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Phenolic Compounds and Antioxidant Activity of Some Citruses

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Abstract: Citrus fruits (Rutaceae) are important source of phenolic compounds and their glycosides. These compounds are phenolic acids for bioactive responsible for the antioxidant and many other biological activities. In this paper five extracts from juice and peel of different types of citruses (tangerine, lemon, pink grapefruit, white grapefruit, and orange) were analyzed for total phenolic content and antioxidant activity. Total phenolic content was determined by spectrophotometric Folin-Ciocalteu method, and the values varied from 0.192 ± 0.015 mg GAE/mL for white grapefruit peel to 0747 ± 0098 mg GAE/mL for white grapefruit juice. Antioxidant activity of samples was tested using the total antioxidant method that implies reduction of molybdenum ions and it is expressed as IC_{50}. The IC_{50} values were ranged from 6.00 ± 0.50 mg/mL for orange juice to 78.11 ± 6.70 mg/mL sample of lemon juice.

INTRODUCTION

Phenolic compounds, as secondary metabolites, are widely distributed in plants. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites, as well as contributing to plants' colors. They are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.), and partially responsible for the overall organoleptic properties of plant foods (Shahidi & Naczak, 2004).

Despite their wide distribution, the health effects of dietary polyphenols have come to the attention of nutritionists only in recent years. Researchers and food manufacturers have become more interested in polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases (Gornistein *et al*., 2001; Manach *et al*., 2004; Li *et al*., 2006). The preventive effects of these second plant metabolites in terms of cardiovascular, neurodegenerative diseases and cancer are deduced from epidemiologic data as well as *in vitro* and *in vivo* (Hertog *et al.*, 1994; Cole *et al.*, 2005) and result in respective nutritional recommendations.

Phenolic compounds are excellent antioxidants due to their ability to donate an electron or hydrogen from phenolic hydroxyl groups. Resulting phenoxy radical tends to be poorly reactive because of electron delocalization in the aromatic ring, and therefore reactive radical is replaced by a radical of limited activity (Li *et al*., 2006; Shahidi and Naczak 2004; Topčagić, 2009).

Citrus is the general term for plants belonging to the family Rutaceae. Plants have large shrubs or small trees that can reach a height of 5-15 m with branches covered with thorns and evergreen leaves (Ladaniya, 2008). They are important source of many bioactive compounds, such as phenolic acids and flavanone glycosides. Naringin and hesperidin, so-called citrus flavonoids, are the two main glycosidic flavanones presented in citruses (Abeysinghe *et al*., 2007). Caffeic, chlorogenic, ferulic, sinapic and *p*-coumaric acid are the most abundant phenolic acids present in citruses (Tokusoglu & Hall, 2011).

The aim of this study was to determine the content of phenolic compounds and total antioxidant activity in extracts isolated from several species of citruses (tangerine, orange, red grapefruit, white grapefruit, and lemon).

EXPERIMENTAL

Isolation

Mandarin, orange, white grapefruit, pink grapefruit, and lemon, were purchased at a local supermarket in October 2012.The peel is separated from the edible part, dried at room temperature and grinded. Then, 1 g of each sample was extracted with ethanol using ultrasonic bath. All samples were filtered and stored in a refrigerator at 4°C in glass bottles, until analysis.

Edible part was squashed and the juice was filtered through gauze and stored in a refrigerator at 4°C in plastic bottles, until analysis.

Determination of total phenolics

Total phenolic content was measured using Folin-Ciocalteu spectrophotometric method (Singleton & Rossi, 1965), using gallic acid for calibration curve. All tests were performed in triplicates, and results are presented as gallic acid equivalents.

Antioxidant activity

Antioxidant activity of isolated extracts was tested using total antioxidant capacity spectrophotometric method (Prieto *et al*., 1999). Method is based on the ability of potent antioxidant to reduce molybdenum ions. All tests were performed in triplicates, and results are presented as IC_{50} values that indicate the concentration of extracts that reduces the 50% of molybdenum. Catechin was as standard probe.

RESULTS AND DISCUSSION

Isolation of phenolic compounds from peel and juice of citrus fruits was performed using ultrasonic extraction. The yields isolated from the peel were in the range from 4.91% to 7.44%, for orange and white grapefruit, respectively.

Determination of the total content of phenolic compounds was performed using spectrophotometric Folin-Cicolateu method.

Results from spectrophotometric determination of total phenolic content in isolated extracts are summarized in Table 1 as mg of gallic acid equivalent per gram of extract, and as content of phenolic compounds in extract. Values are represented as the mean taking into account the standard deviation. The total content of phenolic compounds of peels of citrus fruits ranging from 0.192 ± 0.015 mg GAE/mL for white grapefruit to 0.480 ± 0.007 mg GAE/mL for lemon, while total phenolics for juices were ranged from0.322 ± 0.002 mg GAE/mL for lemon to 0.747 ± 0.098 mg GAE/mL for white grapefruit.

