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Antioxidant and antibacterial activity of safflower (*Carthamus tinctorius* L.) extract from four different cultivars

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RESEARCH ARTICLE

Abstract

Antioxidant and antimicrobial activities were studied in safflower methanolic extracts of four different cultivars (IL111, Padide, Isfahan-28 and Mahali). The total phenolic and flavonoid content of the plant extracts ranged from 46.2 to 62.3 mg gallic acid equivalent/g dry extract and 7.5 to 9.6 mg catechin equivalent/g dry extract, respectively. The antioxidant capacity of methanolic extracts was assessed by reducing power assay, DPPH• (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and β-carotene bleaching methods. Results showed that cultivar 'IL111' had the highest phenolic and flavonoid content, as well as an antioxidant activity comparable to synthetic antioxidants. In addition, the antioxidant activity of the safflower extracts was evaluated in crude soybean oil by monitoring peroxide and thiobarbituric acid values, which showed a stabilisation effect on the soybean oil in the accelerated condition at 60 °C (oven test). Moreover, the analysis of phenolic compounds by HPLC showed that gallic acid was the predominant phenolic in safflower cultivar 'IL111'. The Isfahan-28 cultivar showed the best antimicrobial activity; the minimum inhibitory concentration against *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi were 30 and 60 mg/ml, respectively.

Keywords: flavonoids, inhibition zone, oil stability, phenolic compounds, safflower

1. Introduction

Antioxidants can break the chain reaction of the oxidation processes or inhibit the initial steps of them (Pan *et al.*, 2007). Synthetic antioxidants, such as butylated hydroxyanisole (BHA) have a widespread application in the food industry, however recent approaches have been oriented toward natural antioxidants due to safety concerns and beneficial effects against some diseases, such as cancer, coronary heart disease, diabetes, neurodegenerative disorders and aging (Pan *et al.*, 2007). Consumers have been concerned about the safety of foods containing synthetic preservatives. Therefore, there is an increasing interest in the development of new types of effective and nontoxic antimicrobial compounds, such as natural antibacterial compounds originating from extracts of spices and herbs, for food preservation (Smid and Gorris, 1999).

Herbs and spices have been widely used both traditionally and commercially to increase the shelf-life and safety of foods (Brul and Coote, 1999). Many plant phenols are known to possess antimicrobial properties, so they might change the composition of microflora in any environment where they are applied and/or induced, depending on the compound and concentration involved (Heinaaho *et al.*, 2006). Consequently, several plant extracts have been tested for these activities in edible oils. Currently, the most studied extracts are sage (*Salvia officinalis* L.) and rosemary extract (*Rosemarinus officinalis* L.), coffee beans, vegetables – especially onions and peppers (Chammem *et al.*, 2015), tea

leaves (Oh *et al.,* 2013), safflower (*Carthamus tinctorius* L.) extract (Qazi *et al.,* 2013; Salem *et al.,* 2014) and olive leaves (Chiou *et al.,* 2009). Supposedly, these extracts are more impressive in the prevention of oil oxidation than synthetic antioxidants, such as BHA and butylated hydroxytoluene (BHT) (Chammem *et al.,* 2015).

Safflower belongs to the *Compositae,* a family consisting of about 1000 genera and 20,000 species. It has been cultivated in many regions of the world, such as Iran, where about 50 cultivars are currently used in production (Daadrasan, 2004). The species has been used traditionally for staining cotton and silk textiles and adding flavour to foods (Weiss, 1983). Today it is used in foods, pharmaceutics and cosmetics as a natural pigment (Salem *et al.*, 2011). The main pharmacological effects of the herb include the reduction of lipid levels, treatment of dysmenorrhea, as well as anti-inflammatory, anti-constipation and analgesic activities (Weiss, 1983), increase of peripheral blood flow (Todoki *et al.*, 1983), antibacterial activity (Ogata *et al.*, 1990) and even inhibition of platelet aggregation (Kutsuna *et al.*, 1998). The seeds are used for the production of edible oil (Koyama *et al.*, 2006). Previous studies have revealed some pharmacological activities of safflower (Guimiao and Yili, 1985; Sun, 1955). Up to now, the major chemical constituents isolated from safflower are flavonoids, including 6-hydroxykaempferol 3-O-glucoside, quercetin 3-O-rutinoside, quercetin 3-O-glucoside, kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside, alongside the main coloured compound in flowers, carthamin. Also condensed tannins and other phenolic compounds (e.g. caffeic acid), gamma linolenic acid, alkaloids as well as lignans were isolated (Jun *et al.*, 2011; Salem *et al.*, 2011).

