volatile content of the sample.

Sample preparation and HPLC analysis

50 mg of saffron stigmas were suspended in 10 ml of deionised water, ethanol and methanol and magnetically stirred for 24 h at 4 °C in the dark. After extraction, samples were centrifuged at 14,000 rpm for 15 min to eliminate plant residues and then the supernatant filtered through a PTFE

syringe filter (0.45 μm pore size × 13 mm diameter; Capital, London, UK) before use. A Eurospher 100-5 C_{18} column (25 cm length, 4.6 mm internal diameter; Knauer, Berlin, Germany) with photo diode array detector was employed for all analysis. The mobile phase was a linear gradient of methanol from 10 to 100% in water (15% of acetonitrile) with a flow-rate of l.0 ml/min for a maximum elution time of 60 min at room temperature (Caballero-Ortega *et al.*, 2007). The sample size was 10 μl of the test solution. The range of injected crocin standard concentrations was between 0.1-1 mg/ml.

Determination of total phenolic compounds and flavonoid content

The total phenolic content (TPC) was determined spectrophotometrically according to the Folin-Ciocalteau method. The extract samples (0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min then 2.0 ml of 75 g/l sodium carbonate were added. The absorbance of the reaction was measured at 765 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid (Gal) equivalents (Nabavi *et al.*, 2009; Pourmorad *et al.*, 2006).

Aluminium chloride colourimetric was used for flavonoids determination. Briefly, each of saffron extracts (0.5 ml) were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M of potassium acetate and 2.8 ml of deionised water. It incubated at room temperature for 30 min. Then, the absorbance of the reaction mixture was measured at 415 nm using spectrophotometer. Total flavonoid content (TFC) was calculated as quercetin (QE) equivalents (Pourmorad *et al.*, 2006).

DPPH scavenging assay

The capacity of the extracts and crocin to scavenge the stable DPPH free radical were measured (Chen *et al.*, 2008). Briefly, a 0.5 ml each extract was mixed with 3.5 ml of 0.06 mM ethanolic DPPH solution. The change in absorbance at 517 nm was measured at 30 min and the radical scavenging activity was calculated in terms of α-tocopherol equivalent antioxidant capacity using the following formula:

$$\text{Radical scavenging activity} = \frac{A0 - (A1 - As)}{A0 \times 100} \quad (3)$$

Where A_0 is the absorbance of the control solution containing only DPPH; A_1 is the absorbance of the samples and DPPH and A_s is the absorbance of the sample solution without DPPH.

The quantity of the extract needed to reduce the initial DPPH concentration by 50% was evaluated. This characteristic parameter is called half-inhibition concentration (IC_{50}). The lower the IC_{50}, the higher is the antioxidant activity of the examined compound.

Statistical analysis

All analytical experiments were carried out in three replicates and the results presented as a mean of the obtained values with the standard deviation. Analysis of variance (ANOVA) procedure followed by Duncan's test using SPSS 16 (SPSS Inc., Chicago, IL, USA) software was applied to determine the significant difference ($P<0.05$) between treatment means.

3. Results and discussion

Effect of the solvent on the yield of extract

The yields of the extracts attained with the different solvents after 1 and 24 h of conventional extraction. The highest yield of the extract (57%) was related to extraction with 80% ethanol for 24 h. The yield of ethanolic extract (with ethanol 80% and 24 h extraction) was higher than yield of aqueous extract and methanolic extracts (50 and 80%) for both extraction times. This observation may be explained by the fact that ethanol has two functional groups of CH_3 and CH_2-OH that could be lead to attract more polar and non polar molecules than water and methanol in saffron extract.

Colouring strength of saffron

Colouring strength is one of the main parameter employed by saffron trade companies in order to determine the price. According to our experimental results, saffron sample used in this study belonged to category I, with average values noticeably higher than the limit (190 UCS) (Table 1). The UV-visible spectra of aqueous saffron extracts had three absorption bands which were obtained simultaneously at 257 nm (related to picrocrocin), 330 nm (associated with safranal) and 440 nm (related to crocins).

