In Vitro Antioxidant Activity of Extracts from Wastes of Five Iranian Citrus Species

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Summary

The fact that many health problems arise from synthetic ingredients has directed research to investigate natural products. Many fruits and vegetables are safe and health-promoting due to the presence of various phytochemicals. It is known that citrus fruits and juices contain secondary metabolites including antioxidants that are important to human health. However, very little is known about nutritional and medicinal value of their wastes. Due to the presence of many citrus processing industries in our part of Iran, in the present study, we have investigated the medicinal value of mixed wastes from 5 citrus species. Antioxidant fractions were extracted from wastes produced in the laboratory using different methanolic solvents by Soxhlet extractor. The total phenolic content of the extracts was determined by Folin–Ciocalteu method. The dried fractions were screened for their antioxidant activity potential by 1-diphenyl-2-picryl hydrazyl (DPPH), ferric reducing power (FRAP). The results showed that waste extracts obtained from all citrus species exhibited remarkable antioxidant activity. We concluded that fruit wastes and possibly vegetable by-products have good potential as sources of polyphenols. However, more research should be carried out to identify other possible antioxidants and bioactive compounds in these resources.

Keywords: Antioxidant activity; DPPH; ABTS; total phenol content.

Introduction

The demand for natural sources of supplements is increasing every day due to the more awareness about the dangers and side effects of synthetic counterparts. Natural food supplements are not only healthier, but they are also less contaminated with by products (1). The presence of various antioxidants together with their composition has been reported in various fruits and vegetables (2, 3). In our research laboratory, special attention is also made within our investigations on antioxidant activity of extracts and essential oils of plants growing in our part of the world [4-10]. On the other hand, the effect of various environmental stresses on the activity of antioxidant enzymes has been studied [11, 12]. The presence of a novel tyrosinase was also found and reported in Iranian peanut [13]. It must be emphasized that when using natural sources, a very careful attention should be made, as only little is known about their exact composition and possible presence of allergy causing ingredients (14).

Due to the presence of many citrus trees and gardens in North part of Iran, a large body of wastes is produced each year. These left over wastes come either from citrus gardens after harvest or from juicing factories present in this part of Iran. We have previously obtained reliable results from our *in-vitro* experiments on the antioxidant activity of lemon wastes [10]. Therefore, the aim of this study was to assess the antioxidant activity present in mixed wastes from Thomson, Hamlin, Moro and Mars oranges together with one type of one species of Satsuma, page, all grown in Northern Iran.

Materials and methods

Selected citrus species were all collected from public and home gardens in Gilan or purchased from local market. 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH$^{\circ}$), potassium persulfate, phosphate buffer and 2,4,6-tripridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich Chemie, Steinheim, Germany. All solvents were of reagent grade and obtained from Merck representative in Iran.

Preparation of methanolic extract from citrus wastes

Mixed sample of citrus wastes were produced in the laboratory, either by juicing of eating the flesh and leaving the peels. The citrus species were all collected from gardens in Gilan or purchased from local market. The mixed wastes obtained from each citrus species were ground and stirred thoroughly with extracting solvent in a food processor. The mixture was filtered using Whatman filter paper followed by centrifuge at high speed. Two types of solvent were used both containing methanol and water with varying water contents. The extracted mixture was then dried 40 °C and weighed and the extraction yield was determined accordingly (15).

DPPH radical scavenging assay

This method is based on the reduction of stable DPPH$^{\bullet}$ when it accepts a hydrogen from an antioxidant compound. Radical scavenging activity of extracts from mixed citrus wastes extracts against stable DPPH$^{\circ}$ was determined spectrophotometrically. The changes in color (from deep-violet to light-yellow) were measured at 515 nm using a UV/visible light spectrophotometer.

The method was a modification of the procedure described by Moure (16). In brief, 0.025 g of dried extracts was dissolved in 10 ml of methanol. A freshly prepared solution of DPPH$^{\circ}$ in methanol (6×10^{-5} M) was always used for UV measurements. 3 ml of methanolic solution of DPPH$^{\bullet}$ was then added to 77 µl extract solution in 1 cm path length disposable microcuvettes and mixed. The final mass ratio of extracts with DPPH$^{\bullet}$ was approximately 3:1, 1.5:1, 0.75:1. The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. The following equation was then used to calculate radical scavenging activity:

$$\% \text{ Inhibition} = \left[(A_B - A_A)/A_B \right] \times 100,$$

where: A_B-absorption of blank (t=0 min); A_A-absorption of sample (t=15 min).