Other studies have demonstrated antioxidant properties of safflower (Hiramatsu *et al.*, 2009; Jin *et al.*, 2004; Salem *et al.*, 2014; Zhao *et al.*, 2006). Flavonoids and other polyphenolic compounds were found to possess antioxidant effects (Jin *et al.*, 2004; Jun *et al.*, 2011). Previous studies suggested that the phenolic content of safflower contained the most efficient antioxidants of the herb (Kruawan and Kangsadalampai, 2006; Salem *et al.*, 2011). However, some data suggest that a synergistic interaction between the antioxidants in the phenolic compounds, might be responsible for the good antioxidant activity of safflower (Salem *et al.*, 2011). Among 10 aqueous extracts of herbs tested for antioxidant and antimutagenic effects, *C. tinctorius* exhibited remarkable ferric reducing antioxidant power (FRAP) and scavenging activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals (Kruawan and Kangsadalampai, 2006). Chalcone flavonoids, which are the main components of water extracts, were likely responsible for the good antioxidant activity of the sample (Salem *et al.*, 2011).

Soybean oil is one of the most widely used cooking oils in the world. Due to the high content of unsaturated fatty acids in this oil, especially linoleic acid with an amount of 51%, the stabilisation against oxidation has attracted attention. Accordingly, soybean oil has been used as a model to verify the ability of different plant extracts in the prevention of peroxidation (Mohdaly *et al.*, 2011; Ye *et al.*, 2015).

Although in recent years some studies have been carried out to evaluate the bioactivity of natural compounds extracted from plant substances, but there is still a limited amount of published results comparing natural antioxidants with synthetic antioxidants in edible oils. Also there are no studies in order to use of safflower extract against oxidation of soybean oil. Therefore, the principal objective of this study was to determine antioxidant and antimicrobial activities of different safflower cultivars and to examine the use of safflower total extract in edible oil as a natural antioxidant.

2. Materials and methods

Materials

Different cultivars of *C. tinctorius* L. were collected from various parts of Iran: IL111 from Toroq plantation (Ferdowsi university plantation, Mashhad, June 2009), Padide from Jihad-e-agriculture plantation (Neyshabur, Khorasan razavi Province, June 2009), Isfahan-28 and Mahali from Emamzade Abdolaziz (Ghamshan village, Isfahan province, July, 2008); all cultivars were authorised by SPII-oil crop (Iran). All chemicals and culture media were of analytical grade and obtained from Merck (Darmstadt, Germany) or Sigma-Aldrich Company Ltd. (Gillingham, UK) and used without further purification. Refined, bleached, deodorised soybean oil, without any added synthetic antioxidant (Shadgol, Neyshabur, Iran) was used for storage studies.

Preparation of samples

Flowers were fully dried at room temperature, away from sunlight, powdered by a CG 100 miller (Kenwood Ltd., New Lane, UK) and kept in a cold, dry and dark place. 30 g of powder was macerated two times with 300 ml of 80% (w/v) methanol for 24 h to extract antioxidant flavonoids (Hajimehdipoor *et al.*, 2012). The macerate was filtered and evaporated to dryness by a Hei-vap Precision MLG3B rotary evaporator (Heidolph, Schwabach, Germany) at 38 °C.

Sample analysis

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Hayouni *et al.*, 2007). An UV-Vis spectrophotometer (Seinco, Seoul, South Korea) was used to measure absorbance. Measurements were carried out in triplicate and calculations were based on a calibration curve obtained with gallic acid (Sigma-Aldrich).

Total phenolic contents were expressed as mg of gallic acid equivalents (GAE)/g dry matter (DM).

Total flavonoid content was determined according to the colorimetric method described by Yoo *et al.* (2008). Standard solutions (50, 100, 150, 200, 250 and 300 mg/l) of catechin were prepared to obtain a calibration curve. Results were reported as mg catechin equivalent/g DM.

DPPH radical scavenging activity was done according to Kukic *et al.* (2008). Also, BHT was used for comparison. Reducing power was determined according to the colorimetric method described by Thitilertdecha *et al.* (2008) using BHT as standard.

β-carotene bleaching assay was determined according to the colorimetric method described by Shahsavari *et al.* (2008) using BHT as the positive control. In the negative control, the extract was substituted with an equal volume of ethanol.

HPLC analysis

Identification of major phenolic compounds was performed using high performance ternary gradient liquid chromatography (HPLC; Agilent 1100 series with a diode-array detector; Agilent, Tokyo, Japan). For this purpose, extracts were filtered through a 0.45 μm nylon filter (Agilent) prior to HPLC analysis to obtain clear extracts. The chromatographic system comprised a C18 column (Zorbax Extend-C18, 5μm, 46×150 mm, Agilent). The analytical conditions include two mobile phases: (A) water:trifluroacetic acid (99.08:0.02, v/v) and (B) methanol:trifluroacetic acid (99.08:0.02, v/v). The gradient conditions were as follows: 0-5 min, 25% B; 5-10 min, 25-30% B; 10-16 min, 30-45% B; 16-18 min, 45% B; 18-25 min, 45-80% B; 25-28 min, 80% B and 28-30 min, 80-25% B. The flow rate was 10 μl/s and diode-array detection was performed at 254, 275, 305 and 320 nm (Wen *et al.*, 2005).

