

Full Length Research Paper

Total phenolic compounds and antioxidant capacity of leaf, dry fruit and fresh fruit of feijoa (*Acca sellowiana*, Myrtaceae)

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The methanol extract of fresh fruit (MEFF), dry fruit (MEDF) and leaf of feijoa were analysed for antioxidant capacity in different systems including reducing power, free radical scavenging, total antioxidant activity, and metal chelating activities. Those various antioxidant activities were compared to standard antioxidants. The percentage inhibitions of 40 µg/ml concentration of MEFF, MEDF and leaf on peroxidation in linoleic acid system were 74, 75 and 87%, respectively, and the same concentration (40 µg/ml) of α-tocopherol was showed similar activity. On the other hand, MEFF, MEDF and leaf had effective reducing power, free radical scavenging, and metal chelating activities at same concentrations (40 µg/ml). In addition, total phenolic compounds in ME of FF, DF and leaf were determined as gallic acid equivalent. As conclusion feijoa both fruit and leaf have antioxidant capacity but the activity of leaf greater than fruit. This difference may be phenolic compounds content.

Key words: Antioxidant capacity, feijoa, *Acca sellowiana*, phenolic compounds, antioxidant activity, free radical, functional compounds.

INTRODUCTION

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS) (Barros et al., 2006). ROS, which include free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals (OH^\cdot) and non free-radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2), are various forms of activated oxygen. The interaction of these species with molecules of a lipid nature produces new radicals: hydroperoxides and different peroxides (Halliwell, 2006). ROS are continuously produced during normal physiologic events and

can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. ROS is capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins and carbohydrates. If ROS are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell and Gutteridge, 1990; Elmastaş et al., 2006).

Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food and pharmaceutical products during processing and storage. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods (Kumaran and Karunakaran, 2006; Gülçin et al., 2004). At the present time, the most commonly used antioxidants are BHA, BHT, propyl gallate and *tert*-butylhydroquinone. Besides that BHA and BHT have suspected of being responsible for liver damage and carcinogenesis (Wichi, 1988).

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Therefore, there is a growing interest on natural and safer antioxidants from natural sources (Moure et al., 2001; Elmastaş et al., 2005; 2007).

Acca sellowiana, Myrtaceae (syn *Feijoa sellowiana* Berg.) is an evergreen bush 5 - 8 m high with gray branches, elliptical buds, white and red flowers, and sweet-smelling leaves, originally native to South America. But today, *Acca sellowiana* grows throughout the Mediterranean area including Turkey. The edible fruit (Feijoa) is used for human food widely. Its fruits are rich in vitamin C, polyphenols, terpenes, tannins, steroidal saponins, flavonoids hydrocarbons, minerals, iodine and both methyl and ethyl benzoate (Binder and Flath, 1989; Ruberto and Tringali, 2004; Lintas and Cappelloni, 1992). On the whole, the fresh feijoa fruit is well appreciated for its good nutritional characteristics and for its pleasant flavor and aroma (Homnava, 1990). For these reasons it is widely present on the market. In the literature, various biological activities especially antimicrobial activity of different extracts from feijoa whole fruit or peel are described (Basile et al., 1997). It has been reported that *F. sellowiana* shows potent antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi. Moreover, an antioxidant activity of an aqueous extract on oxidative burst of human whole blood phagocytes and on isolated polymorphonuclear leukocytes has been described (Vuotto et al., 2000). Although there are many studies on feijoa (Basile et al., 1997; Vuotto et al., 2000; Ruberto and Tringali, 2004; Rossi et al., 2007), the antioxidant capacity of feijoa has not been well documented yet.

The aim of this study was to evaluate antioxidant activity tests such as, the total antioxidant activity, ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP) using the potassium ferricyanide reduction method, DPPH $^{\cdot}$ radical scavenging and metal chelating activities of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa *in vitro*. Additionally, an important goal of this research was *in vitro* antioxidative effects of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were compared with commercial and standard antioxidants such as BHA, BHT, α -tocopherol and trolox commonly used by the food and pharmaceutical industry. In addition to those antioxidant tests, total phenolic compounds in fresh fruit, dry fruit and leaf of feijoa were determined.

MATERIALS AND METHODS

Chemicals

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH $^{\cdot}$), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), linoleic acid, α -tocopherol, polyoxyethylenesorbitan monolaurate (Tween-20) and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma-Aldrich or Merck.

