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ORIGINAL ARTICLE

Study of antioxidant activity of non-conventional Brazilian fruits

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Abstract This study aimed to analyze the proximate composition of seeds from some non-conventional Brazilian fruits, as well as to evaluate the antioxidant activity through DPPH$^{•}$ free radical and to quantify the total phenolic compounds. To obtain the extracts, dried and crushed seeds were extracted with ethanol for 30 min, in a ratio of 1:3 (seeds:ethyl alcohol), under continuous agitation, at room temperature. Then, the mixtures were filtered and the supernatants were subjected to rotary evaporator under pressure reduced to 40 °C. The results report that the seeds of non-conventional fruits are remarkable sources of lipids, and the extraction of oil from these seeds could be an alternative for the commercial utilization of waste. They also presented significant percentages of protein and carbohydrates. Ethanol extracts of seeds from non-conventional Brazilian fruits showed relevant antioxidant activity and high amount of phenolic compounds. Therefore Brazilian non-conventional fruits can be used as functional food products or feed.

Keywords DPPH$^{•}$ · Total phenolic compounds · Fruit · Extracts · Seeds

Introduction

There are several concepts concerning the term antioxidant. However, in general, it can be defined as a heterogeneous family of natural molecules, which can prevent or reduce the extent of oxidative damage, when present in low concentrations, compared to the biomolecules that they would supposedly protect (Halliwell and Gutteridge 2007).

Antioxidants of low molecular weight can either be synthesized in the organism itself or come from the diet. They are present in greater number and concentration than enzymatic antioxidants and are distributed in lipophilic and hydrophilic environments. No antioxidant by itself gathers all the characteristics of a good antioxidant, which are of: being an organic compound naturally present in animal tissues, being active in protecting molecules of proteins and lipids, having a good bioavailability after oral and parenteral administration, having long life, being active in intra and extracellular space and being able to cross the cell membrane and remain intact (Vendemiale et al. 1999).

The fruits, besides containing essential nutrients, have a number of micronutrients such as minerals, fiber, vitamins and antioxidants. Among the most important natural antioxidants are the phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen compounds (alkaloids, amino acids, peptides, amines and derivatives of chlorophyll), carotenoids, tocopherols and ascorbic acid (Hassimotto et al. 2009).

Phenolic antioxidants act as free radical scavengers, donating a hydrogen atom to a lipid radical and, sometimes, as metal chelators. The intermediate products, formed by the action of these antioxidants, are relatively stable due to the resonance of the aromatic ring presented by these substances (Lee et al. 2005, Shahidi et al. 1992).

In addition to preventing the oxidative deterioration of lipids, antioxidants act beneficially in health by preventing the outbreak of age-related diseases, such as cancer, heart and inflammatory diseases (Krishnaiah et al. 2010). The formation of free radicals is associated with the normal metabolism of aerobic cells. The oxygen consumption inherent in cell multiplication leads to the generation of a

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series of these radicals. The interaction between these species and molecules of lipid nature to excess produces new hydroperoxide radicals and different peroxides. The production of free radicals in living organisms is controlled by several antioxidant compounds, which may be of endogenous origin or obtained through diet. When there is limited availability of antioxidants, oxidative damage of cumulative nature may occur, and the groups of radicals can interact with biological systems in cytotoxic manners (Uchida 2000).

There are different methods to evaluate the antioxidant capacity in vitro of isolated compounds, mixtures, biological fluids and tissues. The methods most commonly used, due to their easiness, speed and sensitivity of accomplishment, are those involving the measurement of the disappearance of colored free radicals, such as DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl), by spectrophotometry (Arnao 2000).

In the method of scavenging the DPPH$^{\bullet}$ radical, the antioxidant power of a given compound is determined by measuring the decrease in absorbance of the stable radical at 515 nm (Matthaus 2002). When the DPPH$^{\bullet}$ radical reacts with an antioxidant compound (HA), which can donate a hydrogen atom, it is reduced to the stable DPPH-H molecule. Such reduction is accompanied by the change of the radical color, from purple to yellow, leading to a decrease in absorbance. The higher the speed at which the absorbance decreases, the greater the ability of the antioxidant to donate hydrogen is (Miliauskas et al. 2004).

The study of the composition of seeds from non-conventional Brazilian fruits will help the professionals of food field so they can provide adequate dietary guidance, as well as to obtain data that can be used in tables of proximate composition. The obtainment of such data has been stimulated in order to gather updated, reliable and adequate information for the population.