Effect of safflower extracts on soybean oil oxidation

Four different concentrations of safflower cultivars 'IL111' and 'Mahali' extracts (200, 400, 800 and 1,600 mg/l) as natural antioxidants were evaluated for antioxidant activity in soybean oil. Experiments were also carried out with BHT and BHA (100 and 200 mg/l) as synthetic antioxidants and a control set without added antioxidants. The oxidation process was carried out at (60 °C) for 28 days and oxidation progress was quantified on day 0, 7, 14, 21 and 28 by measuring peroxide (AOCS, 1989) and thiobarbituric acid (TBA) (Sidewell, 1954) values.

Determination of antibacterial activity

Activation of strains

Lyophilised *Staphylococcus aureus* ATCC 25923 and *Salmonella enterica* subsp. *enterica* serovar Typhi PTCC 1609 ampoules (Bacteria and Fungi collection Center, Iranian Institute of Industrial and Scientific Research, Tehran, Iran) were opened in sterile conditions and cultivated in tryptone soy broth. Master and sub-master cultures were prepared on tryptone soy agar.

Preparation of 0.5 McFarland suspensions

Bacterial strains from sub-master cultures were cultivated in nutrient agar slant tubes and incubated at 37 °C for 24 h. Microorganisms were then washed from the surface of slants using saline solution. The suspensions were diluted with saline until their absorbance at 530 nm was equivalent to 0.5 McFarland standard (1.5×10^8 cfu/ml) (Mahon and Manuselis, 1995).

The antibacterial effect of extracts

The Broth Dilution method with Muller Hinton Broth was used to evaluate antibacterial activity. Extracts were added to the Muller Hinton broth at a concentration of 0-120 mg/l in 16×80 mm tubes and inoculated with 5×10^5 cfu/ml of a bacterial strain. Incubation was carried out at 35-37 °C for 18-24 h. The least concentration of extract that showed no turbidity was determined as minimum inhibitory concentration (MIC). Tubes with no turbidity were used as inoculum on nutrient agar and incubated at 37 °C for 24 h. The enumerated minimal bactericidal concentration (MBC) was concentration where no growth was observed in nutrient agar (Rezaei and Rasouli, 2001).

Statistical analysis

All experiments were carried out in triplicate and the results were presented as the mean and standard deviation. Statistical variance analysis of independent data with three replicates was performed by ANOVA using a completely randomised factorial design and compared with least significant differences (LSD) ($P<0.05$). All statistical analyses were performed with SAS 9.1 software (SAS, Cary, NC, USA). Pearson's correlations between measurements were made using Minitab for Windows, version 13.0 (Minitab Inc., State College, PA, USA).

3. Results and discussion

Total phenolic and flavonoid contents

As mentioned earlier, antioxidant activity of safflower cultivars mainly refers to flavonoids and other polyphenolic compounds. Alcohols (methanol, ethanol), acetone, diethyl

ether, and ethyl acetate are frequently used solvents for extraction of flavonoids (Hajimehdipoor *et al.*, 2012). In our experiment, the very polar flavonol glycosides and the even more polar aglycones could not entirely be extracted with pure organic solvents, therefore a methanol:water mixture (80:20, v/v) was used as an alternative. In addition, it proved less interfering in comparison to the other solvents mading it a suitable solvent for extraction of all flavonoids (Hajimehdipoor *et al.*, 2012).

Total phenolic and flavonoid content of safflower cultivars was determined by drawing a calibration curve of gallic acid and catechin, respectively. The total phenolic content in safflower methanolic extracts was different in each cultivar (Table 1). Cultivar IL111 showed the highest amount of these compounds, with 62.3±0.6 mg GAE/g DM. In addition, the lowest amount was obtained for cultivar Mahali with 46.2±0.6 mg GAE/g DM. The difference between phenolic compounds of IL111 and Mahali was significant (P<0.05).

Total flavonoid content of safflower cultivars varied from 7.5 to 9.6 mg catechin equivalent/g DM (Table 1). IL111 and Mahali cultivars had the highest and lowest amount of these compounds, with 9.6 and 7.5 mg of catechin equivalents/g DM, respectively. A significant difference was observed between cultivar IL111 and cultivar Mahali (P<0.05). Baydar and Ozkan (2005) reported that the total phenolic content in safflower petal of cultivars 'Yenice', '5-154' and 'Dinçer' in methanol:water (80:20) were 20.92, 16.62 and 9.06 mg GAE/g DM, respectively. Also, the results of Salem *et al.* (2011) showed that extraction with acetone:water (2:8) had the highest flower polyphenolic content (15.09 mg GAE/g dry weight).

Antioxidant activity

In this study, the antioxidant capacity of safflower petal extracts was measured by different chemical assays including the DPPH assay, the reducing power assay and lipid peroxidation inhibition by β-carotene bleaching method.