Plant materials and extraction procedures

For methanol extraction 100 g air-dried fresh fruit (FF), dry fruit (DF) and leaf of feijoa samples ground into a fine powder in a mill and was mixed with 500 mL methanol. The residue was re-extracted under some condition until extraction solvents became colourless. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected, then methanol was removed by a rotary evaporator at 50°C to obtain dry extract (Gulcin et al., 2008). Those extracts were used for antioxidant capacity tests.

Determination of total phenolic compounds by folin-ciocalteu reagent

The amount of total phenolic contents in the methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977). Folin-Ciocalteu is a method used for the determination of total phenolic compounds. Gallic acid was used as a standard phenolic compound. Briefly, 1 mL of extract solution contains 1 mg extracts, in a volumetric flask diluted with distilled water (46 mL). One millilitre of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. After 3 min, 3 mL of Na_2CO_3 (2%) was added and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The amount of total phenolic compounds in methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa determined as microgram of gallic acid equivalent (GAE) using an equation that was obtained from calibration curve of gallic acid graph ($r^2:0.9938$).

$$\text{Absorbance}_{(760 \text{ nm})} = 0.104 \times \text{Total phenols (GAE } \mu\text{g)}$$

Total antioxidant activity determination by ferric thiocyanate method

The total antioxidant activities were determined according to the thiocyanate method (Mitsuda et al., 1996). For stock solutions, 10 mg of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were dissolved in 10 mL methanol. Then, the solution which contains different concentration of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa (from 25 - 75 $\mu\text{g/mL}$) solution in 2.5 mL of sodium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL of linoleic acid emulsion in sodium phosphate buffer (0.04 M, pH 7.0). Therefore, 5 mL of the linoleic acid emulsion was prepared by mixing and homogenising 15.5 μL of linoleic acid, 17.5 mg of tween-20 as emulsifier, and 5 ml phosphate buffer (pH 7.0). On the other hand, 5 mL of control was composed of 2.5 mL of linoleic acid emulsion and 2.5 mL, 0.04 M sodium phosphate buffer (pH 7.0). The mixed solution (5 mL) was incubated at 37°C in glass flask. The peroxide levels were determined by reading the absorbance at 500 nm in a spectrophotometer (Jasco V-530 UV/VIS Spectrophotometer), after reaction with FeCl_2 and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides formed and these compounds oxidize Fe^{+2} - Fe^{+3} . The latter Fe^{+3} ions form complex with SCN^- and this complex has maximum absorbance at 500 nm. The solutions without added extract used as blank samples. All data about total antioxidant activity are the average of triplicate analyses. In this test BHT and α -tocopherol were used as standards. The inhibition of lipid peroxidation in % was calculated by following equation:

$$\% \text{ Inhibition} = 100 - [(A_1/A_0) \times 100]$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample (Gülçin et al., 2009).

Table 1. Yield and total phenolic contents in percent of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa.

	Yield (%)	Total phenolic compounds (µg)
FF	13.17	17.68
DF	32.39	8.69
Leaf	22.12	68.69

Ferric ions (Fe³⁺) reducing antioxidant power assay (FRAP)

The reducing power of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were determined by the method of Oyaizu (1986) with slight modification (Elmastas et al. 2006). Different concentrations of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa (25 - 75 µg/mL) in 1 mL of distilled water were mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. Aliquots (2.5 mL) of trichloroacetic acid (10%) were added to the mixture. The 2.5 mL of this solution was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates an increase of reduction capability.

Ferrous ions (Fe²⁺) chelating activity

The chelating of ferrous ions by methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa was estimated by the method of Dinis (1994), wherein the Fe²⁺-chelating ability of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were monitored by the absorbance of the ferrous iron-ferrozine complex at 562 nm. Briefly, MEF (from 25 - 75 µg/mL) in 0.4 ml was added to a solution of 2 mM FeCl₂ (0.2 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.4 ml) and total volume was adjusted to 4 ml of ethanol. Then, the mixture was shaken vigorously and left at room temperature for ten minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm.