Ferric reducing power (FRAP) assay

This method is based on the activity of extract to reduce Fe^{3+} to Fe^{2+} ions in the presence of 2,4,6-tripridyl-s-triazine (TPTZ). The later reacts with Fe^{2+} ions to produce a blue colored complex with maximum absorption at 593 nm. The FRAP reagent was freshly prepared by mixing 10 mM TPTZ, 40 mM HCl and 20 mM ferric chloride (10:1:1). After 10 minutes mixing the freshly prepared ingredients at 37°C, 10 ml of the reagent was added to 100 µl of the extract sample. The blank was also prepared exactly the same way with pure solvent replacing the extract. The 593 nm absorbance was then measured after 30 minutes

Determination of total phenolics

The total phenolic contents of extracts was determined by a modified by (17). In practice, the dried extracts were dissolved in mixtures of methanol and water. To this solution, 1 ml of 10-fold diluted Folin–Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate solution were added, mixed well and incubated at room temperature for one hour. The absorbance of the solution was then measured at 765 nm using Shimadzu UV-Visible spectrophotometer.

Statistical analysis

All determinations were carried out in three triplicate and data were subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the MSTATC software. Significant differences between means were determined by Duncan's multiple range test. *P* values less than 0.05 were considered statistically significant.

Results and discussion

Total antioxidant capacity in various extracts from citrus wastes measured by FRAP method were compared and the results are presented in Figure 1. It was observed that among various species tested, the highest activity was shown by methanol extracts of Hamlin, while Mars showed the lowest antioxidant power.

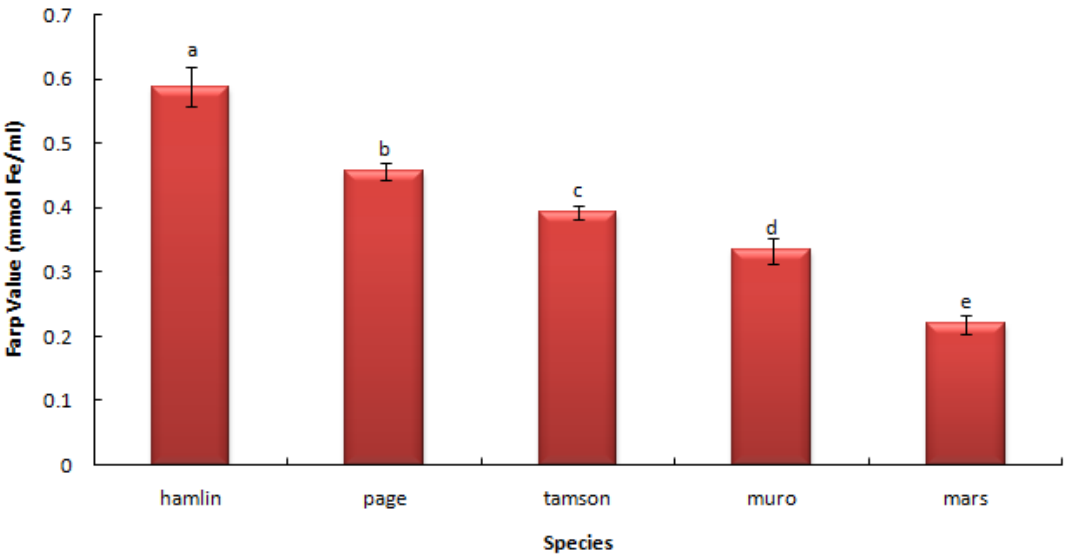

Figure 1. Total antioxidant power obtained from FRAP assay. The results are mean value of 3 experiments (P<0.05).

The results obtained from DPPH tests also showed a similar behavior in waste extracts from various citrus species (Figure 2).

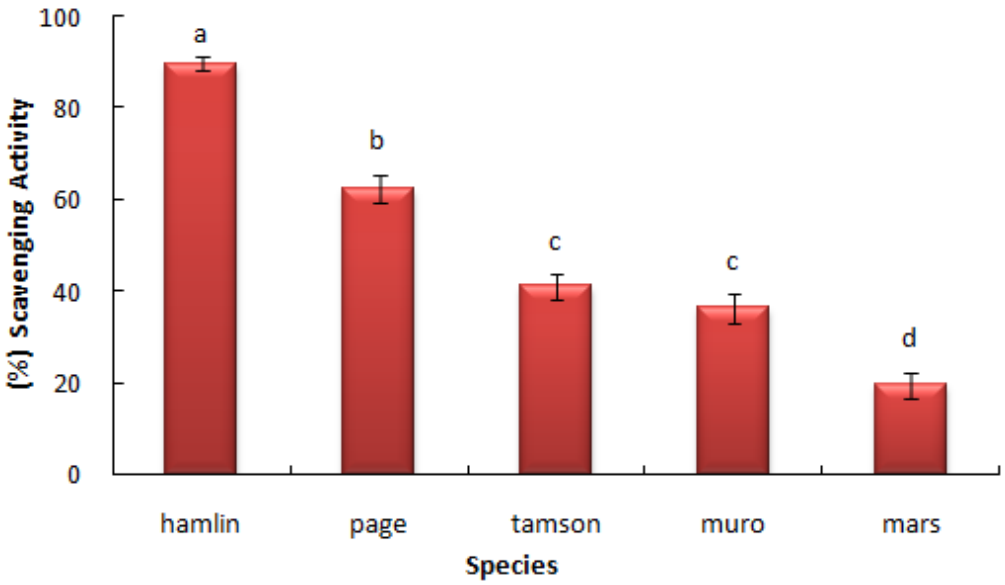

Figure 2. Percent of radical scavenging of methanol extracts of citrus wastes. The results are the mean value of three repeated experiments ($P<0.05$).

