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PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF PEELS AND SEEDS OF ORANGE (*CITRUS SINENSIS*) CULTIVATED IN IRAQ

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ABSTRACT

Four different extracts of Iraqi sweet orange (*Citrus sinensis*) peels and seeds were evaluated for their total phenolic content (TPC) using the Folin–Ciocalteu method. In addition, the scavenging activity against the DPPH radical was evaluated by the DPPH method. The results showed the presence of phenolic compounds in peels and seeds with varying proportions. The TPC of the peels were significantly ($P < 0.05$) higher than that of the seeds. The highest TPC and radical scavenging activities were found in both peels and seeds prepared in 5% hydrochloric acid (HCl) solvent, followed by those prepared in boiling water, 50% ethanol and then cold water. The antioxidant activity found in the peels and seeds of *Citrus sinensis*, should be attributed to the presence of phenolic compounds as evidenced by the positive correlation between the radical scavenging activity and TPC. In conclusion, citrus by-products (peels and seeds) possess relatively high antioxidant activity and may represent very rich sources of natural low-cost antioxidants which can be used for various purposes. Moreover, the results of the present study recommend using diluted solution (5%) of HCl for optimum extraction of phenolic compounds and consequently high antioxidant activity.

KEYWORDS: Orange (*Citrus sinensis*), Phenolic contents, Antioxidant activity, Peels, seeds.

INTRODUCTION

The oxidative species, which are produced in the body either naturally or artificially, can damage many biological molecules including fatty acids, proteins, deoxyribonucleic acid and

carbohydrates[1] and accordingly may lead to the development of various physiological and pathological syndromes such as aging, neurodegenerative diseases, cardiovascular diseases and various cancers.[2,3]

The fruits are rich sources of various vitamins, minerals and fibers required by human body for optimal health. It has been reported that consumption of fruits was associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer and these health benefits were attributed mainly to the antioxidants found in the fruits.[4, 5]

Phenolic compounds have been identified as important antioxidants found in fruits and are even more powerful as antioxidants than vitamin C, E both *in vitro* and *in vivo.*[6, 7, 8, 9]

Moreover, our previous studies and other studies have showed a strong correlation between the contents of the phenolic compounds and the antioxidant activity.[6, 10, 11, 12]

Our interest in the health benefits of the agricultural and industrial waste products steamed from the fact that the peel and seed fractions of some fruits possess higher antioxidant activity than the pulp fractions.[13, 14] This means we can achieve dual benefits by dealing with the agricultural wastes; health benefits and also minimizing their harmful effects on environment.

The objectives of this study were to determine and to compare the content of phenolic compounds and the antioxidant capacity of peels and seeds of Iraqi orange, using different solvents. This study is part of a program aiming at the determination of health benefits of agricultural and industrial waste products via determining the phenolic contents and the antioxidant activity.

MATERIALS AND METHODS

Sample preparation and extraction

The orange (*Citrus sinensis*) fruits were purchased from the local markets of Diyala Province, middle of Iraq. The fruits were first flushed by tap water and then washed in distilled water for three times before the peel, pulp and seed fractions were carefully separated. Only the peels and seeds were used in the current study. They were dried at 50 C using an electric oven and then grinded using coffee grinder. A portion of 5 grams was weighed and mixed with 50 ml of the selected solvents [cold distilled water, boiling distilled water, hydro-ethanolic solution (1: 1 v/v) and diluted hydrochloric acid (5% with cold distilled water)].

The mixtures were left for 24 hours and then centrifuged at 5000g for 10 minutes. The supernatants were recovered and used directly for the determination of the total phenolic contents and the antioxidant activity.

Measurement of total phenolic contents (TPC)

The amount of total phenolic content (TPC) in the extracts was determined according to the Folin-Ciocalteu procedure as used by Molan et al. [10] with some modifications. Briefly, an aliquot of 12 µL of water-soluble extract was mixed with 200 µL of 2% sodium carbonate solution in 96-well microplates and allowed to react for 5 min at room temperature. Then, 12 µL of Folin-Ciocalteu phenol reagent (50%) was added and allowed to stand for 30 min at room temperature before the absorbance of the reaction mixture was read at 650 nm using a plate reader. Calibration was achieved with an aqueous gallic acid solution (100–1000 µg/ml). The TPC of the extract was expressed as mg gallic acid equivalent (GAE) per gram of peels and seeds on a dry basis.

Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals

This assay detects scavenging of free radicals by the tested compound through the scavenging activity of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical. This assay was performed using a previously described method[15] with some minor modifications.[10] Briefly, 20 µL of each extract was allowed to react with 200 µL of 0.2 mM DPPH in 95% ethanol in a 96-well microplate. The plate was then incubated at 37 C for 30 min after which the absorbance was measured at 550 nm using a microplate reader.

The antiradical activity was calculated as a percentage of DPPH decolouration relative to a negative control using the following equation.