DPPH free radical scavenging activity

The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple coloured methanol solution of DPPH. The free radical scavenging activities of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois (1958). Wherein the bleaching rate of a stable free radical, DPPH· is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH· absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. When a hydrogen atom or electron was transferred to the odd electron in DPPH·, the absorbance at 517 nm decreased proportionally to the increases of non-radical forms of DPPH. Briefly, 0.1 mM solution of DPPH· in methanol was prepared and 1 ml of this solution was added 3 ml of methanolic extract mushroom species at different concentrations (25 - 75 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity (Gulcin and Ak, 2008). The capability to scavenge the DPPH· radical was calculated using the following equation:

$$\text{DPPH}\cdot \text{ scavenging effect (\%)} = \left(1 - \frac{A_s}{A_c}\right) \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of samples or standards (Gülçin et al., 2004; Elmastas et al., 2006).

Statistical analysis

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation and analysed by SPSS (version 11.5 for Windows 2000, SPSS Inc.). One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests. P < 0.05 was regarded as significant and p < 0.01 was very significant.

RESULTS AND DISCUSSION

Natural antioxidants were closely related to their bio-functionalities. Antioxidant capacity is widely used as a parameter for medicinal bioactive and functional components in food. In this study, the antioxidant activity of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were compared to BHA, BHT, trolox and α-tocopherol. Table 1 shows the yields and total phenolic contents of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa. The antioxidant effect of plant phenolics has been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders (Parr and Bolwell, 2000). In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Yen et al., 1993; Gülçin, 2005). As it can be seen in Table 1, 17.68, 8.69 and 68.69 µg GAE of phenols was detected in 1 mg of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa. According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species (Velioglu et al. 1998).

Total antioxidant activity determination in linoleic acid emulsion

Lipid peroxidation contains a series of free radical-mediated chain reaction processes and is also associated with several types of biological damage (Perry et al., 2000). The ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation which is the primary product of lipid oxidation. In this assay, hydroperoxide produced by linoleic acid added to the reaction mixture, which has oxidized by air during the experimental period, was indirectly measured. Ferrous chloride and thiocyanate react with each other to produce ferrous thiocyanate (red colour) by means of hydroperoxide.

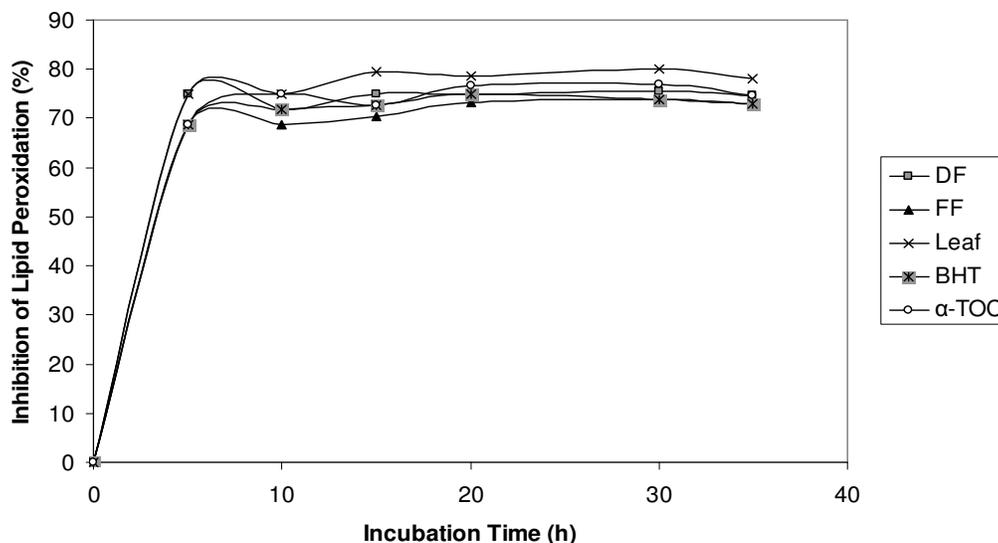


Figure 1. Total antioxidant activities of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and BHT and α -tocopherol.

Total antioxidant activity of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa, BHT and α -tocopherol were determined by the ferric thiocyanate method in the linoleic acid system. Methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa exhibited effective antioxidant activity in this system. The effect of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and standards on lipid peroxidation of linoleic acid emulsion are shown in Figure 1. The effect of 75 μ g/mL concentration of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were found to be 73.8, 75.4 and 80.0% respectively and their activities are greater than same concentration of BHT (73.0%). Consequently, these results clearly indicated that methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa had effective and powerful antioxidant activity by ferric thiocyanate.

Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP)

Different studies have indicated that the electron donation capacity, reflecting the reducing power, of bioactive compounds is associated with antioxidant activity (Siddhuraju et al., 2002). The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung et al., 2002; Gülçin 2009). There are a number of assays designed to measure overall antioxidant activity or reducing potential, as an indication of host total capacity to withstand free radical stress (Wood et al., 2006). In this assay, the yellow colour of the

test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

As can be seen from Figure 2, methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa had effective reducing power using the potassium ferricyanide reduction method when compared to the standards (BHA, trolox and α -tocopherol). For the measurements of the reductive ability of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa, the Fe^{3+} - Fe^{2+} transformation was investigated using the method of Oyaizu (1986). At different concentrations (10 - 60 μ g/mL), methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa demonstrated reducing ability. The reducing power of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and α -tocopherol increased steadily with increasing concentration of samples. Reducing power of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and standard compounds exhibited the following order: BHA > trolox > leaf > α -tocopherol > DF > FF. The results on reducing power demonstrate the electron donor properties of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa thereby neutralizing free radicals by forming stable products.

Ferrous ions (Fe^{2+}) chelating capacity

Transition metal species such as ferrous iron (Fe^{2+}) can facilitate the production of ROS within animal and human systems, the ability of substances to chelate iron can be a valuable antioxidant capability (Halliwell and Gutteridge, 1984). Iron, in nature, can be found as either

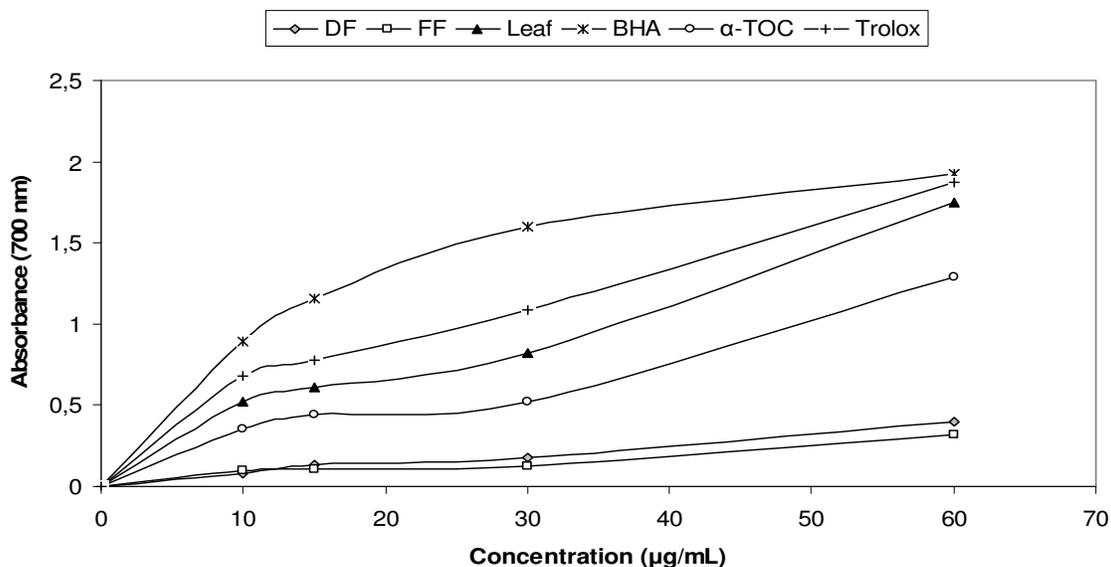


Figure 2. Total reductive potential of different concentrations (10-60 µg/mL) of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and reference antioxidant; BHT, BHA, Trolox and α-tocopherol.