The scavenging effect on the DPPH radicals assay showed concentration-dependent activity (Figure 1A). For example, cultivars IL111 and Mahali at 80 mg/l, presented a scavenging effect of 28.5 and 24.1% that increased to 79.8 and 70.1% at 480 mg/l, respectively. Also cultivars Padide and Isfahan-28, at 80 mg/l, presented a scavenging effect of 27.9 and 27.1% that increased to 79.84 and 72.99% at 480 mg/l, respectively. Figure 1A shows a positive relationship between increased concentration and increased antioxidant capacity. EC_{50} values of extracts ranged from 232.9 to 299.1 mg/l (Table 1). Among them, the extract from cultivar IL111 was the most powerful (EC_{50}=232.9 mg/l) and that from cultivar Mahali the weakest (EC_{50}=299.1 mg/l). The synthetic antioxidant BHT showed higher antioxidant activity (EC_{50}=63.0 mg/l) compared to safflower extracts (Table 1). Samples with higher total phenols showed the strongest free radical scavenging effect (lower EC_{50} values). The addition of safflower extracts to the $DPPH^{\bullet}$ solution caused a rapid decrease in the absorption at 517 nm. The degree of discoloration indicate the radical scavenging capacity of the safflower extracts. Baydar and Ozkan (2005) studied antioxidant activities of safflower petal extracts. Their results showed that the methanolic extract of cultivar '5-154' had 29.85% antiradical activity at 50 mg/l using the DPPH model system.

Figure 1B shows that the reducing power of different extracts of safflower cultivars was dependent on their concentration. According to the results, cultivar IL111

Table 1. Extraction yield and antioxidant activity of safflower petal methanolic extracts. Results of EC_{50} values (mg/l) are compared with synthetic antioxidant (butylated hydroxytoluene; BHT).[1]

Cultivar	Total extract yield (w/w)[2]	TPC[3]	TFC[4]	DPPH[5]	Reducing power[5]	β-carotene bleaching[5]
IL111	27.5	62.3±0.6^{a}	9.6±0.4^{a4}	232.9±3.5^{b}	938.4±25.0^{b}	2,068.4±15.6^{b}
Padide	26.7	57.4±0.9^{b}	8.6±0.5^{b}	245.1±5.3^{c}	958.0±28.2^{c}	2,133.8±29.8^{c}
Isfahan-28	25.0	52.6±0.8^{c}	8.0±0.6^{c}	267.1±9.9^{d}	988.2±12.9^{d}	2,222.3±33.5^{d}
Mahali	24.2	46.2±0.6^{d}	7.5±0.6^{d}	299.1±6.8^{e}	1,058.0±28.9^{e}	2,317.1±27.8^{e}
BHT				63.0±1.9^{a}	62.9±2.5^{a}	311.5±4.4^{a}

[1] Values correspond to mean ± standard error of three (n=3) measurements. Within each column, different capital letters indicate significant difference according to least significant difference (α=0.05).
[2] Extract yields expressed as mg of extract per g dry weight of safflowers.
[3] TPC = total phenolic content (mg gallic acid equivalent/g dry extract).
[4] TFC = total flavonoid content (mg catechin equivalent/g dry extract).
[5] DPPH = 2,2-diphenyl-1-picrylhydrazyl. According to EC_{50} (mg/l).