Antiradical activity (%) = absorbance of control incubation - absorbance of the extract/ absorbance of control incubation X 100.

The scavenging activity against the DPPH radical was compared with that of Gallic acid, which is known of its antioxidant activity.[16]

Statistical analysis

All measurements were performed in triplicates. The results were expressed as mean ± SEM and analyzed using SAS version 9.2 for windows (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) followed by Tukey's post test was used to test for

significant differences among means. Linear regression analysis was performed to determine the correlation between total phenolic contents and the scavenging activity against DPPH radical. The differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

The TPC values of the orange peel extracts ranged from 15.1 to 53.1 mg GAE/g dry peels and from 10.9 to 39.4 mg GAE/g dry seeds (Table 1). The TPC of the four samples decreased in the following order: 5% HCl extract > boiling water > 50% ethanol extract > cold distilled water extract. The levels of phenolic compounds change significantly ($P< 0.05$) depending on the type of the solvent and the nature of the plant part used (peels or seeds). In all solvents, the TPC values of the peels were significantly higher ($P< 0.05$-0.001) than those of the seeds (Table 1). Similarly, many previous studies have shown that Citrus peels contain high concentrations of phenolic compounds.[16, 17, 18]

Acidification of cold distilled water with HCl (5%) resulted in a significant increase in TPC in comparison with other solvents. Recently, Yang et al.[19] investigated the effect of acid and alkali hydrolysis on total phenolic content in microwave-assisted extraction and found that acid hydrolysis was suitable for the extraction of phenols from *Geranium sibiricum* and hydrochloric acid (HCl) hydrolysis was more efficient than alkali hydrolysis in this regard. The authors concluded that HCl hydrolysis in microwave-assisted extraction provides an efficient and rapid approach for the natural products extraction and the research also provides a valuable nature resource for healthy food industry. Although the mechanism of action of acid hydrolysis is not fully known, HCl can improve the extraction of phenolic compounds via the cleavage of the ether and/or ester linkages.[20]

It is interesting to find that boiling water is better than hydroethanolic mixture (50:50, v/v with distilled water) for extracting phenolic compounds. Some studies have shown that hot water extraction of tea has been reported to provide the highest extraction yield of phenolic compounds and antioxidant activity than that of ethyl acetate and methanolic extractions.[21,22]

The TPC values of seed samples extracted by the four solvents follow the same order as in the peel samples mentioned above. Accordingly, the 5% HCl extract seems to be the best solvent for the extraction of TPC as evidenced by the highest values of TPC in both the peels and seeds of orange in comparison with the other solvents. The results showed clearly that the

TPC values of the seed extracts were significantly lower ($P< 0.05$) than those of the peel extracts and this trend was seen in all the solvents used.

In comparison with the results of other studies conducted on oranges cultivated in other countries, the TPC values of the present study were lower than those reported by Kamran et al.[23] who reported that the orange peels contain 132.2–223.2 mg GAE/g DM while our values are much higher than those reported by Anagnostopoulou et al.[24] and Li et al.[25] who reported values ranged between 0.036 and 2.54 mg GAE/ g DM. Recently, Lagha-Benamrouche and Madani[16] reported that the peels of the oranges cultivated in Algeria contain 9.608–31.623 mg GAE/g DM of polyphenolic compounds.

Free radical scavenging activity

The highest free radical scavenging activity, expressed as the percentage inhibition, was found for both peels and seeds prepared in 5% hydrochloric acid (HCl) solvent, followed by those prepared in boiling water, 50% ethanol, and cold water (Fig. 1). As with the TPC, the results showed clearly that the free radical scavenging activity of the seed extracts against DPPH were significantly lower ($P< 0.05$) than those of the peel extracts and this trend was seen in all the solvents used (Fig. 1).

The scavenging activity against the DPPH radical was compared with that of Gallic acid, which is known of its antioxidant activity.[16] It is very important to mention that the scavenging activity against the DPPH radical of the peels and seeds of Iraqi sweet orange should be considered high in comparison with the gallic acid because only crude extracts (powdered dry peels and seeds) while in other studies purified or semi-purified extracts have been used.

The antioxidant activity found in the peels and seeds of *Citrus sinensis*, should be attributed to the presence of phenolic compounds as evidenced by the positive correlation between the radical scavenging activity and TPC. In addition, the orange peel contains other phytochemicals such as hesperidin which may contribute to the antioxidant activity.[26]

Several studies have reported on the correlation between total phenol contents and the scavenging activity against the stable DPPH radical with varying outcomes. In this study, the results have shown a positive correlation between the scavenging activity against the stable DPPH radical and total phenolic contents and the correlation depends mainly on the solvent

and the part of the fruit (peels or seeds) used (Table 2). The range of correlation coefficient between total phenolics and DPPH for peels and seeds found to be 0.4252-0.9999 and 0.7882-0.9999, respectively (Table 2). Similarly, the results of the study conducted by Lagha-Benamrouche and Madani[16] indicate the presence of a strong correlation between the scavenging activity against the stable DPPH radical and total phenol for peels (R^2 = 0.92) of oranges cultivated in Algeria. In contrast, Anagnostopoulou et al.[24] evaluated the radical scavenging activity of various extracts and fractions of Greek sweet orange peel (*Citrus sinensis*) and reported that the total phenolic content did not correlate well with the radical scavenging activity.