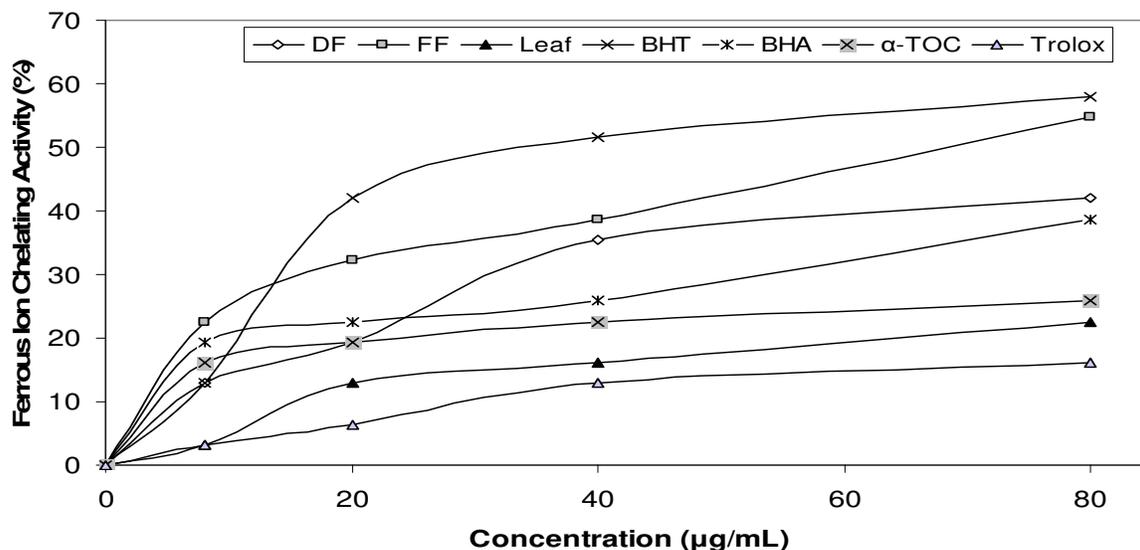
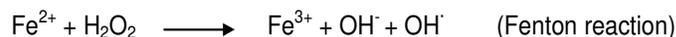


Figure 3. Metal chelating effect of different concentrations (10-80 µg/mL) of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and standards (BHT, BHA, Trolox and α-tocopherol) on ferrous ions (Fe^{2+}).

ferrous (Fe^{2+}) or ferric ion (Fe^{3+}), with the latter form of ferric ion predominating in foods. Ferrous ions (Fe^{2+}) chelation may render important antioxidative effects by retarding metal-catalyzed oxidation.

Ferrous ions (Fe^{2+}) chelating activities of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa, BHA, BHT, α-tocopherol and trolox are shown in Figure 3. The chelating effect of ferrous ions (Fe^{2+}) by the methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and standards was determined according to the method of Dinis (1994). Iron is known as the most

important lipid oxidation pro-oxidant due to its high reactivity among transition metals. The effective ferrous ions (Fe^{2+}) chelators may also afford protection against oxidative damage by removing iron (Fe^{2+}) that may otherwise participate in $\text{HO}\cdot$ generating Fenton type reactions.



Ferrous ions (Fe^{2+}) are the most powerful pro-oxidant among the various species of metal ions (Halliwell and

Gutteridge, 1984). Minimizing ferrous (Fe^{2+}) ions may afford protection against oxidative damage by inhibiting production of ROS and lipid peroxidation. Ferrozine can quantitatively form complexes with Fe^{2+} in this method. In the presence of chelating agents the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity of the coexisting chelator. Metal chelation is an important antioxidant property and hence of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were assessed for its ability to compete with ferrozine for ferrous ions (Fe^{2+}) in the solution. In this assay, of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa are interfered with the formation of ferrous ions (Fe^{2+}) and ferrozine complex. It was suggesting that they have chelating activity and are able to capture ferrous ion before ferrozine.

As can be seen in Figure 3, of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa exhibited marked chelation of ferrous ion at all used concentrations ($p < 0.01$). On the other hand, the percentages of ferrous ions (Fe^{2+}) chelating capacity of same concentration (40 $\mu\text{g/mL}$) of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and standards BHT, BHA, trolox and α -tocopherol were found as 38.71, 35.48, 16.13, 51.61, 25.81, 12.9 and 22.58%, respectively. These results show that the ferrous ion (Fe^{2+}) chelating effect of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa were higher than Trolox and lesser than BHT ($p < 0.05$).

Metal chelating capacity was significant since it reduced the concentration of the catalysing transition metal in lipid peroxidation. 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. The data obtained from Figure 3 reveal that methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa demonstrate a marked capacity for iron binding, suggesting that their main action as peroxidation protector may be related to its iron binding capacity.

