ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF FEIJOA SELLOWIANA FRUITS PEEL AND LEAVES

Ebrahimzadeh M. A.*, Hosseinimehr S. J., Hamidinia A. and Jafari M.

Pharmaceutical Sciences Research Center, School of Pharmacy, Medical Sciences University of Mazandaran, Sari, Iran. Tel.: +98 151 3543081-3; fax: +98 151 3543084. zadeh20@yahoo.com.

Summary

Antioxidant activity of Feijoa sellowiana leaves and fruit peels was investigated employing various in vitro assay systems. IC_{50} for DPPH radical-scavenging activity was in the order: leaf aqueous extract, LW (7.25 ± 0.31) > leaf methanolic extract, LM (12.5 ± 0.34) > fruit peels aqueous extract, FW (20.25 ± 0.52) > fruit peels methanolic extract, FM (34.5.29 ± 0.44) μg ml^{-1}, respectively. The extracts also showed weak nitric oxide-scavenging activity between 0.2 and 3.2 mg ml^{-1}. Nearly all tested extracts exhibited good Fe^{2+} chelating ability. IC50 for Fe^{2+} chelating ability were LM (2.40 ± 0.11), LW (0.24 ± 0.02) and FM (1.51 ± 0.12) mg ml^{-1} respectively. FW had shown only 19% inhibition at 3.2 mg ml^{-1}. The peroxidation inhibition of extracts exhibited values from 91.9 to 98% (at 72^{nd} hrs). All tested extracts exhibited high antioxidant activity and manifested almost the same pattern of activity as Vit C at different incubation times ($p > 0.05$). The extracts exhibited different levels of antioxidant activity in all the models studied. The extracts had good reductive capability for reducing Fe^{3+} to Fe^{2+} by donating an electron Fe^{2+} chelating activity and anti-lipid peroxidation activity.

Key words: Antioxidant activity, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH), Feijoa, Fruit peels

Introduction

The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (1). Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers (2,3) and neurodegenerative diseases, including Parkinson_s and Alzheimer`s diseases (4), as well as inflammation and problems caused by cell and cutaneous aging (5). Feijoa sellowiana (Myrtaceae) is an evergreen bush native to southern of South America, where it is widely distributed.

Owing to its easy adaptability in subtropical regions, nowadays it is extensively cultivated in many countries (6) and also in Iran where the fruits are very popular. Although the chemical composition of Feijoa has been clearly reported (7-11) pharmaceutical studies of its constituents have barely been carried out. Feijoa showed potent antimicrobial and antifungal activity and a sensible activity against H. pylori (12-14). Some anti-cancer activities of the full Feijoa extract have been reported (15,16). Moreover, antioxidant activities of an aqueous extract on oxidative burst of human whole blood phagocytes have been reported (10, 16). Yet little information is available about Feijoa antioxidative activity. In this study, we examined the antioxidant activity of Feijoa leaves and fruit peels, employing various in vitro assay systems, such as DPPH and nitric oxide radical scavenging, reducing power, linoleic acid and iron ion chelating power, in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

Materials and methods

Plant material and preparation of freeze-dried extract: Feijoa fruits and fresh leaves were collected from Fajr citrus experimental institute in autumn 2006. Leaves and fruit peels were dried at room temperature and coarsely ground before extraction. A known amount of each part was extracted at room temperature by percolation method using methanol and water separately. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal. The fruit peels aqueous extract, FW (15.6%), fruit peels methanolic extract, FM (20.5%), leaf aqueous extract, LW (15.3%), leaf methanolic extract, LM (10.7%) were obtained, respectively

Determination of total phenolic compounds and flavonoid content: Total phenolic compound contents were determined by the Folin-Ciocalteau method (17). The extract samples (0.5 ml of different dilutions) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Flavonoid content of each extract was determined by following colorimetric method (18). 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 (UV- Visible EZ201, Perkin Elmer: USA). Total flavonoid contents were calculated as quercetin from a calibration curve.

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 (19). 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. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Reducing power determination: Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (20). The reducing power of feijoa was determined according to the method of Yen and Chen (21). Different amounts of each extracts (25-800 μg ml^{-1}) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%).

The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

Assay of nitric oxide-scavenging activity: The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each extracts dissolved in water and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm.Quercetin was used as positive control (22).