Table 1. Total phenol content (TPC) of the peels and seeds of Iraqi sweet orange (*Citrus sinensis*) extracted by four different solvents as determined by the colorimetric Folin–Ciocalteu method. The TPC values are expressed in mg Gallic acid equivalent (GAE) per gram dry ground peels and seeds.

Solvent/extract	TPC in mg GAE/g dry weight	
	Peels	Seeds
Cold distilled water	15.1 ± 1.7*	10.9 ± 1.1
Boiling distilled water	38.4 ± 5.2*	26.1 ± 2.1
50% ethanol	25.9 ± 1.7*	18.6 ± 1.1
5% Hydrochloric acid	53.1 ± 3.1*	39.4 ± 1.1
Range	15.1-53.1	10.9-39.4

Values are the mean of three determinations ± SE (standard error of the mean).

* indicates a significant difference (P< 0.05-0.001) between the peels and seeds.

Table 2. Correlation (R^2 value) between the total phenolic content (TPC) and the scavenging activity against DPPH radical of extracts prepared from the peels and seeds of Iraqi sweet orange (*Citrus sinensis*) using four different solvents.

Solvent/extract	Correlation (R^2 value) between total phenolic content and scavenging activity against DPPH radical.	
	Peels	Seeds
Cold distilled water	0.8192	0.4252
Boiling distilled water	0.9222	0.9999
50% ethanol	0.7882	0.4775
5% Hydrochloric acid	0.9999	0.8337
Range	0.7882-0.9999	0.4252-0.9999

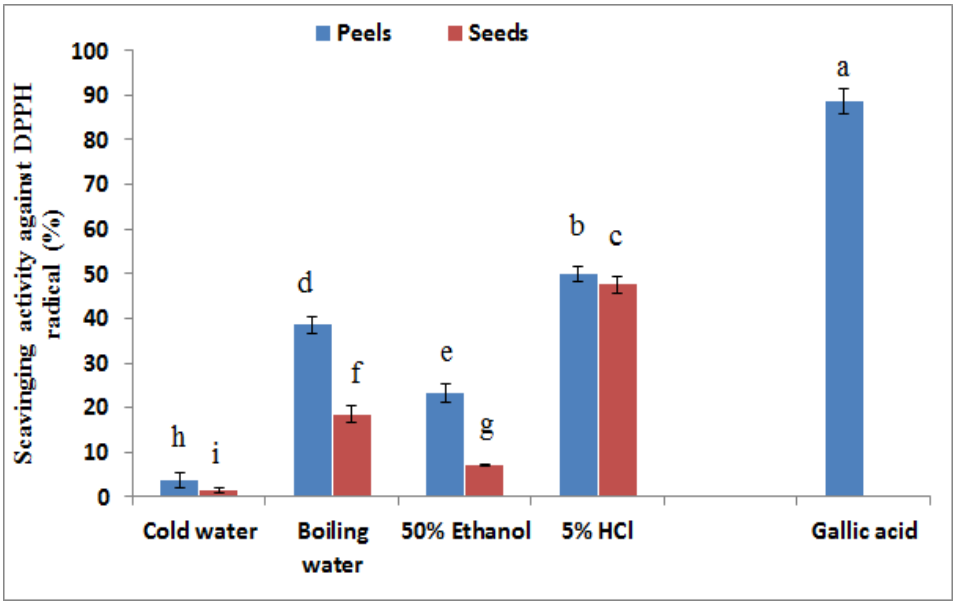

Figure 1. Scavenging activity (%) against DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical of peels and seeds extracts at 10 mg/ml and the standard (Gallic acid) at 50 µg/ml. Values are the mean of three determinations ± SE (standard error of the mean). Columns with the same letters showed no significant difference at a level of P < 0.05.

CONCLUSION

Extracts of Iraqi sweet orange peels and seeds were examined by DPPH method for their free radical activity, as well as for their total phenolic content (TPC) by the Folin–Ciocalteu test. Results showed clearly that the TPC and the scavenging activity against the DPPH radical depend mainly on the solvent and the part of the fruit used. The results showed that the diluted hydrochloric acid (5% HCl) extracted significantly more phenolic compounds from both the peels and seeds than the other solvents and consequently showed the best radical scavenging activity. It is important to mention that the radical scavenging activity correlates well with TPC. Further studies on the effective antioxidants contained in the peels and seeds and the mode of their action are highly warranted.

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