Radical scavenging activity

The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products. Assay based upon the use of DPPH \cdot is the most popular spectrophotometric methods for determination of the antioxidant capacity of food, beverages and vegetable extracts (Bendini et al., 2006). This chromogen radical compound can directly react with antioxidants.

Additionally, DPPH \cdot scavenging method have been used to evaluate the antioxidant activity of compounds

due to the simple, rapid, sensitive, and reproducible procedure (Özçelik et al., 2003).

Radical scavenging activity is very important due to the deleterious role of free radicals in foods and in biological systems. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point.

In this study, DPPH radical scavenging method was used to assess the deterrmination of potential radical scavenging activities of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

With this method it was possible to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH \cdot at 517 nm. Resulting a color change from purple to yellow, the absorbance decreased when the DPPH \cdot was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH \cdot molecule. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. Figure 4 illustrates a significant decrease ($p < 0.01$) in the concentration of DPPH radical due to the scavenging ability of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa, BHT, BHA, trolox and α -tocopherol. The scavenging effect of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa, BHT, BHA, trolox and α -tocopherol on the DPPH radical decreased in the order of trolox > Leaf > DF > α -tocopherol > BHA > FF > BHT, which were 98.69, 96.73, 95.91, 94.6, 93.78, 92.96 and 86.42%, at the concentration of 80 $\mu\text{g/mL}$, respectively. DPPH free radical scavenging activity of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa also increased with an increasing concentration. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity of specific compounds (Amarowicz et al., 2004).

Conclusion

According to obtained data in the present study, methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa was found to be an effective antioxidant and

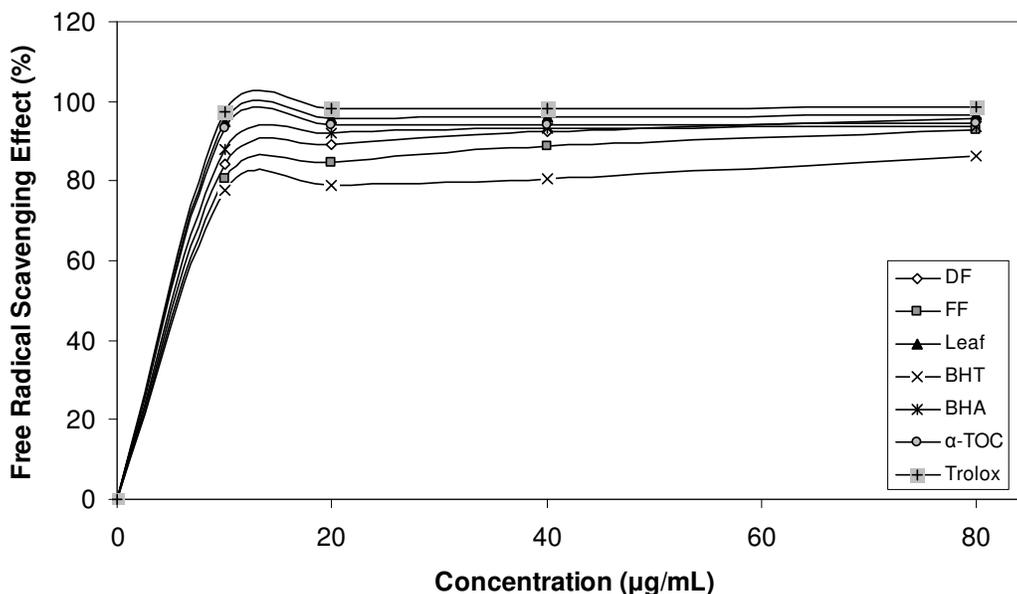


Figure 4. DPPH free radical scavenging activity of different concentrations (10-80 µg/mL) of methanol extract of fresh fruit (FF), dry fruit (DF) and leaf of feijoa and standards (BHT, BHA, Trolox and α-tocopherol) [BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene; DPPH•: 1,1-diphenyl-2-picryl-hydrazyl free radical].

radical scavenging activity in different *in vitro* assay such as inhibition of linoleic acid peroxidation, ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP), ferrous ions (Fe^{2+}) chelating activities, and DPPH• radical scavenging when it is compared to standard antioxidant compounds such as BHA, BHT, trolox and α-tocopherol. Based on the discussion above, it can be us for minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of pharmaceuticals.

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