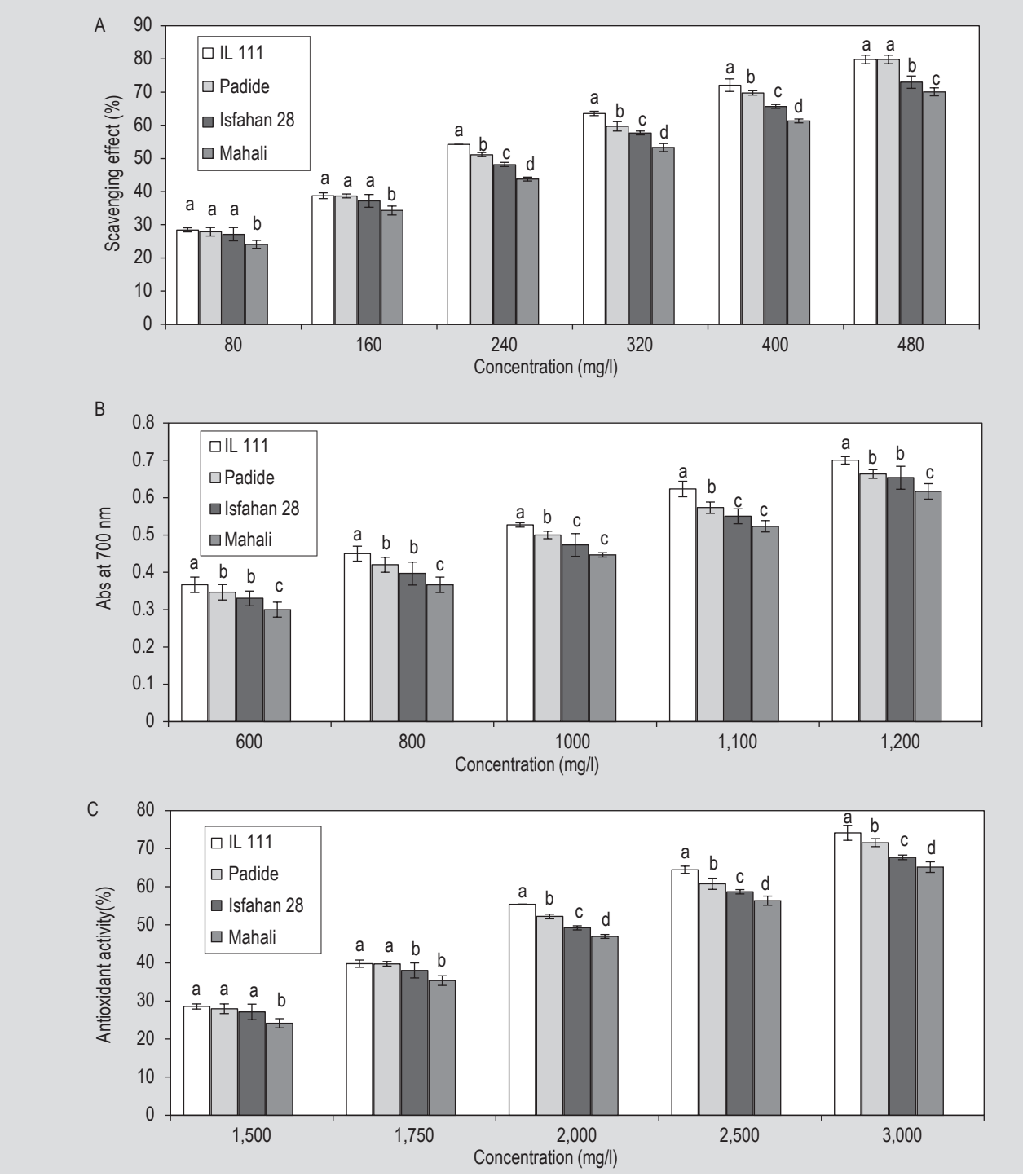

Figure 1. (A) DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging effect (%), (B) reducing power values, and (C) antioxidant activity (%) by β-carotene bleaching method of methanolic extracts of safflower petals from cultivars IL111, Padide, Isfahan-28 and Mahali. Each value is expressed as mean ± standard deviation.

exhibited the strongest activity, while cultivar Mahali showed the weakest. As the concentration of extracts increased, the reducing power enhanced too. EC_{50} values obtained from aqueous safflower extracts were lower than 1,058 mg/l. In general, extracts with a high total phenolic content presented lower EC_{50} values in the reducing power assay and could be arranged in the following order: IL111 < Padide < Isfahan-28 < Mahali. Moreover, the differences in the reducing power were statistically significant between the different cultivars (P<0.05) (Table 1).

In the β-carotene bleaching method, free radicals arise from oxidation of linoleic acid and attacks the highly unsaturated β-carotene molecules. As a result, β-carotene is oxidised and breaks down in part, subsequently losing its chromophore and characteristic orange colour, which is monitored spectrophotometrically at 470 nm. (Cao *et al.*, 2009). The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralising the linoleate-free radical and other free radicals formed in the system (Jayaprakasha *et al.*, 2001). The EC_{50} of the extract is a measure of its antioxidant activity as it is the concentration that inhibits oxidation of linoleic by 50% (Shaddel *et al.*, 2014). The antioxidant activity of safflower extracts, measured by the inhibition of β-carotene bleaching is shown in Figure 1C. Generally, all the extracts displayed concentration-dependent antioxidant capacity. EC_{50} values of safflower extracts ranged from 2,068.44 to 2,317.11 mg/l for the different cultivars (Table 1). EC_{50} for cultivar IL111 was 2,068.4±15.6 mg/l. This was dramatically higher than the EC_{50} of BHT (311.5±4.4 mg/l). It is a fact that the antioxidant activity of most plant extracts is much lower than that of synthetic compounds. Similar to this finding, Shaddel *et al.* (2014) reported that BHT scavenging ability as a pure antioxidant was more than subcritical water extracts from bene hull. Salem *et al.* (2011) studied phenolic composition and antioxidant activity during flower development of safflower. These authors reported that phenolic contents and antioxidant activities varied considerably as a function of solvent polarity. The extraction with acetone:water (2:8) showed the highest flower polyphenol content and antiradical capacities against $DPPH^{\bullet}$, chelating power and lipid peroxidation assay.

