

Phenolic Compounds and Antioxidant Activity from Saffron (*Crocus sativus* L.) Petal

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Abstract

Saffron petal is the main by-product of saffron processing which is producing in large amounts annually. The purposes of this work were to determine total phenolics content in the methanolic extract of saffron petal using Folin-ciocalteu reagent and to measure their antioxidant activity in various *in vitro* models, such as β -carotene-linoleate and 1,1-diphenyl-2-picryl hydrazyl (DPPH). Saffron petal extract in different concentrations (0.5-5 mg ml⁻¹) were compared with standard antioxidants of ascorbic acid, α -tocopherol and TBHQ (0.5-1 mg ml⁻¹). Total phenolics content was 3.42 mg gallic acid/g dry weight. In model systems of β -carotene-linoleate and DPPH, the extract at 500 ppm concentration showed 91.4% and 74.2% antioxidant activity which was comparable with that of TBHQ (93.1% and 77.9%) at 100 ppm. The results showed that saffron petal could be considered as a bioresource of phenolic compounds with high antioxidant activity.

Keywords: saffron petal, phenolic, β -carotene-linoleate, DPPH

1. Introduction

Nowadays, synthetic antioxidants such as BHA, BHT and TBHQ are widely used in food industry. However, it is arguable whether these compounds are safe and their use in food products is questioned (Konczak, Zabaras, Dunstan & Aguas, 2010; Kosar, Goger & Baser, 2011). In recent years there has been an interest to determine total phenolics (as natural antioxidant) content and their antioxidant activities in various plants specifically by-products of agriculture and even marine microorganisms (Negro, Tommasi & Miceli, 2003; Lee & Lee, 2010; Fu et al., 2011; Goh, Yusoff & Loh, 2010). Phenolic compounds of edible and inedible plants have multiple biological effects such as anti-inflammatory, bactericidal as well as antioxidant properties because of their ability to neutralize free radicals (Wojdylo, Oszmianski & Czemerz, 2007; Temerdashev, Frolova & Kolychev, 2011). Herbs are used in many industries such as medicine, food, fragrance and cosmetics. Crude extracts of herbs and spices have been reported to be rich in phenolics and attracted more attention in food industry because of their antioxidant capacity (Wojdylo et al., 2007).

Crocus sativus L. commonly known as saffron, belonging to Iridaceae family, is a perennial plant widely cultivated in different parts of the world, particularly in Iran (Esmaeili, Ebrahimzadeh, Abdi & Safarian, 2011). Although the source of saffron is obscure, it is apparently originated from Asia Minor and Iran. The name of saffron is derived from Arabic word of za-faran meaning "be yellow" (Winterhalter & Straubinger, 2000; Cabellero-Ortega, Pereda-Miranda & Abdullaev, 2007). Iran is the major producer of saffron in the world market, but its quality is reported not to be as well as saffron of other main supplier, Spain (Winterhalter & Straubinger, 2000; Cabellero-Ortega et al., 2007). Although the most usage of saffron is as a food coloring and flavoring agent in food industry, but it is utilized in folk medicine as antispasmodic, carminative, stomachic, expectorant, aphrodisiac, cardiogenic and stimulant. Modern pharmacological studies have reported that saffron extract have antitumor, anticonvulsant, antidepressant, anti-inflammatory, anti-hyperlipidemic, free radical scavenging and antioxidant effects (Hadizadeh, Khalili, Hosseinzadeh & Khair-Aldine, 2003; Asdaq & Inamdar, 2010; Melnyk, Wang & Marcone, 2010). Moreover, chemopreventive and protective effects of saffron extract on genotoxins-induced oxidative stress in animals have been reported (Asdaq & Inamdar, 2010).

Since one dry stigma in saffron plant weighs about 2 mg and each flower contains three of them, approximately 150,000 saffron flowers must be carefully picked for the production of 1 kg of the spice. Harvesting the flowers

and separating the stigmas is very time consuming. Consequently, saffron is still the world's most expensive spice (Winterhalter & Straubinger, 2000; Melnyk et al., 2010). Saffron petal is the main by-product of saffron harvesting which is not usable for the farmers. The amount of saffron petal is more than 10000 tons each year (Kafi, Kakhki & Karbasi, 2000). Nowadays, saffron petals are only used for dye extraction, which is not flourished yet.