Thus, the objective of this study was to analyze the proximate composition of seeds from some non-conventional Brazilian fruits, as well as to evaluate the antioxidant activity through free radical DPPH$^{\bullet}$ and quantify the total phenolic compounds.

Experimental procedures

Materials

Standards and reagents

DPPH$^{\bullet}$ and galic acid as standards; petroleum ether, hydrochloric acid and sulfuric acid, Folin-Ciocalteau, sodium carbonate, methyl alcohol and ethyl alcohol as reagents.

Raw materials

The seeds of non-conventional Brazilian fruits (Table 1) originating from plantations located in São José do Rio Preto–SP, Southeastern Brazil, were acquired during the 2007/2008 harvest. Once selected, the seeds were rinsed lightly with distilled water to remove residual pulp and soluble sugars, placed in an oven at 35 °C, for a period of 2 h to reduce the moisture content, and then homogenized for further analysis, performed in triplicate.

Ethanolic extracts

The dried and triturated seeds were extracted with ethyl alcohol for 30 min, at a ratio of 1:3 seed:ethyl alcohol, under continuous agitation at room temperature. Then, the mixture was filtered and the supernatant subjected to rotary evaporator under pressure reduced to 40 °C, second method described by Roesler et al. (2007).

Methods

Proximate composition

The analytical determinations of moisture, lipids and ashes in seeds were performed according to the official methods of AOCS (2009). The proteins were determined by the Kjeldahl method, described by AOAC (1995) and total carbohydrates were quantified by the difference of the values obtained by the sum of moisture, lipids, proteins and ashes. The estimated caloric value of seeds was calculated using the conversion factor of 4 kcal g^{-1} for protein and carbohydrate and 9 kcal g^{-1} for lipids.

Radical scavenging capacity (DPPH$^{\bullet}$)

This procedure was described by Brand-Williams et al (1995). An ethanolic solution with 500 mg mL^{-1} concentration of fruit

Table 1 List of nine non-conventional Brazilian fruits

Common name	Species	Family
Cagaita	*Eugenia dysenterica* DC	Myrtaceae
Cashew	*Anacardium occidentale* L.	Anacardiaceae
Soursop	*Annona muricata* L.	Annonaceae
Jabuticaba	*Myrciaria cauliflora* Berg	Myrtaceae
Black plum	*Syzygium cumini* L.	Myrtaceae
Lychee	*Litchi chinensis* Sonn	Sapindaceae
Sweetsop	*Annona squamosa* L.	Annonaceae
Pitanga	*Eugenia uniflora* L.	Myrtaceae
Tamarind	*Tamarindus indica* L.	Leguminosae

seeds extract was prepared. Each sample of this solution (0.3 mL) was added to 2.7 mL of DPPH• standard solution (40 mg mL^{-1}) in different concentrations (50, 100, 200, 300 and 400 mg mL^{-1}). After 30 min of reaction, the absorbance was read at 515 nm and converted into percentage of antioxidant activity (AA) by using the following formula: $AA(\%) = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\}$, Abs = absorbance. Control was done with 2.7 mL of DPPH• and the blank was performed with 0.3 mL of ethanolic solution of the extract and 2.7 mL of ethanol for each concentration. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of AA percentage against extract concentration.

Total phenolic compounds

The quantification of total phenolic compounds was determined by spectrophotometry, using the Folin-Ciocalteau reagent, according to the methodology described by Singleton and Rossi (1965). In this procedure, 100 μL of natural extract solution was put in test tubes from a pipette and then 500 μL of the Folin-Ciocalteau reagent was added. Next, 1.5 mL of sodium carbonate 20% saturated solution and 6 mL of distilled water were also added. This mixture remained at rest for 2 h at room temperature and the absorbance was determined at 765 nm. The gallic acid standard was used to make the calibration curve and the result was expressed in milligrams of equivalents of gallic acid per gram of extract (mg g^{-1}). The equation of the gallic acid calibration curve was $C=0.001\ A+0.012$, in which C is the concentration of gallic acid, A is the absorbance at 765 nm and the coefficient of determination $R^2=0.9985$.

Statistical analysis

The analytical determinations were performed in triplicate. The results presented correspond to mean±standard deviation. Analyses of variance were carried out at 5% of significance by using the ESTAT program for Statistical Analysis System, version 2.0.