Major phenolic compounds determined by HPLC

Phenolic compounds were identified by comparing retention times as well as quantified using external standards and plotting peak areas against known concentrations. The results are presented in Table 2. Repeatability is shown as the relative standard deviation. Four phenolic compounds of gallic acid, *p*-coumaric acid, caffeic acid and ferulic acid were identified in cultivar IL111. The results of analysis of phenolic compounds by HPLC showed that gallic acid was the predominant phenolic compound in cultivar IL111 extracts. Salem *et al.* (2011) showed that gallic acid was the most abundant phenolic compound in safflower flowers, accounting for about 102.57 (µg/g DW). However, serotonin derivatives are major antioxidants in safflower seed; these compounds have shown an inhibitory effect to low-density lipoprotein (LDL) oxidation and atherosclerosis (Koyama *et al.*, 2006).

Table 2. Major phenolic compounds in safflower petal methanolic extracts from cultivar IL111 identified by HPLC analysis.

Analyte	Retention time (min)	RSD%[1]	mg/100 g of dry herb
Gallic acid	4.0	3.5	28.5
p-coumaric acid	17.0	5.1	12.7
Caffeic acid	11.1	1.5	2.9
Ferulic acid	19.2	6.0	2.0

[1] Relative standard deviation of three (n=3) measurements.

Antioxidant activity in soybean oil

In a period of 28 days, lipid peroxidation of soybean oil, treated by two different safflower extracts (cultivars with the highest and lowest content of phenolics and flavonoids were chosen) was evaluated (Table 3). Peroxide and TBA values were used to measure primary and secondary lipid oxidation products, respectively (Shahsavari *et al.*, 2008). By surveying oxidation rate of soybean oil between days 0 and 28 it was concluded that the oxidation procedure is related to time, its antioxidants and their concentrations. Natural antioxidants (safflower extracts) were tested at four different concentrations (200, 400, 800 and 1,600 mg/l). Additional treatments were done with BHT and BHA as synthetic antioxidants at 100 and 200 mg/l, as well as a control containing no additives. Among the samples, the lowest peroxide and TBA values were observed in BHT at 200 mg/l, closely followed by 'IL111' at 800 mg/l, which suggested it has the highest antioxidant activity. Anusuya *et al.* (2013) reported that polyphenols from pseudostem of banana cultivars might serve as a substitute for synthetic antioxidants in sunflower oil stability. Soybean oil with cultivars 'Mahali' and 'IL111' extracts at 1,600 mg/l had a maximum peroxide value among all other treatments. It seems that cultivars 'Mahali' and 'IL111' have pro-oxidant activity at 1,600 mg/l. Sometimes, the antioxidant activity of phenolic compounds is decreased at high concentrations and they instead become pro-oxidants. Huang *et al.* (1995) showed that the increased addition of α-tocopherol to oil might increase the peroxidase value, while reducing the hexanal formation. Also, a reduction was found in antioxidant activity of samples treated with safflower extracts during 28 days of oven test. This was probably because of more susceptible structures of natural antioxidants compared to synthetics. In all experiments, BHT (200 mg/l) and cultivar IL111 (800 mg/l) had the lowest peroxide and TBA values and were comparable in antioxidant activity with nearly significant difference (P<0.05).

Antimicrobial activity

S. aureus is a gram-positive bacterium and both a commensal and opportunistic pathogen in humans with a wide range of infections (Jamkhandea *et al.*, 2014). Typhoid fever, a

Table 3. Effect of different concentrations (in mg/l) of cultivars 'IL111' and 'Mahali' extracts on soybean oil oxidation expressed as peroxide values and thiobarbituric acid values during accelerated storage at 60 °C in comparison with synthetic antioxidants.[1]