There are limited reports on phenolic compounds of saffron and their antioxidant activity. Ordoudi, Befani, Nenadis, Koliakos and Tsimidou (2009) investigated antiradical capacity of saffron stigma extract and its bioactive constituents using the Folin-ciocalteu reagent and various free radical species produced in cell-free or cell model system. They concluded that saffron extracts exhibit a considerable intracellular antioxidant activity. In other study, the phenolic and flavonoid compounds of saffron stigma were determined by HPLC. The results showed that saffron stigma had antioxidant activity which was lower than that of BHT and α -tocopherol. Total phenolics content of stigma was 6.55 mg gallic acid equivalent/g dry weight for methanolic saffron extract (Karimi, Oskoueian, Hendra & Jaafar, 2010). Esmaceli et al. (2011) evaluated total phenolics content in saffron corms in dormancy and waking stages. Gentisic and gallic acids were the highest and lowest phenolic compounds in dormant and waking corms, respectively. Hadizadeh et al. (2003) isolated kaempferol from saffron petal and determined its structure by chemical and spectroscopic methods. There is only one report in which phenolic compounds of saffron petal was investigated. Termentzi and Kokkalou (2008) isolated various fractions from the petals of *crocus sativus* cultivated in Greece and determined its phenolics content. They also reported the content of flavonoids, acids, crocetin and alkaloids, but in their study no reports were found about antioxidant activity of saffron petal in different model systems. However, no research has been reported on phenolics content and their antioxidant capacity of Iranian saffron petal.

The objectives of present study were (1) to determine total phenolics content of saffron petal using Folin-Ciocalteu reagent and (2) to evaluate its antioxidant activity by various model systems and 3) to determine the suitable concentration of saffron petal extract which is equivalent in antioxidant activity with standard antioxidants.

2. Materials and Method

2.1 Chemicals

All solvents and chemicals were analytical grade and obtained from Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH) and tert-Butylhydroquinone (TBHQ) were purchased from Sigma Chemical Co. (Sigma-Aldrich, Germany).

2.2 Plant Material

Saffron petals were collected from Kesheh, Karkas region, Isfahan province, Iran in 2011. The petals were air-dried in shadow and ground into fine powder by a laboratory mill.

2.3 Methanol Extract

One gram of the fine ground sample was extracted with 10 methanol in a flask placed in an ultrasonic bath (Hilsonic, UK) at 65 °C for 2 h. The sample was cooled at room temperature and centrifuged (Sigma, Germany) at 1500rpm for 15min (Budrat & Shotipruk, 2009).

2.4 Total Phenolics Content

The total phenolics were determined colorimetrically by means of the Folin-Ciocalteu method, as described by Pinelo, Rubilar, Sineiro and Nunez (2004). 2.5 ml of ten-fold diluted Folin-Ciocalteu reagent, 2 ml of 7.5% sodium carbonate, and 0.5 ml of phenolic extract were mixed. After heating at 45°C for 15 min, the absorbance was measured at 765 nm against a blank. The phenolics content was expressed as gallic acid equivalent/g dry weight of sample.

2.5 Antioxidant Activity by β -carotene-linoleic Acid Method

This assay was conducted according to method of Gursoy, Sarikurkcu, cengiz and Solak (2009) by minor modifications. 0.5 mg β -carotene was dissolved in 1 ml of chloroform and 25 μ l linoleic acid and 200 mg Tween 80 was added to prepare stock solution. The solvent was evaporated by a vacuum evaporator and 100 ml of oxygenated distilled water was added with vigorous shaking. 2.5 ml of reaction mixture was dispersed to test tubes and 0.5 ml of various concentrations (0.5-5 mg ml⁻¹) of the extract (for control antioxidants, 0.5 and 1 mg ml⁻¹) was added and the mixture was incubated at 50 °C. The absorbance was measured at zero time (t=0) at 490 nm. Absorbance reading was continued at an interval of 15 min until the color of β -carotene disappeared in the control tubes (t = 120 min).

The antioxidant activity (AA) of the extracts was calculated using the following formula:

$$AA = 100[1 - (A_0 - A_t) / (A_0^\circ - A_t^\circ)]$$

Where A_0 and A_0° are the absorbance values measured at zero time and A_t and A_t° are the absorbance values measured after incubation of 120 min for test sample and control, respectively.

2.6 Scavenging Activity on DPPH

Different concentrations of saffron petal extract (equivalent to 50, 100, 250 and 500 ppm) and TBHQ, ascorbic acid and α -tocopherol (50 and 100 ppm) were taken in different test tubes. The volume was adjusted to 100 μ L by adding methanol. Five milliliters of a 0.1 mM methanolic solution of DPPH was added to tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared without any extract, and methanol was used for the baseline correction. The absorbance of the samples was measured at 517 nm. Radical scavenging activity of the extracts was calculated by the following formula:

% radical scavenging activity = (control OD - sample OD/control OD) \times 100 (Singh, Murthy & Jayaprakasha, 2002).

2.7 Reducing Power

The extracts (2.5 ml) were mixed with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and incubated at 50°C for 20 min. Then 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 200g for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The absorbance at 700 nm was measured against a blank (Gursoy et al., 2009).