Results and discussion

Proximate composition

The proximate or percent composition expresses, in general, the nutritive value of food and corresponds to the proportion of homogeneous groups of substances present in 100 g of the considered food. The groups of substances considered to be homogeneous are those that are present in all foods, namely, moisture, lipid or fat, protein, fiber, ash and carbohydrates.

Table 2 reports the average of the proximate composition of seeds from non-conventional fruits. According to the results, the seeds presented from 1.9 to 32.1% of moisture, once they had previously been dried.

It is noted that the data obtained from the seeds of non-conventional fruits differ significantly by Tukey test ($p<0.05$), regarding the proximate composition, of which analyzed parameters reported percentages of coefficient of variation (CV), which showed good reproducibility, ranging from 0.70% for ash to 3.2% for moisture.

The percentages of lipids in the seeds ranged from 0.64% for lychee to 50.2% for cashew. The values obtained for lipids indicate that the seeds from cashew, sweetsop and soursop are

Table 2 Proximate composition of the seeds from non-conventional Brazilian fruits

Fruit seeds	Macronutrients (%)[c]					
	Moisture	Lipids	Proteins	Ash	Total Carbohydrates[d]	Energy kcal 100 g^{-1}
Cagaita	32.1±0.28[a]	2.4±0.31[e]	4.9±0.16[fg]	1.2±0.16[b]	59.4[e]	278.8[i]
Cashew	1.9±0.02[h]	50.5±3.79[a]	21.7±0.28[a]	2.8±0.16[b]	23.1[h]	633.7[a]
Soursop	5.8±0.06[f]	23.5±0.16[b]	11.5±2.00[d]	1.5±0.16[b]	57.7[f]	488.3[b]
Jabuticaba	8.9±0.07[e]	0.5±0.07[g]	5.7±0.24[f]	3.2±0.16[a]	81.7[a]	354.1[g]
Black plum	11.3±0.10[c]	1.4±0.04[f]	4.3±0.02[g]	1.7±0.16[b]	81.3[a]	355.0[f]
Lychee	19.3±0.05[b]	0.6±0.06[g]	5.4±0.26[f]	1.8±0.16[b]	72.9[c]	318.6[h]
Sweetsop	6.1±0.06[g]	20.4±0.33[c]	15.0±2.13[b]	1.7±0.16[b]	56.8[g]	470.8[c]
Pitanga	6.4±0.41[g]	3.2±0.36[d]	8.2±0.26[e]	2.6±0.16[b]	79.6[b]	380.0[d]
Tamarind	9.8±0.05[d]	3.4±0.01[d]	12.7±0.06[c]	2.2±0.16[b]	71.9[d]	369.0[e]
CV (%)	3.2	1.9	2.8	0.7	1.5	1.7

[a, b] … (column)—averages followed by the same small do not differ by the Tukey test ($p>0.05$).

[c] Mean±standard deviation ($n=3$).

[d] Total carbohydrates obtained by difference.

good sources of oil when compared to soybean seeds, which contain approximately 20% of lipids (Orthoefer 1996). However, the seeds from the other non-conventional fruits analyzed obtained lipid percentages lower than 20%.

The amount of proteins in the seeds ranged from 4.9 to 21.7%. In general, these values are higher for some types of cereals such as rice (8.1%), corn (10.2%), oats (11.3%) and wheat (12.2%) (Lasztity 1996).

The highest percentage of ash (3.2%) was found in jabuticaba seeds, while the lowest percentage (1.2%) was found in cashew. In a study carried out by Lima et al. (2008) the seeds of jabuticaba presented values for lipids (0.5%), protein (1.1%) and ash (2.8%) which were similar to the ones presented in this study.

The amount of total carbohydrates in the seeds ranged from 23.1 to 81.7%. High amounts of carbohydrates are an alternative source of fiber in food.

Brazilian non-conventional fruits may constitute important energy source, once included in the diet. As expected, in samples with higher lipid content, the caloric value was also higher. Therefore, the amount of calories was higher in the seeds of cashews, soursop and sweetsop, which presented about 634, 488 and 471 kcal 100 g^{-1}, respectively.

According to Yu et al. (2002), the proximate composition of fruits can be influenced by several factors, including variety, cultivar, maturity, climate and geographical conditions of production, handling during and post-harvest, processing and storage. Furthermore, the species genotype, growing conditions and the interaction between genotype and environmental characteristics may also influence directly in the composition of fruits.