Antioxydants/extracts[2]	Concentration (mg/l)	Day 0	Day 7	Day 14	Day 21	Day 28
Peroxidase value						
Control		0.45±0.01^{a}	26.02±0.05^{c}	45.40±0.23^{c}	67.32±0.24^{c}	95.18±0.04^{c}
BHA	100	0.45±0.01^{a}	19.09±0.10^{f}	30.11±0.08^{g}	42.16±0.18^{h}	53.75±0.34^{h}
	200	0.45±0.01^{a}	16.40±0.09^{j}	26.41±0.21^{k}	37.16±0.23^{k}	48.62±0.19^{k}
BHT	100	0.45±0.01^{a}	18.04±0.04^{h}	27.28±0.29^{i}	40.50±0.08^{i}	51.70±0.16^{i}
	200	0.45±0.01^{a}	14.44±0.07^{k}	24.57±0.20^{m}	35.24±0.12^{m}	45.12±0.21^{m}
IL111	200	0.45±0.01^{a}	20.44±0.14^{e}	33.06±0.09^{e}	52.45±0.27^{e}	74.12±0.18^{e}
	400	0.45±0.01^{a}	18.52±0.16^{g}	29.12±0.13^{h}	44.45±0.08^{g}	61.20±0.09^{g}
	800	0.45±0.01^{a}	15.30±0.06^{j}	25.44±0.13^{l}	36.61±0.13^{l}	47.36±0.11^{l}
	1,600	0.45±0.01^{a}	28.36±0.10^{b}	48.81±0.16^{b}	70.56±0.35^{b}	96.35±0.07^{b}
Mahali	200	0.45±0.01^{a}	22.38±0.06^{d}	36.08±0.04^{d}	55.58±0.12^{d}	76.34±0.22^{d}
	400	0.45±0.01^{a}	20.16±0.01^{e}	31.26±0.13^{f}	46.26±0.14^{f}	63.21±0.03^{f}
	800	0.45±0.01^{a}	17.76±0.06^{h}	26.86±0.06^{j}	39.45±0.13^{j}	49.43±0.15^{j}
	1,600	0.45±0.01^{a}	30.31±0.09^{a}	51.48±0.11^{a}	73.62±0.25^{a}	98.44±0.20^{a}
Thiobarbituric acid value						
Control		0.008±0.00^{a}	0.051±0.00^{b}	0.091±0.00^{b}	0.182±0.00^{c}	0.481±0.00^{c}
BHA	100	0.008±0.00^{a}	0.037±0.00^{e}	0.069±0.00^{f}	0.104±0.00^{i}	0.331±0.00^{h}
	200	0.008±0.00^{a}	0.027±0.00^{i}	0.054±0.00^{i}	0.094±0.00^{k}	0.271±0.00^{k}
BHT	100	0.008±0.00^{a}	0.031±0.00^{g}	0.058±0.00^{h}	0.113±0.00^{h}	0.301±0.00^{i}
	200	0.008±0.00^{a}	0.015±0.00^{k}	0.045±0.00^{k}	0.08±0.00^{m}	0.234±0.01^{m}
IL111	200	0.008±0.00^{a}	0.041±0.00^{d}	0.080±0.00^{d}	0.140±0.00^{e}	0.398±0.00^{e}
	400	0.008±0.00^{a}	0.035±0.00^{f}	0.066±0.00^{g}	0.125±0.01^{g}	0.351±0.01^{g}
	800	0.008±0.00^{a}	0.021±0.01^{j}	0.049±0.00^{j}	0.088±0.00^{l}	0.252±0.00^{l}
	1,600	0.008±0.00^{a}	0.052±0.01^{b}	0.091±0.00^{b}	0.189±0.01^{b}	0.491±0.00^{b}
Mahali	200	0.008±0.00^{a}	0.045±0.00^{c}	0.083±0.01^{c}	0.151±0.01^{d}	0.430±0.00^{d}
	400	0.008±0.00^{a}	0.040±0.00^{d}	0.072±0.01^{e}	0.130±0.00^{f}	0.379±0.01^{f}
	800	0.008±0.00^{a}	0.029±0.00^{h}	0.057±0.00^{h}	0.102±0.00^{j}	0.283±0.00^{j}
	1,600	0.008±0.00^{a}	0.056±0.00^{a}	0.093±0.00^{a}	0.194±0.00^{a}	0.499±0.00^{a}

[1] Values are means ± standard deviations of three (n=3) measurements. Values with different superscript letters within a column are significantly different at $P<0.05$.

[2] Control = antioxidant free soybean oil; BHA = butylated hydroxyanisole (synthetic antioxydant); BHT = butylated hydroxytoluene (synthetic antioxydant).

Table 4. Enumeration of bacterial strains in presence of safflower petal methanolic extracts (log cfu/ml).

Bacterial strain	Extract	Concentration (mg/ml)[1]						
		0	7.5	15	30	60	120	240
Staphylococcus aureus	Isfahan-28	8.66±0.08	7.80±0.05	6.79±0.04	5.64±0.08	3.84±0.05	0	0
	Padide	8.73±0.03	7.85±0.06	6.85±0.05	5.67±0.08	3.88±0.04	0	0
	IL111	8.80±0.04	7.90±0.05	6.90±0.03	5.69±0.06	3.91±0.04	0	0
	Mahali	8.83±0.05	7.94±0.03	6.94±0.04	5.70±0.06	3.96±0.03	0	0
Salmonella Typhi	Isfahan-28	8.78±0.06	8.10±0.03	7.62±0.08	6.79±0.05	5.63±0.08	3.77±0.05	0
	Padide	8.84±0.05	8.13±0.02	7.69±0.07	6.84±0.04	5.67±0.04	3.83±0.05	0
	IL111	8.88±0.04	8.15±0.04	7.75±0.06	6.89±0.04	5.69±0.05	3.87±0.03	0
	Mahali	8.90±0.05	8.18±0.03	7.81±0.05	6.92±0.03	5.70±0.05	3.91±0.03	0

[1] Values correspond to mean ± standard error of three (n=3) measurements.