Main components of saffron extracts by HPLC

The chromatographic conditions employed allowed identification of major components in each sample and a distinct baseline separation was obtained. Figure 1 shows the results for an aqueous extract, a methanolic extract and an ethanolic extract of saffron detected at 257, 310 and 440 nm, respectively. According to our results, it seemed that different extracts did not differ in their chemical composition, but did differ in the concentration of each component. Safranal, the main volatile oil, is responsible for the saffron aroma and to obtain and quantify this component sensitive methods such as gas chromatography, steam distillation and vacuum head space method have been employed (Kanakis *et al.*, 2004). Therefore, the peak related to safranal has not seen in studied saffron aqueous

Table 1. Saffron spice characterisation based on ISO 3632 trade standard (ISO, 2003).

Parameter[1]	Saffron spice studied	Commercial categories (ISO 3632)		
		I	II	III
Moisture and volatile content, % max	6.5	12	12	12
$E^{1\%}$ 257 nm dry basis, min	105.45	70	55	40
$E^{1\%}$ 330 nm dry Basis, min	32.71	20	20	20
$E^{1\%}$ 330 nm dry Basis, max	32.71	50	50	50
$E^{1\%}$ 440 nm dry Basis, min	251.33	190	150	100

[1] $E^{1\%}$ is the specific extinction coefficient.

