

ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOID CONTENTS OF 13 CITRUS SPECIES PEELS AND TISSUES

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ABSTRACT

Methanolic extracts of 13 commercially available citrus spp., peels and tissues growing in Iran were investigated for their antioxidant activity by DPPH method. IC₅₀ for antioxidant activity ranged from 0.6-3.8 mg ml⁻¹. Total phenolic content of the citrus spp. samples (based on folin Ciocalteu method) varied from 66.5 to 396.8 mg gallic acid equivalent/g of extract and flavonoids content (based on colorimetric AlCl₃ method) varied from 0.3 to 31.1 mg quercetin equivalent/g of extract. There were no correlation between the total phenolic and/ or flavonoids contents and antioxidant activity in tissues and/or peels.

Keywords: Antioxidant activity; phenolic content; flavonoids content; citrus; peels.

INTRODUCTION

Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Fernandez-Lopez *et al.*, 2005; Jayaprakasha and Patil, 2007; Ebrahimzadeh *et al.*, 2004). Flavanones, flavones and flavonols are three types of flavonoids which occur in Citrus fruit (Calabro *et al.*, 2004). The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin (Mouly *et al.*, 1994; Schieber *et al.*, 2001). Epidemiological studies on dietary Citrus flavonoids improved a reduction in risk of coronary heart disease (Di Majo *et al.*, 2005; Hertog *et al.*, 1993) and is attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Stavric, 1993; Elangovan *et al.*, 1994; Martin *et al.*, 2002). The interest in these classes of compounds is due to their pharmacological activity as radical scavengers (Cotelle *et al.*, 1996). Several studies have demonstrated the antibacterial and/or antioxidant properties of these plants, mainly using in vitro assays. Moreover, some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the plants and their above mentioned functional properties (Dean and Svoboda, 1989; Farag *et al.*, 1989). In addition, Citrus byproducts also represent a rich source of naturally occurring flavonoids (Horowitz, 1961). The peel which represents almost one half of the fruit mass, contains the highest concentrations of flavonoids in the Citrus fruit (Anagnostopoulou *et al.*, 2006; Mantley and Grohmann, 1996 and 2001). Many papers have reported

antioxidants in juice and edible parts of oranges of different origin and from different varieties (Miller and Rice-Evans, 1997; Rapisarda *et al.*, 1999; Roberts and Gordon, 2003; Vinson *et al.*, 2001). As far as the peel is concerned, extracts from this part of the fruit were found to have a good total radical antioxidative potential (TRAP) (Gorinstein *et al.*, 2001). Also Larrauri *et al.*, (1996) compared lime and orange peel fibre with α -tocopherol and BHA.

The objectives of this study were to investigate and comparison of (I) the radical scavenging activity of 13 commercially available citrus spp., peels and tissues, separately (II) determination of their phenol and flavonoids contents and (III) analyses of correlation between them. The DPPH method was used to determine the free radical scavenging of each sample. The Folin–Ciocalteu and Colorimetric aluminum chloride method were used to determine the total phenolic and flavonoids contents of each sample, respectively.

MATERIALS AND METHODS

Chemicals

Gallic acid, DPPH, Quercetin, BHA and Vitamin C were purchased from Merck and Fluka companies. All other chemicals and reagents used were of the highest commercially available purity.

Plant material

Fruits of Citrus sinensis var. Washington Navel, C. sinensis var. Sanguinello, C. sinensis var. Valencia, C. reticulata var. Ponkan, C. reticulata var. Page, C. reticulata var. Clementine, C. unshiu var. Mahalli, C.

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unshiu var. Sugiyama, C. unshiu var. Ishikawa, C. limon, C. paradisi, C. aurantium and C. aurantium var. Khosheii were collected at the ripening stage. All the trees were cultivated in the experimental fields, Fajr citrus experimental institute. Edible parts of the fruits were cut into small slices (0.5-1.5 cm) and lyophilized. Resulting dried material was powdered using blender. Fruits peels were dried at room temperature and coarsely ground before extraction. Dried powdered samples were extracted at room temperature by percolation with methanol. All extracts were concentrated over a rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extract was freeze-dried.

Determination of total flavonoid content

Colorimetric aluminum chloride method was used for flavonoid determination (Ebrahimzaded *et al.*, 2008a, b; Nabavi *et al.*, 2008). Briefly, 0.5 mL solution of each plant extracts in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg ml⁻¹ in methanol.

Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteu method (Ebrahimzaded *et al.*, 2008a, b; Nabavi *et al.*, 2008). The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na₂CO₃ (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetry at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg ml⁻¹ solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound.

DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Ebrahimzadeh *et al.*, 2008a, 2008b, 2008c). Different concentrations of each extracts were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamine C, BHA and quercetin were used as standard controls. IC₅₀ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

STATISTICAL ANALYSIS

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance (p < 0.05) and the means separated by Duncan's multiple range test. The IC₅₀ values were calculated from linear regression analysis.

RESULTS

Total phenol compounds, as determined by folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y = 0.0063x$, $r^2 = 0.987$). The total phenolic contents were usually higher in peels with the range of 104.2-223.2 respect to tissues with the range of 66.5-396.8 mg gallic acid equivalent/g of extract powder. C. reticulata var. Clementine and C. reticulata var. Page tissues showed the highest values (396.8 and 226.2 mg gallic acid equivalent/g of extract powder, respectively).

The total flavonoid contents were usually higher in peels with the range of 0.3- 31.1 respect to tissues with the range of 0.3-17.1 mg quercetin equivalent/g of extract powder by reference to standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$). C. unshiu var. Mahalli and C. sinensis var. Washington Navel peels showed the highest values (31.1 and 23.2 mg quercetin equivalent/g of extract powder, respectively).

The extracts showed weak antioxidant activities. C. reticulata var. Ponkan peels showed highest activity (IC₅₀ = 0.6 mg ml⁻¹) and C. aurantium tissues showed the weakest one (IC₅₀ = 3.9 ml⁻¹). The IC₅₀ values for Ascorbic acid, quercetin and BHA were 5.05 ± 0.12, 5.28 ± 0.43 and 53.96 ± 2.13 µg ml⁻¹, respectively. Results summarized in table 1 and figs. 1 and 2.

DISCUSSION

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee *et al.*, 2003). It was found that the radical- scavenging activities of all the extracts increased with increasing concentration. The high phenol and flavonoids contents of Peels and tissue of C. reticulata var. Ponkan may cause high antioxidant activity of this plant. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (van Acker *et al.*, 1996). The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables (Klimczak *et al.*, 2007; Kiselova *et al.*, 2006; Jayaprakasha *et al.*, 2008; Kedage *et al.*, 2007). As reported, antioxidant activity of

Table 1: Radical scavenging activity and total phenol and flavonoids contents in peels and tissues of 13 most commonly used citrus spp.

No.	plant	Phenol content *	Flavonoids content **	DPPH IC ₅₀ ***
	<i>C. sinensis</i> var. Washington Navel			
1	peel	160.3	23.2	1.1
2	tissues	232.5	1.2	2.8
	<i>C. reticulata</i> var. Ponkan			
3	peel	172.1	5.2	0.6
4	tissues	197.4	0.6	2.2
	<i>C. unshiu</i> var. Mahalli			
5	peel	170.5	31.1	1.9
6	tissues	66.5	6.4	3.9
	<i>C. unshiu</i> var. Sugiyama			
7	peel	195.5	19.8	1.3
8	tissues	140.9	2.2	3.6
	<i>C. sinensis</i> var. Sungin			
9	peel	153.8	2.1	1.7
10	tissues	136.9	4.3	3.7
	<i>C. unshiu</i> var. Ishikawa			
11	peel	148.8	4.8	1.8
12	tissues	144.9	5.3	3.8
	<i>C. limon</i>			
13	peel	131.0	16.2	1.4
14	tissues	102.2	2.0	3.4
	<i>C. reticulata</i> var. Clementine			
15	peel	161.7	5.7	1.7
16	tissues	396.8	17.1	3.2
	<i>C. paradisi</i>			
17	peel	222.2	23.2	2.1
18	tissues	112.1	0.3	3.7
	<i>C. aurantium</i>			
19	peel	223.2	7.7	1.9
20	tissues	122.0	3.3	3.9
	<i>C. sinensis</i> var. Valencia			
21	peel	132.9	7.2	2.1
22	tissues	124.0	3.1	3.8
	<i>C. aurantium</i> var. Khosheii			
23	peel	164.7	7.9	1.8
24	tissues	90.3	3.5	3.5
	<i>C. reticulata</i> var. Page			
25	peel	104.2	0.3	2.9
26	tissues	226.2	6.8	3.7