Based on the results, it can be concluded that the content of phenolic compounds is generally higher in juice samples.

Table 1: The phenolic content of extracts of citrus fruits.

Sample	Total phenolic content mg GA/mL	%
Lemon peel	0.480 ± 0.007	0.89 ± 0.01
Lemon juice	0.322 ± 0.002	0.46 ± 0.04
Orange peel	0.452 ± 0.027	0.61 ± 0.04
Orange juice	0.437 ± 0.002	1.02 ± 0.04
Mandarin peel	0.334 ± 0.014	0.49 ± 0.02
Mandarin juice	0.357 ± 0.033	0.51 ± 0.05
Red grapefruit peel	0.283 ± 0.018	0.55 ± 0.03
Red grapefruit juice	0.359 ± 0.029	0.44 ± 0.04
White grapefruit peel	0.192 ± 0.015	0.39 ± 0.03
White grapefruit juice	0.747 ± 0.098	1.00 ± 0.08

According to available literature data for samples of peel of citrus fruits, there are certain differences in the results. In a study of Ghasemi *et al*., (2009) who used percolation with methanol, the highest content of phenolics was in the orange peel (232.5 mg GAE/g), while the lowest content was found in the use of lemon peel (102.2 mg GAE/g). Moreover, the content of phenolic compound detected in juices of citruses was also lower in comparison with the literature. Petchlert *et. al.* (2013) reported the values ranging from 5.71 ± 0.01 mgGAE/mL to 10.57 ± 0.17 mg GAE/mL for mandarin and orange, respectively.

Reason for these differences might be in diverse types of extraction and solvent used, as well as the different origin of the samples.

Results of determination of total antioxidant activity using molybdenum reduction method are shown in Table 2, where the IC_{50} present the concentration of extract to reduce 50% of molybdenum cation. Values are represented as the mean taking into account the standard deviation.

Table 2: Antioxidant activity of extracts of citrus fruits.

Sample	IC_{50} (mg/mL)
Chlorogenic acid	3.11 ± 0.22
Orange juice	6.00 ± 0.50
Mandarin peel	9.13 ± 0.28
Orange peel	19.15 ± 0.24
Lemon peel	20.30 ± 1.98
Red grapefruit peel	24.52 ± 1.33
White grapefruit peel	30.93 ± 0.86
Red grapefruit juice	33.55 ± 0.60
Mandarin juice	36.82 ± 1.82
White grapefruit juice	39.46 ± 0.57
Lemon juice	78.11 ± 6.70

The IC_{50} values are in the range of 6.00 ± 0.50 mg/mL to 39.46 ± 0.57 mg/mL for juices, and from 9.13 ± 0.28 mg/mL to 30.93 ± 0.86 mg/mL for peels. Results are in comparable range with the IC_{50} value chlorogenic acid (3.11 ± 0.22 mg/mL) which was used as standard probe.

Since the lower IC_{50} value indicates better antioxidant activity, it might be concluded that peels of citrus fruits possess higher antioxidant activity that juices.

Presented results are in agreement with those published earlier (Ghasemi *et al*., 2009).

It is known that phenolic compounds are carriers of antioxidant activity in plant extracts (Zheng & Wang, 2001). Based on the data reported in this paper, it can be seen that there is a slight correlation between the content of total phenolics and antioxidant activity. The reason lies in complexity of isolated extracts, and as well as in the fact that antioxidant activity cannot be defined on the basis of one method used.

CONCLUSIONS

Citrus fruits contain a large variety of bioactive components and are considered as potential sources of functional components. The citrus fruits purchased in market were evaluated for the total phenolics and antioxidant activity. Presented results reveal that citrus fruits are of good quality and a valuable source of health promoting constituents.

Presented results suggest further analysis of the chemical composition of isolated extracts to identify phenolic compounds that could be responsible for the antioxidant activity, as well as more diferent asssays of antioxidant activity.

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Summary / Sažetak

Citrus je uobičajni termin za biljke iz porodice *Rutaceae.* Citrusi su važan izvor bioaktivnish spojeva koji su odgovorni za antioksidativnu aktivnost kao što su fenolski spojevi i njihovi glikozidi. U ovom radu je vršena analiza pet ekstrakata soka i kore različitih vrsta citrusa (mandarina, limun, crveni grejp, bijeli grejp i narandža) na sadržaj ukupnih fenola i antioksidativnu aktivnost. Ukupan sadržaj fenola određen je Folin-Ciocalteu metodom, dobivene vrijednosti variraju od 0.192 ± 0.015 mg EGK/mL za koru bijelog grejpa do 0.747 ± 0.098 mg EGK/mL za sok bijelog grejpa. Antioksidativna aktivnost ekstrakata ispitana je pomoću spektrofotometrijske metode bazirane na redukciji molibdena i izražena kao IC_{50}. Vrijednost IC_{50} se kreće od 6.00 ± 0.50 mg/mL za uzorak soka narandže do 78.11 ± 6.70 mg/mL za uzorak soka limuna.