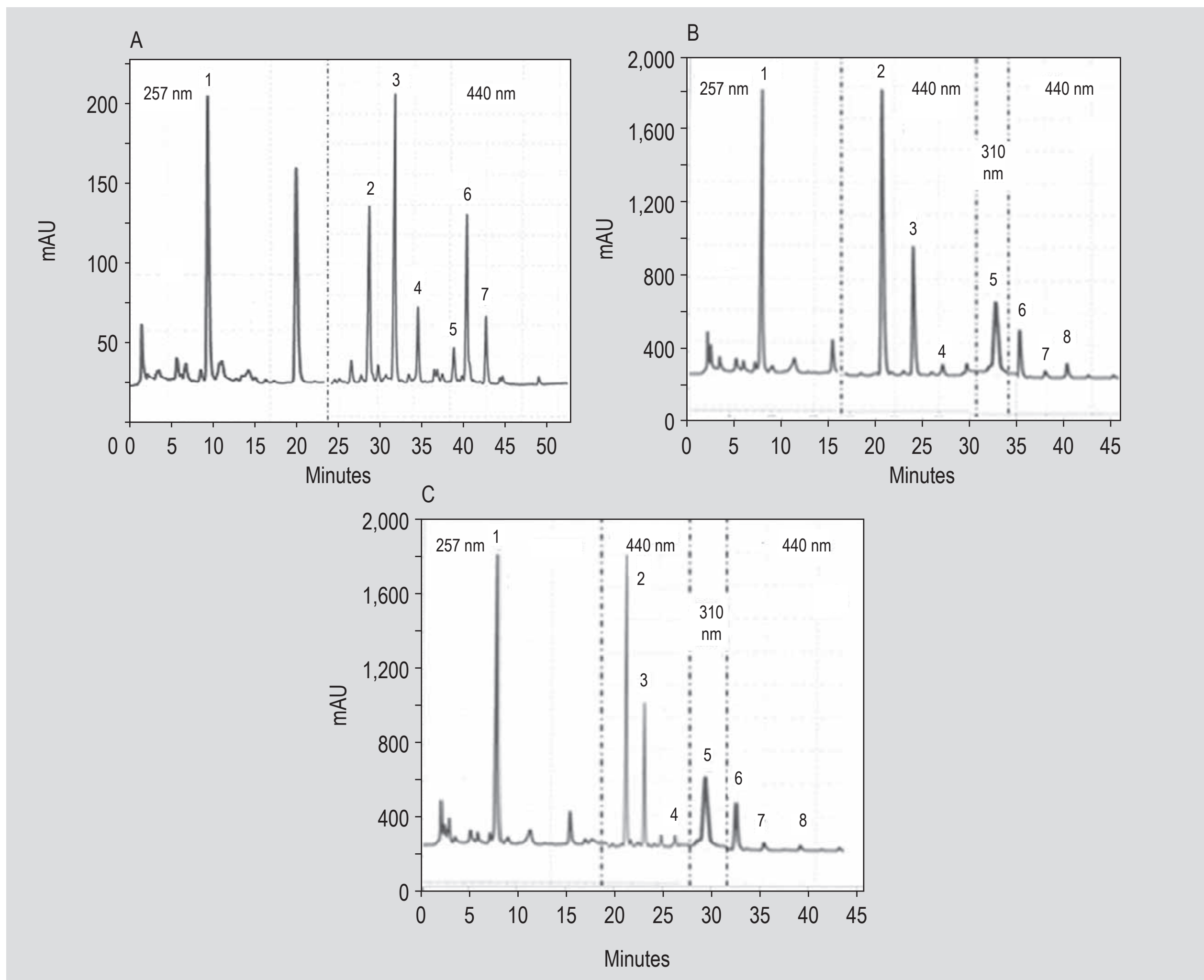

Figure 1. High performance liquid chromatography chromatograms of (A) saffron aqueous extract, (B) saffron methanolic extract and (C) saffron ethanolic extract recorded at three wavelengths (picrocrocin at 257 nm, safranal at 310 nm and crocins at 440 nm).

extract. Based on HPLC analysis and the calibration curve of the standard sample, the peak area of the investigated compounds were determined in all the extracts. A comparison between profiles A, B and C shows that the ethanolic extract contains more concentration of major components of saffron than the other extracts. Our results are in line with Lozano *et al.* (1999) conducted on using HPLC method to check components and purify in commercial saffron by photodiode array detection. They identified and quantified 10 saffron metabolites responsible

for the taste, flavour and colour. Separation of picrocrocin, crocins and safranal of saffron using HPLC was reported by Tarantilis *et al.* (1994). They detected and identified above mentioned components from methanolic extract of saffron. Their results are in agreement with our results.

Total phenolic and flavonoid content of different extracts

The extracts obtained by different solvents were subjected to screening for their possible antioxidant activity. Three complementary test systems namely TPC, TFC and DPPH free radical scavenging were used for this purpose. The TPC and TFC of the saffron extracts are presented in Table 2. The highest value was obtained for overnight extracted saffron in 80% ethanol and the lowest by the extract done with methanol as solvent. It was found that the 80% ethanolic extract showed the highest phenolic concentration of 29.2 mg Gal/g DW followed by 50% ethanolic and aqueous extracts which were 18.86 to 24.2 mg Gal/g DW and 15.54 to 17.53 mg Gal/g DW, respectively. The lowest phenolic concentration was found for methanolic extract (12.67 to 14.50 mg Gal/g DW). The TFC were usually higher in ethanolic extract with the range of 0.45 to 0.56 mg QE/g DW respect to methanolic extract with the range of 0.2-0.43 mg QE/g DW and aqueous extract with the range of 0.28 to 0.44 mg QE/g DW. The variation of TPC and TFC of the tested extracts could be related to the difference of the solvents used for the extraction and the time of extraction. A general principle in solvent extraction is 'like dissolves like', which means that solvents only extracts those phytochemicals which have similar polarity with themselves (Zhang *et al.*, 2007). Thus, the authors suggested that saffron contained diverse phenolic compounds with different polarity. However, extraction time is crucial in minimising energy and cost of the extraction process. Table 2 shows the effect of extraction time on phenolic and flavonoid content of crude extract. Overall, extraction time had significant effect ($P<0.05$) on TPC and TFC. In general, the maximum concentration of phenolic and flavonoid compounds in extracts was achieved at extraction time of 60 min except for 50% and 80% ethanolic concentrations in TPC and 80% methanolic and ethanolic extraxts in TFC, respectively. Apart from exposure of more oxygen for prolonged extraction time, reduction of phenolic and flavonoid content with longer time could also be due to the endogenous enzymes in stigma tissues degraded the phenolic compounds in saffron extracts.