DPPH$^•$ and total phenolic compounds

The yields of dry extracts ranged from 3.7% for cagaita seeds to 38.2% for the ones of tamarind, depending on the solvent used. According to Parry et al. (2006), the yield of extracts may vary depending on the fruit species and the technique used for extraction.

Figure 1 shows the percentage of antioxidant activity of extracts from the non-conventional fruits seeds determined as a function of concentration. From among the seeds analyzed in this study, the sweetsop seed extract was the one that showed the lowest percentage of antioxidant activity in all determined concentrations, and the values obtained for concentrations of 50 mg mL^{-1}, 100 mg mL^{-1}, 200 mg mL^{-1}, 300 mg mL^{-1} and 400 mg mL^{-1} were 6.1%, 6.9%, 8.8%, 9.5% and 10.8%, respectively.

Table 3 can be observed that the non-conventional fruits seed extracts differed significantly by Tukey test ($p<0.05$) for the determinations analyzed in this study. All parameters analyzed showed coefficients of variation (CV), which showed good reproducibility of the results, ranging from 0.1–1.4%.

As shown in Table 3, all the non-conventional fruits seed extracts presented scavenging activity of DPPH$^•$ free radical higher than 70%. According to Mensor et al. (2001), the extracts that showed DPPH$^•$ scavenging activity above 70% are considered to be effective, while the extracts presenting activity between 60–70% are classified as of moderate action and those showing lower than 60% activity are considered to be of poor antioxidant activity.

In the present study, the highest values of antioxidant activity achieved by seed extracts decreased in the following

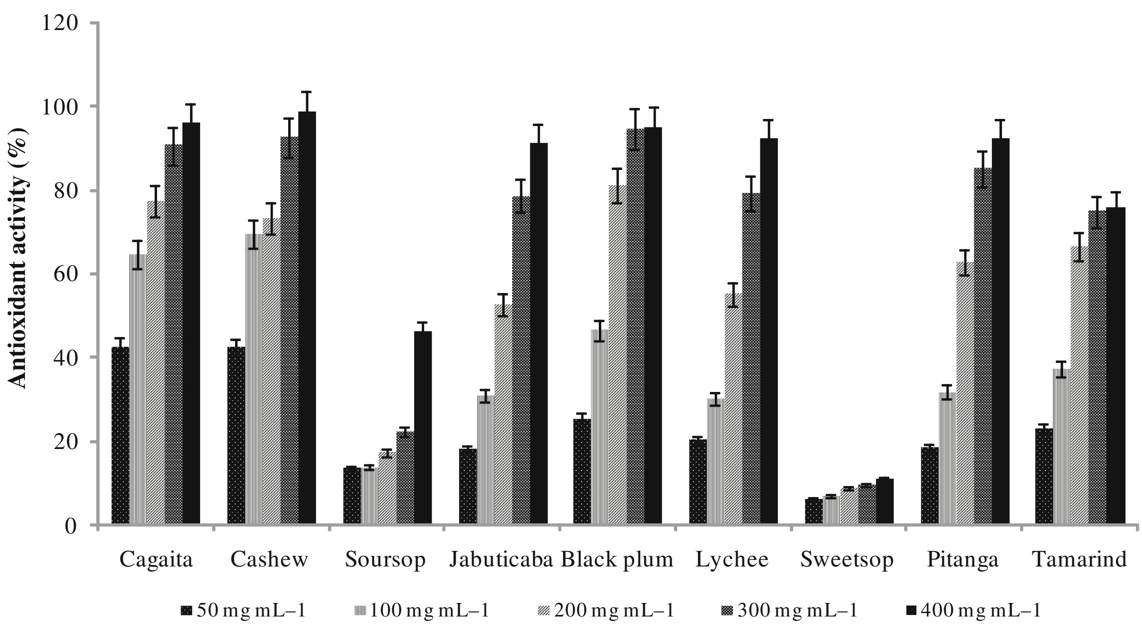

Fig. 1 Percentage of antioxidant activity, in different concentrations, of non-conventional Brazilian fruits seed extracts. Mean±standard deviation (n = 3)

Table 3 Determination of yield, $AA_{maximum}$, EC_{50} and total phenolic compounds of non-conventional Brazilian fruits seed extracts