* mg gallic acid equivalent/g of extract powder, ** mg quercetin equivalent/g of extract powder

***mg/ ml. The IC₅₀ values for ascorbic acid, quercetin and BHA were 17.3 ± 0.12 , 19.3 ± 0.43 and $73.6 \pm 2.13 \mu\text{g ml}^{-1}$, respectively

fruits and vegetables significantly increases with the presence of high concentration of total polyphenol content. In the present study, the correlation between total phenolic and flavonoids contents and radical scavenging activity of 26 methanolic extracts from 13 citrus species were analyzed. The correlation graphs are depicted in figs. 1 and 2. In general, extracts or fractions with a high radical scavenging activity showed a high phenolic content as well, but good correlations could not be found

among them (fig. 1). A direct correlation between radical scavenging activity and phenolic content of the samples was failed to demonstrate by linear regression analysis. There was no correlation between total flavonoids and radical scavenging activity, too (fig. 2). This lack of relationship is in agreement with other literature (Heinonen *et al.*, 1998; Anagnostopoulou *et al.*, 2006; Nickavar *et al.*, 2007). It is known that only flavonoids with a certain structure and particularly hydroxyl position

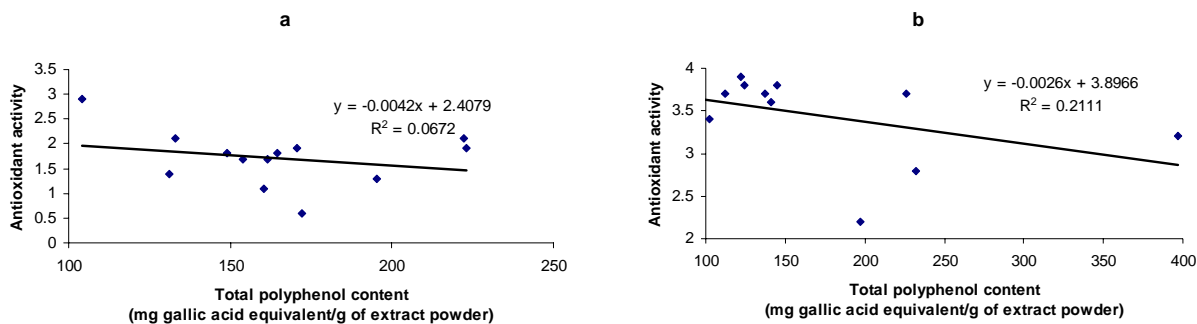


Fig. 1: Correlation between antioxidant activity (DPPH method) and polyphenol content of methanolic (a) peels and (b) tissues extracts of 13 citrus spp.

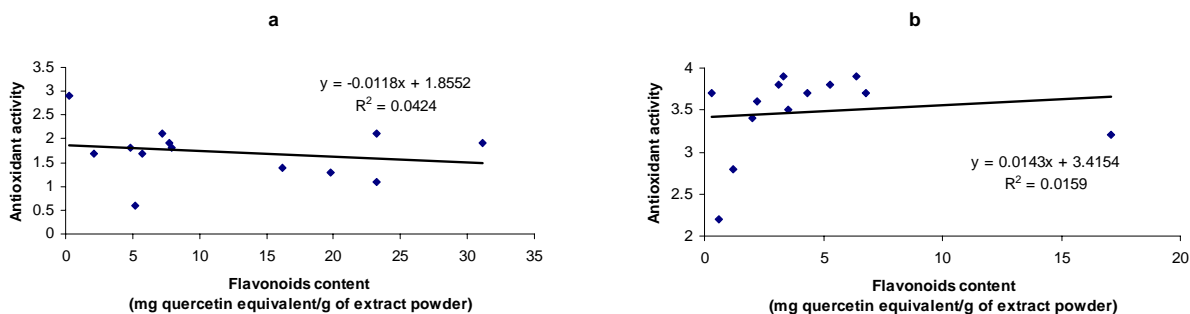


Fig. 2: Correlation between antioxidant activity (DPPH method) and flavonoids content of methanolic (a) peels and (b) tissues extracts of 13 citrus spp.

in the molecule can act as proton donating and show radical scavenging activity (Mensor *et al.*, 2001; Hou *et al.*, 2003). Furthermore, the extracts are very complex mixtures of many different compounds with distinct activities (Mensor *et al.*, 2001; Hou *et al.*, 2003).

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