The relationship between total phenol content and antioxidant power was obtained by plotting the two values against each other (Figures 3 and 4). Although we found a good relationship between total phenol content and radical scavenging activity in citrus waste extracts (Figure 3), the results of similar studies reported in literature are slightly different. For example the results reported by some other scientists have shown no relationship between the two factors (18).

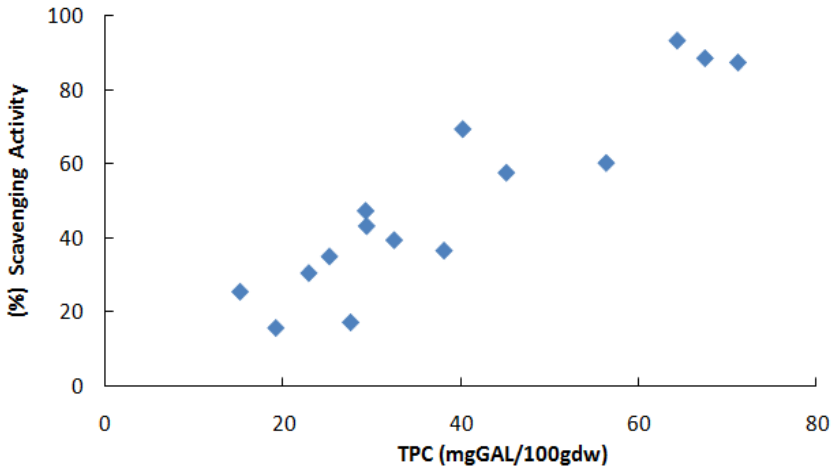

Figure 3. The relationship between total phenol content (TPC) in terms in milligrams of gallic acid and % of radical scavenging activity (DDPH).

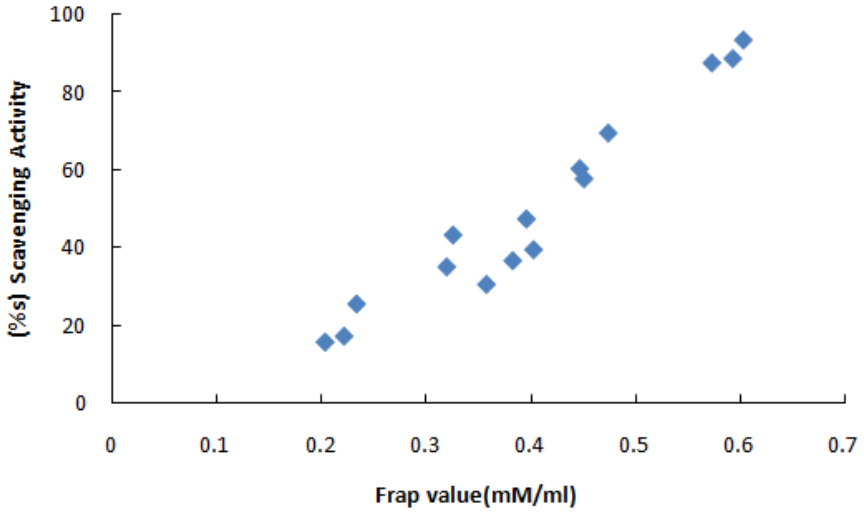

Figure 4. The relationship FRAP assay results and % of radical scavenging activity obtained by DDPH.

It has been reported that citrus fruits and juices contain many important antioxidants including ascorbic acid, flavanones, phenolics and pectin. For example, inhibitory activity of citrus limonoids on human cancer cell proliferation has been reported (19). The antiproliferative activity of citrus components such as pectin, pulp, naringin and limonin against colon cancer in vivo systems has been studied (20). According to the results of this study, the wastes have almost the same value for antioxidant activity compared to the fruit itself. It is needed to examine the exact content and chemical composition in citrus wastes in order to make best use of these valuable sources in food and pharmaceutical industries.

However, it must be reminded that various methods used to measure antioxidant activity may exhibit some limitations (18). Some scientists have reported that antioxidant activity of natural extracts depend on the assay (21). We found that the assay methods could also support results from other methods. It was obtained that the antioxidant properties exhibited by extracts of wastes from all five citrus species were similar by FRAP and DPPH assays. Based on results obtained from the present study, the highest activity was found in methanol extracts of Hamlin oranges and the Mars species showed the lowest antioxidant power confirmed by both assay methods.

It has been reported that phenolic compounds could scavenge radicals and to chelate metals (22), while ascorbic acid can play a pro-oxidant role in the presence of transition metals (23). It must be emphasized that, the phenolic compounds at low concentrations could exhibit antioxidant activity, while in higher concentrations; they are pro-oxidants (24). Although, the results of present study is promising, but in future, the *in vivo* power of waste extracts should be examined before introducing the extracts for possible industrial applications.

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