Metal chelating activity: Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (23). The chelating of ferrous ions by Feijoa was estimated by the method of Dinis (24). Briefly, the extract (0.2–3.2 mg/ml) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as $[(A_0- A_s)/A_s] \times 100$, where A_0 was the absorbance of the control, and A_s was the absorbance of the extract/ standard. Na_2EDTA was used as positive control.

Determination of Antioxidant Activity by the FTC Method: Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation (25). The inhibitory capacity of Feijoa extracts was tested against oxidation of linoleic acid by FTC method. This method was adopted from Osawa and Namiki (26). Twenty mg/mL of samples dissolved in 4 mL of 95% (w/v) ethanol were mixed with linoleic acid (2.51%, v/v) in 99.5% (w/v) ethanol (4.1 mL), 0.05 M phosphate buffer pH 7.0 (8 mL), and distilled water (3.9 mL) and kept in screwcap containers at 40°C in the dark. To 0.1 mL of this solution was then added 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance at 500 nm of the resulting red solution was measured, and it was measured again every 24 h until the day when the absorbance of the control reached the maximum value. The percent inhibition of linoleic acid peroxidation was calculated as: (%) inhibition = 100 - [(absorbance increase of the sample/absorbance increase of the control) × 100]. All tests were run in duplicate, and analyses of all samples were run in triplicate and averaged. Vit C and BHA used as positive control.

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 EC_{50} values were calculated from linear regression analysis.

Results and Discussion

Total phenol and flavonoid contents: 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 of FW, FM, LW and LM were 89.07 ± 0.54, 69.14 ± 0.39, 92.09 ± 0.75 and 44.17 ± 0.28 mg gallic acid equivalent/g of extract powder, respectively. The total flavonoid contents of FW, FM, LW and LM were 18.62 ± 0.75, 43.45 ± 1.75, 59.52 ± 1.03 and 55.83 ± 1.29 mg quercetin equivalent/g of extract powder, respectively, by reference to standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$). It was noted that aqueous extracts had significant higher total phenol contents than did methanolic extracts. In other hand, leaf extracts had significant flavonoid contents than did fruits extracts. 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 (27). In this study, the total phenolic compounds of the various extracts from different tissues of Feijoa were found to be in the ranges of 220.2– 460.5 mg/g (as gallic acid equivalent), and they may cause the antioxidative activities of the Feijoa extracts.

DPPH radical-scavenging activity: The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (28). It was found that the radical- scavenging activities of all the extracts increased with increasing concentration. IC_{50} for DPPH radical-scavenging activity was in the order: LW (7.25 ± 0.31) > LM (12.5 ± 0.34) > FW (20.25 ± 0.52) > FM (34.5.29 ± 0.44) μg ml^{-1}, respectively. Based on the IC_{50} results, it was also shown that leaves extracts had the higher DPPH-scavenging activity than fruits extracts. In other hand, in each part of plant, aqueous extract shown higher activity than methanolic one. The IC_{50} 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.

Reducing power: In the reducing power assay, the presence of reductants (antioxidants) in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 1 shows the dose–response curves for the reducing powers of the extracts from Feijoa. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. There were significant differences ($p < 0.05$) among the different extracts in reducing power. The leaf extracts had shown better reducing power than fruits extracts. The leaf extracts exhibited a good reducing power at 40 and 80 μg ml^{-1} that was comparable with Vit C ($p > 0.05$). It was evident that the extracts from *Feijoa* did show reductive potential and could serve as electron donors, terminating the radical chain reaction.

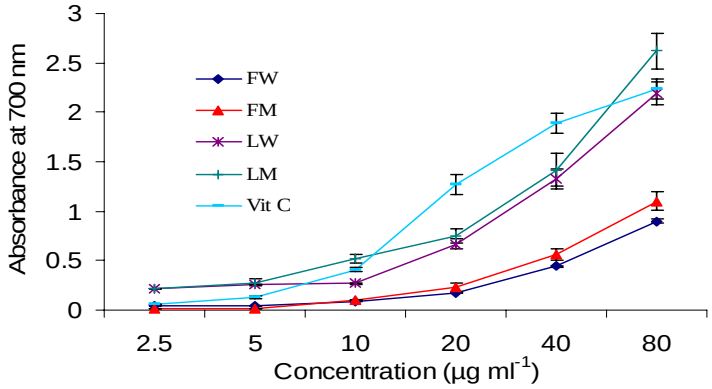

Fig.1. Reducing power of Feijoa fruit peels aqueous extract, FW, fruit peels methanolic extract, FM, leaf aqueous extract, LW and leaf methanolic extract, LM.