2.8 Statistical Analysis

The results were shown as the mean \pm SD of three separate determinations. The data were statistically analyzed by ANOVA program in Statistix 8 software. The means evaluation was done using Least Significant difference (LSD) test at a confidence level of 95%. The dendrogram based on the results were constructed using SPSS ver.17.

3. Results and Discussion

The total phenolics content was determined using Folin-Ciocalteu reagent. The data showed that saffron petal had 3.42 \pm 0.2 mg phenolic content equivalent to gallic acid per g dry weight. This value was higher than amount of phenolic acids (1.38 mg caffeic acid/g dry weight) reported by Termentzi and Kokkalou (2008) for saffron petal harvested in Greece.

Since there are differences between various radical scavenging systems, different *in vitro* model systems have been recently recommended to evaluate antioxidant capacities (Rockenbach et al., 2011). Therefore, in this study, three model systems; β -carotene/linoleic acid, DPPH and reducing power through potassium ferricyanide methods were applied. Food lipids and cell membranes in human body contain unsaturated fatty acids which are in exposure to oxidation reaction. Thus, in evaluation of antioxidant activity, the test based on unsaturated fatty acid oxidation is very important. β -carotene/linoleic acid method is widely used to investigate oxidation progress in polyunsaturated fatty acids. In this method, linoleic acid free radical formed from oxidation of fatty acid, attacks the highly unsaturated β -carotene molecules. As β -carotene loses its double bonds by oxidation development, its color also would disappear which can monitored by spectrophotometry. The presence of extracts with antioxidant activity can hinder β -carotene bleaching through neutralization of free radicals formed in the system (Kosar et al., 2011; Singh et al., 2002; Rockenbach et al., 2011).

Antioxidant effect of petal extracts as well as natural and synthetic antioxidants in model system of β -carotene/linoleic acid is presented in Table 1. For all antioxidants, an increment in activity was observed when the concentration increased (F value= 838, P<0.01). Although TBHQ at 100 ppm concentration showed highest antioxidant activity of %93.07 but petal extract (PE) at 500 ppm (%91.39) had no significant difference with TBHQ (P>0.05). This data showed that petal extract in higher concentration can be competitive with TBHQ as a standard antioxidant. PE with 250 ppm concentration (PE-250) was in second order (%90.02) and then PE-100 and TBHQ-50 were more active antioxidants, respectively. In this method, vitamin C or ascorbic acid in both concentrations had the least activity which might be due to that ascorbic acid is water-soluble and cannot be efficient in reduction of fatty acid oxidation resulting in bleaching of β -carotene. Kosar et al. (2011) have reported the same results in which ascorbic acid showed the weakest antioxidant capacity compared to BHT and *Salvia halophila* extract due to its pro-oxidant activity.

Table 1. Antioxidant activity of different antioxidants measured by the β -carotene/linoleic acid method

Samples	% Antioxidant activity			
	50 ppm	100 ppm	250 ppm	500 ppm
Ascorbic acid	47.54±0.63 ^h	49.07±0.28 ^h	-	-
α -tocopherol	60.33±1.83 ^g	68.62±0.33 ^f	-	-
TBHQ	80.44±0.04 ^d	93.07±0.02 ^a	-	-
Petal extract	78.52±0.49 ^e	88.49±0.75 ^c	90.02±1.55 ^{bc}	91.39±0.08 ^{ab}

Lowercase letters are represented significant difference at 0.05 levels

The DPPH method is a simple, practical and sensitive assay which has been widely used to detect active antioxidants with scavenging capacity even in low concentration (Pinelo et al., 2004; Zhu, Lian, Guo, Peng & Zhou, 2011). The ability of antioxidants to react with DPPH which is a stable free radical and its conversion to α, α -diphenyl- β -picryl hydrazine is expressed in % DPPH inhibition. DPPH by accepting an electron loses its color and changes from purple to yellow. Discoloration degree indicates that the antioxidants possess scavenging potentials. Since DPPH is a stable free radical and does not dimerize as happens with most free radicals, the absorbance diminution depends linearly on the antioxidant concentration (Kosar et al., 2011; Singh et al., 2002; Zhu et al., 2011; Esquivel, Moreno, Álvarez, Álvarez & Giusti, 2011).

As shown in Table 2, in this model system, as well as β -carotene method, a moderate increase in radical scavenging was observed when the concentration of all antioxidants was increased (F value= 394, P<0.01). TBHQ at 100 ppm concentration with %77.81 of radical scavenging was the most active antioxidant. The ability of DPPH scavenging was decreased in the following order; ascorbic acid-100 > TBHQ-50 > ascorbic acid -50 > PE-500, although PE-500 had no significant difference with ascorbic acid-100. Among the samples, α -tocopherol was weak in DPPH radical scavenging (Table 2). The results were in agreement with that of Kosar et al. (2011) and Maksimovic (2008) stated that ascorbic acid along with synthetic antioxidant had the strongest activity in inhibition of DPPH.