Karimi *et al.* (2010) determined the antioxidant activity and TPC and TFC of different extract of saffron. They have determined some phenolic and flavonoids compounds including gallic acid and pyrogallol which have shown antioxidant activity. They reported that the highest phenolic content was found for 80% methanolic extract but for aqueous extracts it was significantly lower. Significant differences in the flavonoid content of methanolic, aqueous and ethanolic extracts was observed, with values of 5.8, 3.8 and 2.9 mg rutin equivalent/g DW, respectively. The TPC of different extracts of saffron in the present work was higher than Karimi *et al.* (2010) results. Makhlouf *et al.* (2011) reported the phenolic content of the saffron extracted with water as 16 mg Gal/g DW. Our results are higher than those reported by Makhlouf *et al.* (2011). In addition to using different solvents in researches, other contributing factors for this difference may be related to genotypic and environmental differences within saffron species, sample preparation and analytical procedure.

Free radical scavenging activity of saffron extracts

The antioxidant activity of various extracts of saffron was investigated through the ability of extracts to scavenge hydroxyl radicals. The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. The scavenging activity of saffron extracts was determined by the DPPH assay and the results are shown in Figure 2. All of the extracts had a radical scavenging activity. Dose response studies showed a positive correlation between antioxidant activity and the concentration of extracts. In general, the scavenging ability of the DPPH free-radical increased gradually with the increase in concentration of extracts to a certain

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of the saffron different extracts.[1]

Extracts	TPC (mg GAL/g)		TFC (mg QE/g)	
	Shaking (1 h)	Shaking (24 h)	Shaking (1 h)	Shaking (24 h)
Aqueous	17.53±0.010^{cd}	15.54±0.011^{de}	0.44±0.010^{c}	0.28±0.005^{f}
50% methanolic	14.50±0.010^{fg}	13.04±0.012^{g}	0.28±0.050^{e}	0.28±0.005^{e}
80% methanolic	13.65±0.015^{g}	12.67±0.003^{g}	0.20±0.007^{g}	0.43±0.030^{c}
50% ethanolic	18.86±0.010^{c}	24.20±0.020^{b}	0.56±0.020^{a}	0.37±0.007^{d}
80% ethanolic	14.20±0.020^{ef}	29.20±0.030^{a}	0.45±0.001^{b}	0.53±0.020^{a}

[1] Mean ± standard deviation. Values with same letter have no significant difference. GAL = gallic acid;. QE = quercetin equivalents.

extent, and then levels off with further increases of extract concentration except at 0.4 mg/ml concentration (P<0.05). DPPH antioxidant activity of saffron extracts slightly differed depending on the solvent and extraction time applied. The lower free radical scavenging activity values in 60 min extraction time samples were obtained from aqueous extract (58.11%) and methanolic extract (60.03%), however the higher activity was related to ethanolic extract (81.63%). As extraction time increased to 24 h, the same tendency of DPPH scavenging ability of each extract was observed. At a dosage of 400 μg/ml extracts, the highest DPPH scavenging ability related to α-tocopherol, which is found to be 95.13% and followed by ethanolic extract, crocin, methanolic and aqueous extracts, respectively. The IC_{50} of saffron extracts for the DPPH free radical scavenging activity ranged from 0.037 to 0.346 mg/ml, depending on the solvent and time of extraction. The 60 min extracted ethanolic sample having the lowest IC_{50} exhibited the highest DPPH free radical scavenging ability compared to aqueous and methanolic extracts. The findings on this work show that extracts of saffron stigmas possess antioxidative potential and are in agreement with data collected by Karimi *et al.* (2010), who reported the antioxidant activity of saffron. However, they reported that the free radical scavenging activity of saffron showed more potency with methanol solvent and the less with ethanol one. In order to study whether the antioxidant activities of the extracts were consistent with the contents of crocin, the relationships between antioxidant capacities and total corcin content were investigated. The crocin content of extracts was determined using UV-visible and direct reading, and the results are shown in Table 3. Quantitative determination by two methods indicated that ethanolic extract significantly increased pigment contents, comparable to another extracts. On the other hand, as shown in Figure 2 as well as Table 3, a correlation was observed between free radical scavenging activity and total crocin in saffron different extracts. Thus, it implies that crocin could be responsible for marked antioxidant effects of saffron extracts. In our work, ethanol extract of saffron exhibited stronger antioxidant potential than did crocin tested by DPPH radical scavenging activity. There are numerous investigations aimed to elucidate antioxidant properties of crocins and extracts of saffron. Radical scavenging activity of saffron extract and its bioactive compounds, safranal and crocin have been shown using DPPH radical scavenging assay (Assimopoulou *et al.*, 2005). They expressed that the