Assay of nitric oxide-scavenging activity: The extracts also showed weak nitric oxide-scavenging activity between 0.2 and 3.2 mg ml^{-1}. The percentage of inhibition was increased with increasing concentration of the extract. The aqueous extracts had shown better reducing power than methanolic extracts (Table 1). FW extract had shown the higher activity. However, activity of quercetin was very more pronounced than that of FW. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (29).

Table 1. In vitro NO-scavenging activity of aqueous and methanolic extracts of Feijoa.

Sample	IC_{50} (mg ml^{-1})
aqueous fruit peels	0.111
methanolic fruit peels	1.843
aqueous leaves	0.135
methanolic leaves	1.225
quercetin	0.017

Fe^{2+} chelating ability: The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease (30). Because Fe^{2+} causes the production of oxyradicals and lipid peroxidation, minimizing its concentration affords protection against oxidative damage. In Fe^{2+} chelating ability test, in the presence of other chelating agents, the ferrozine complex formation is disrupted with the result that the red color of the complexes decreases. The absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently, i.e. the activity was increased on increasing concentration from 0.2 to 3.2 mg ml^{-1}. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (31). Results reveal that Feijoa extracts have effective capacity for iron binding, suggesting that its action as an antioxidant may be related to its iron binding capacity. Nearly all tested extracts exhibited good Fe^{2+} chelating ability. IC50 for Fe^{2+} chelating ability were LM (2.40 ± 0.11), LW (0.24 ± 0.02) and FM (1.51 ± 0.12) mg ml^{-1} respectively. FW had shown only 19 % inhibition at 3.2 mg ml^{-1}. EDTA showed very strong activity (IC50 = 0.018 mg ml^{-1}).

FTC Method: Figure 2 shows the time-course plots for the antioxidative activity of the different extracts of Feijoa using the FTC method. The peroxidation inhibition of extracts exhibited values from 96.1 to 98.4% (at 48th hrs) and from 91.9 to 98% (at 72nd hrs). All tested extracts exhibited high antioxidant activity. There were no significant differences (p> 0.05) among the different extract fractions in antioxidative activity. All of extracts manifested almost the same pattern of activity as Vit C at different incubation times (p> 0.05).

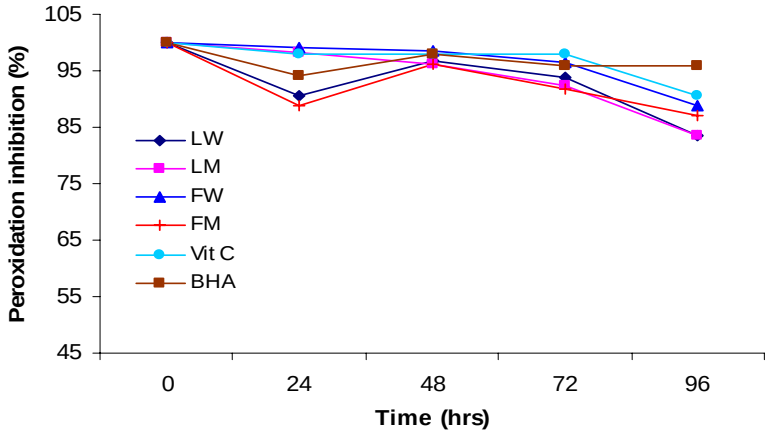

Fig. 2. Antioxidant activity of Feijoa in FTC method at different incubation times. Fruit peels aqueous extract, FW, fruit peels methanolic extract, FM, leaf aqueous extract, LW and leaf methanolic extract, LM (0.4 mg/ml), Vit C and BHA (0.1 mg/ml).

The aqueous and methanolic extracts of Feijoa sellowiana fruits and leaves exhibited different levels of antioxidant activity in all the models studied. The extracts had good reductive capability for reducing Fe^{3+} to Fe^{2+} by donating an electron Fe^{2+} chelating activity and anti-lipid peroxidation activity. Further investigation of individual compounds, their in vivo antioxidant activities and in different antioxidant mechanisms is needed.

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