Table 2. Radical scavenging activity of different antioxidants measured by DPPH method

Samples	% Inhibition of DPPH			
	50 ppm	100 ppm	250 ppm	500 ppm
Ascorbic acid	74.90±0.71 ^{abc}	76.35±0.29 ^{ab}	-	-
α -tocopherol	35.15±1.50 ^e	39.92±1.85 ^d	-	-
TBHQ	75.02±1.59 ^{abc}	77.81±2.14 ^a	-	-
Petal extract	22.45±2.80 ^f	39.80±2.89 ^d	72.94±1.65 ^c	74.23±1.82 ^{bc}

Lowercase letters are represented significant difference at 0.05 levels

The ability of the antioxidant fraction to reduce Fe^{+3} to Fe^{+2} represents the reducing power of the antioxidant. In this method, reducing power could be determined by color changes of the solution from yellow to various shades of green and blue (depending on the activity of the samples) which can be monitored by absorbance measurement at 700 nm (Kosar et al., 2011; Zhu et al., 2011). In this assay (Table 3), contrary to other methods, ascorbic acid had the highest power in reduction of Fe^{+3} (1.2). Then, TBHQ at 100, PE at 500 and ascorbic acid at 50 ppm concentration had more activity (0.92, 0.72 and 0.6, respectively) (F value= 1484, P<0.01). The lowest activity was pertained to α -tocopherol and PE at 50 ppm. Since ascorbic acid is a strong reductive antioxidant, in this model system this compound showed higher activity followed by TBHQ and plant extract which was in agreement with data of Kosar et al. (2011) and Maksimovic (2008).

Table 3. Reducing power of different antioxidants measured by ferricyanide method

Samples	Reducing power (absorbance at 700 nm)			
	50 ppm	100 ppm	250 ppm	500 ppm
Ascorbic acid	0.60±0.00 ^d	1.20±0.02 ^a	-	-
α-tocopherol	0.02±0.00 ⁱ	0.17±0.01 ^g	-	-
TBHQ	0.41±0.03 ^c	0.92±0.01 ^b	-	-
Petal extract	0.10±0.00 ^h	0.19±0.00 ^g	0.30±0.00 ^f	0.72±0.00 ^c

Lowercase letters are represented significant difference at 0.05 levels

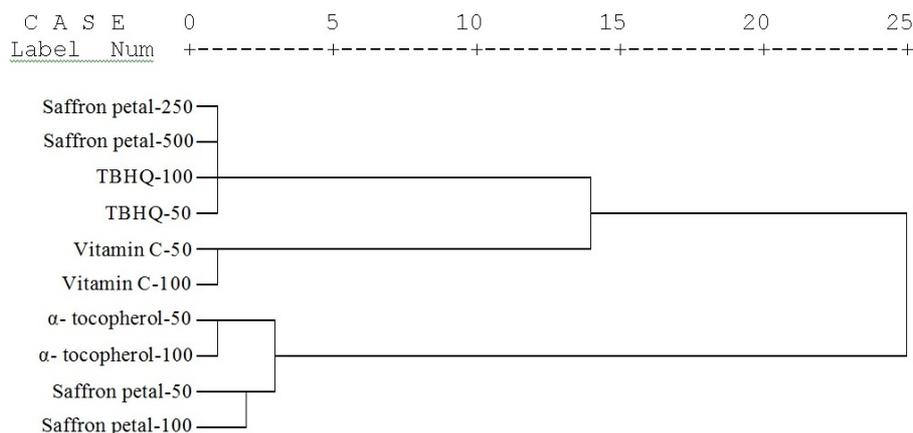


Figure 1. The dendrogram based on antioxidative activity of natural and synthetic antioxidants

To facilitate comparison of different antioxidants activity, the dendrogram constructed based on the results of three antioxidant model systems. The dendrogram classified various antioxidants tested in this research into three major groups (Figure 1). According to cluster analysis and in respect to antioxidant activity, TBHQ-50, TBHQ-100, PE-250 and PE-500 were classified in one group. Ascorbic acid was separated in different group and PE-50 and PE-100 were grouped along with α-tocopherol. The cluster analysis confirmed the results of model systems in which ascorbic acid showed different activity, TBHQ and saffron petal extract at higher concentration (250 and 500 ppm) had high similarity in antioxidant power meanwhile other compounds had the lowest activity.

4. Conclusion

It could be concluded that saffron petal as the main by-product of saffron production possessed considerable phenolic compounds which showed high antioxidant power. Regarding to antioxidant activity, petal extract at 500 ppm was comparable with TBHQ (as a strong synthetic antioxidant) at 100 ppm. Therefore, taking into account that saffron petal is discarding more than thousands tons each year, phenolic compounds extracted from this solid waste might be used as natural antioxidant.

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