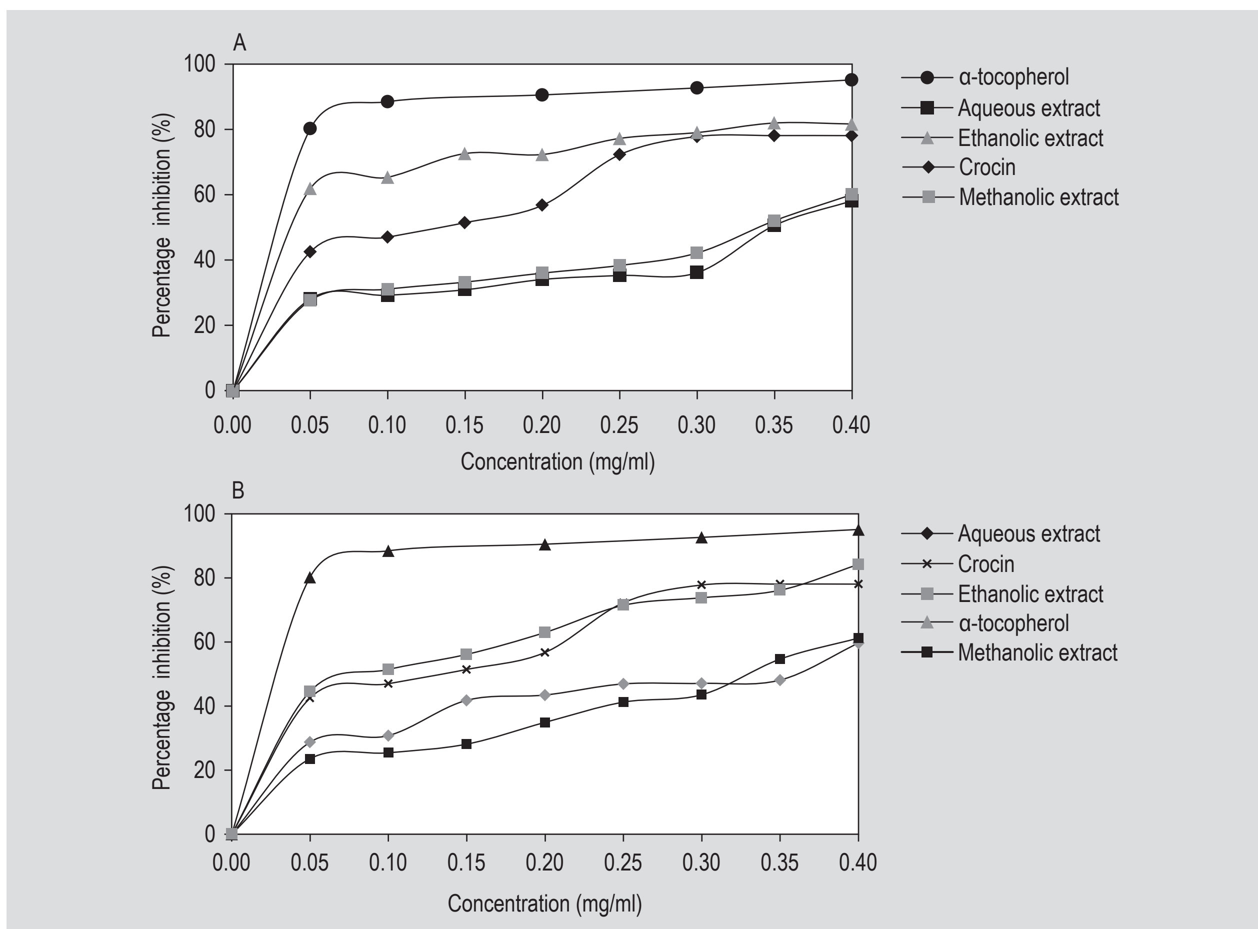

Figure 2. Free radical scavenging activities of saffron extracts and α-tocopherol as reference antioxidant (A) extraction time of 1 h; (B) extraction time of 24 h.