major communicable disease, is caused by infection with *S.* Typhi, a gram-negative bacterium (Jamkhandea *et al.*, 2014). Enumeration of bacterial strains showed an inhibitory effect of safflower petal methanolic extracts (Table 4). No bacterial growth was observed when a concentration of 240 mg/ml was used. At a concentration of 120 mg/ml *S. aureus* was completely inhibited, but the mean population of *S.* Typhi was 3.85 log cfu/ml. The interaction effect of extract concentration and safflower cultivars on *S. aureus* growth was statistically significant; cultivar Isfahan-28 showed the highest inhibitory effect compared to other cultivars (log cfu/ml= 3.84±0.05 at 60 mg/ml concentration). MIC and MBC mean values of the safflower extracts against *S. aureus* were 30 and 120 mg/ml, respectively. Kasra-Kermanshahi *et al.* (2006) reported that MIC and MBC of the safflower plant (seed and root) ethanolic extracts were 31.2 and 125 mg/ml, respectively. The authors used the disc diffusion method for evaluation of antimicrobial activity. MIC and MBC of safflower petal extracts against *S.* Typhi were 60 and 240 mg/ml, respectively. Cultivar Isfahan-28 was most efficient and decreased log cfu/ml to 3.77±0.05.

Correlation between measurements

As shown in Table 5, total phenolic contents (TPC) were highly significantly correlated with the β-carotene bleaching values (r=-0.999, $P \leq 0.001$) and *S. aureus* enumeration (r=-0.999, $P \leq 0.0001$) and also moderate significantly correlated with the DPPH (r=-0.990, $P \leq 0.01$) and reducing power assay (r=-0.976, $P \leq 0.05$) values. Also the antioxidant and antibacterial activities, β-carotene bleaching, TPC, *S. aureus* enumeration ($P \leq 0.05$) and also *S.* Typhi enumeration ($P \leq 0.01$) significantly correlated with TFC. Interestingly, the negative correlation of TPC and also TFC with EC_{50} values of DPPH, reducing power assay and β-carotene bleaching indicated that the higher total phenolic and flavanoid contents of the extracts resulted in lower EC_{50} values. That means a higher total phenolic and flavanoid content of the extract resulted in higher antioxidant (DPPH, reducing power and β-carotene bleaching) activities, because the lower EC_{50} values indicated higher DPPH radical scavenging, reducing power and also β-carotene bleaching activities in extracts of safflower cultivars. On the other hand, the correlation between the DPPH and reducing power values and also between β-carotene bleaching and DPPH values were positively significant ($P \leq 0.05$). Moreover, a positive and significant correlation ($P \leq 0.05$) was observed between β-carotene bleaching values and RP. As a result, they had a significant correlation with each other due to their same values (EC_{50}). Similar results were observed by Azlim Almey *et al.* (2010). In spite of *S.* Typhi, there were a positive significant correlation between *S. aureus* and DPPH or reducing power values ($P \leq 0.05$) and also between *S. aureus* and β-carotene bleaching values ($P \leq 0.01$). A high positive correlation between total phenolic content and antibacterial activity against many bacteria has been reported by Bag and Chattopadhyay (2015).

4. Conclusions

In this research antioxidant and antimicrobial activities of safflower methanolic extracts were studied. We demonstrated that the safflower methanolic extracts present a strong antioxidant and antimicrobial activity. Good correlations were found between antioxidant activities (DPPH, reducing power, and β-carotene bleaching) and active compounds (TPC and TFC). Among 4 phenolic compounds identified by HPLC, gallic acid was the predominant phenolic in safflower cultivar 'IL111'. All extracts had the greatest inhibition zone against pathogenic bacteria *S. aureus* and *S.* Typhi in this study. Moreover, the effect of safflower extracts in stabilizing unstable oils like soybean oil was comparable to that of the typical synthetic antioxidants. This finding was confirmed by the results obtained concerning the two factors of the peroxide and TBA values. Therefore, it can be concluded that safflower petal extract can be used as an alternative natural and effective source of bioactive compounds. On the other

Table 5. Pearson's correlation coefficients between the antioxidant and antibacterial activities of safflower extract.[1]

	DPPH[2]	Reducing power[2]	β-carotene bleaching[2]	*S. aureus*[3]	*S.* Typhi[3]	TPC
Reducing power	0.994**					
β-carotene bleaching	0.993**	0.975*				
Staphylococcus aureus	0.987*	0.975*	0.995**			
Salmonella Typhi	0.885	0.845	0.933	0.942		
Total phenolic contents (TPC)	-0.990**	-0.976*	-0.999***	-0.999***	-0.945*	
Total flavonoid content	-0.936	-0.904	-0.970*	-0.976*	-0.992**	0.975*

[1] * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$.

[2] DPPH = 2,2-diphenyl-1-picrylhydrazyl. According to EC_{50} (mg/l).

[3] Enumeration of bacterial strains in presence of safflower petal methanolic extracts at concentration of 60 mg/ml.

hand, the potential shown by safflower petal extracts can lead to the valorisation of a by-product that nowadays has an inadequate use.

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