Table 3. Total crocin content in the different extracts of saffron.[1]

Extracts	Shaking (1 h)		Shaking (24 h)	
	Direct reading[2]	UV-visible[3]	Direct reading	UV-visible
Aqueous	199±1.0[d]	30.64±0.30[d]	107±1.2[d]	16.40±1.00[d]
50% methanolic	316±5.2[b]	48.68±0.30[c]	261±1.5[c]	40.23±0.60[c]
80% methanolic	205±1.4[c]	29.95±0.90[d]	265±2.1[c]	40.84±0.50[c]
50% ethanolic	367±2.5[a]	56.42±0.80[a]	332±1.4[b]	51.08±0.04[b]
80% ethanolic	329±1.1[b]	50.70±0.01[b]	383±2.7[a]	59.04±0.20[a]

[1] Means followed by different letters in a column are significantly different.
[2] $E^{1\%}$ (1 cm, 440 nm).
[3] Means of triplicate determinations expressed as mg/g dry weight.

ability to scavenge the free radical DPPH was very high in methanolic extract and two components, crocin and safranal, had a high radical scavenging activity 50% and 34% for 500 mg/kg solution in methanol, respectively. Chen *et al.* (2008) determined the free radical scavenging activity of the saffron ethanolic extract and crocin equal to 107 and 98.3 mg α-tocopherol/g, respectively. Their results evidenced that crocin possessed antioxidant capacities assayed in four models, which confirm their roles as antioxidant agents. Although the DPPH assay protocols used in those studies were almost similar, differences in results could be attributed to the extract preparation procedure or the duration of the monitoring period. Ordoudi *et al.* (2009) reported that saffron methanolic extract had a remarkable intracellular antioxidant activity as effectively as the selected well-known phenolic antioxidants. Their findings reinforced the perception of saffron bioactivity through antioxidant mechanism of action. They pointed out that crocins are responsible for the antiradical potential of saffron extract. Papandreou *et al.* (2006) were determined the antioxidant properties of saffron extract by measuring the ferric-reducing antioxidant power and Trolox-equivalent antioxidant capacity. According to their results, the 50% methanolic extract of saffron possessed good antioxidant activities, higher than those of carrot and tomato. Another study by Ochiai *et al.* (2004) documented the most effective of crocin among the crocetin glycosides of saffron in maintaining the normal morphology of PC-12 cells by resisting oxidative stress. Their results revealed that the antioxidant activity of crocins was nearly the same as that of α-tocopherol. However, the synergistic effects of all the bioactive constituents give to saffron spice a significant antioxidant activity.

The relationship between antioxidants activity and phytochemical compounds

Phenolic compounds are believed to account for a major portion of the antioxidant activity in many plants. In general, extracts with a high antioxidant activity showed high phenolic contents. Yet, current data in the literature on the relationship between the polyphenol content of plants and their antioxidant activity are sometimes contradictory. While some authors have observed a linear correlation between content of TPC and antioxidant (Knur and Kapoor, 2002), others found no such correlation exists or only a very weak one (Souri *et al.*, 2008). However the authors found this may be true across the three types of extracts of saffron. In the present study, the correlation between TPC and TFC and radical scavenging activity of extracts from saffron were analysed (Figure 3). The results of this study showed in general, a good correlation between the free radical scavenging activity (antioxidant activity) and the amount of TPC in 24 h extracted samples. The ethanolic extracts with the highest TPC exhibited the highest DPPH free-radical scavenging ability. The same phenomenon was existed in TFC. But, a direct correlation between radical scavenging activity and both of phenolic and flavonoid content of 60 min extracted samples was failed to demonstrate by linear regression analysis. Also, it can be concluded that antioxidant activity of extracts is not limited to phenolic and flavonoid components. Activity may also come from the presence of saffron carotenoids especially crocin because numerous studies have been reported that crocin have antioxidant activities (Assimopoulou *et al.*, 2005; Chen *et al.*, 2008; Ochiai *et al.*, 2004).

4. Conclusions

The importance of natural antioxidants for medical and food application is commonly performed through extraction procedures to provide maximum yield of

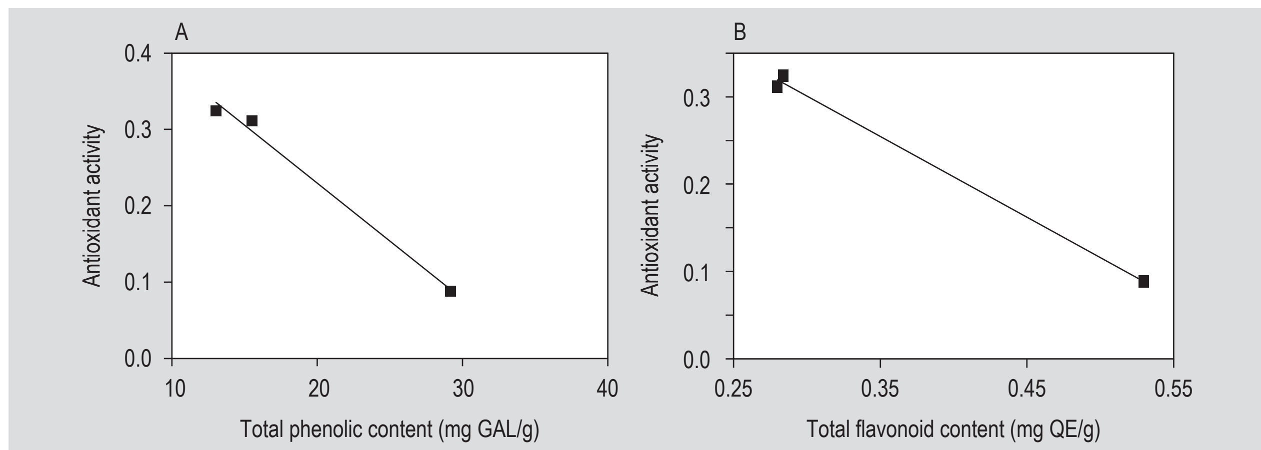

Figure 3. Correlation between antioxidant activity (DPPH method) and (A) phenolic content; or (B) flavonoid content of saffron extracts.

bioactive substances and of the highest yield quality by optimisation of some process parameters, sample/solvent ratio, type of solvent and time and temperature of extraction. Nevertheless, on the basis of the results obtained, and based upon the consumption of saffron, the alleged antioxidant properties might be somewhat beneficial to the antioxidant protection system of the human body, shielding against oxidative damage. It can be said that the polarity of the solvent had significant impact on the extraction of phytochemicals such as antioxidants, crocins content, phenolics and flavonoids from the stigma matrices of *C. sativus*. It appears that the alcoholic and aqueous extracts from stigmas of *C. sativus* possess hydrogen donating capabilities to act as antioxidant. In this study, the antioxidant activity decreasing order among saffron extracts assayed through the DPPH method was found to be ethanol extract > methanol extract > aqueous extract. Similar order to the phenolic contents of the extracts that showed the extent of antioxidant activity of each extract is in accordance with the amount of phenolics content. Our results showed that the highest antioxidant activity, TPC and TFC were exhibited by the extracts obtained by ethanol as solvent. It is interesting to note that ethanol solvent was better than methanol in qualitative and quantitative analytical data. In conclusion, the saffron extracts especially ethanolic extract are a promising natural product with antioxidant activity and it is proposed to use it for its antioxidant activity as a food supplement, in functional foods, beverages, pharmaceutical preparations and cosmetic formulations.

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