Fruits seeds	Yield (%)	$AA_{maximum}$ (%)[c]	EC_{50} (mg mL^{-1})[d]	TPC (mg EAG g^{-1})[e]
Cagaita	3.7±0.13[h]	95.9±0.08[ab]	40.6±0.18[g]	136.7±0.12[a]
Cashew	15.1±0.28[d]	98.6±0.20[a]	36.4±0.19[h]	108.9±0.10[c]
Soursop	6.1±0.11[g]	76.2±0.21[d]	58.1±0.15[e]	78.5±0.20[f]
Jabuticaba	11.5±0.08[e]	91.1±0.18[c]	190.2±0.19[b]	91.6±0.21[e]
Black plum	9.4±0.16[f]	94.9±0.15[a]	118.7±0.10[c]	130.6±0.18[b]
Lychee	18.3±0.13[c]	92.3±0.16[bc]	46.1±0.08[f]	105.2±0.10[d]
Sweetsop	12.1±0.10[e]	70.8±0.11[e]	63.2±0.12[d]	65.5±0.11[h]
Pitanga	21.1±0.18[b]	92.1±0.14[bc]	30.7±0.14[i]	75.6±0.16[g]
Tamarind	38.2±0.14[a]	75.9±0.14[d]	204.7±0.17[a]	49.3±0.14[i]
CV (%)	1.4	0.7	0.7	0.1

[a, b] … (column)—averages followed by the same small do not differ by the Tukey test (p>0.05).

Mean±standard deviation (n=3).

[c] AA—antioxidant activity.

[d] EC_{50} is defined as the sufficient concentration to obtain 50% of maximum effect, estimated at 100%.

[e] TPC (mg EAG g^{-1})—total phenolic compounds, expressed in milligrams of equivalents of gallic acid per gram of extract.

order cashew>cagaita>black plum>lychee>pitanga>jabuticaba>soursop>tamarind>sweetsop.

The antioxidant activities of the compounds, given by the EC_{50} values, are calculated by the reduction of 50% in the initial concentration of DPPH$^•$. It can be pointed out that the lower the EC_{50} values are, the higher the antioxidant activity of the compounds analyzed is. The EC_{50} values obtained by linear regression for the non-conventional fruits seed extracts showed good correlation coefficients ranging from R^2=0.903 for cagaita to R^2=0.989 for lychee.

The amount of extract necessary to decrease the initial DPPH$^•$ concentration by 50% (EC_{50}) ranged from 30.7 to 204.7 mg mL^{-1} DPPH$^•$. These values are much lower than those formerly obtained by Bozan et al. (2008) in extracts of red grapes, melot and canernet varieties, cultivated in Turkey. Such values were of 3.0 and 2.9 mg mL^{-1} DPPH$^•$, respectively.

The phenolic are important compounds that contribute to the antioxidant activity. The concentrations of phenolic compounds found for the extracts of tamarind and cagaita seeds were 49.3 and 136.7 mg EAG per g of extract, respectively. The extraction of phenolic compounds from natural products is strongly influenced by the solvent used. It has been observed that the higher the polarity of the solvent extraction is, the greater the amount of phenolic compounds extracted is (Gaméz-Meza et al. 1999).

The seeds of non-conventional fruits that showed to be richest in total phenolics were cagaita (EAG 136.7 mg g^{-1}), black plum (EAG 130.6 mg g^{-1}), cashew (EAG 108.9 mg g^{-1}) and lychee (105.2 EAG mg g^{-1}).

In a study by Oboh and Ademosun (2011) about characterization of the antioxidant properties of phenolic extracts from some citrus peels, it was high antioxidant properties of the free and bound phenolic extracts from orange peels could be harness in the formulation of nutraceuticals and food preservatives.

Rufino et al. (2010) studied acerola fruits and cashew apple should therefore be considered as new natural sources of phenolic compounds. Already, Sharma et al. (2011) studied the antioxidant capacity, polyphenolics and pigments of broccoli-cheese powder blends and Banu et al. (2011) studied nutritional composition, functional properties and antioxidant activities of multigrain composite mixes.

The $AA_{maximum}$ obtained by the method of free radical DPPH$^•$, correlated significantly with the amount of total phenolics (r=0.80, p<0.05). This correlation shows the efficiency of each method in the extraction of antioxidant compounds in the seeds of the fruits analyzed. In a study by Silva et al. (2007) a positive correlation between total antioxidant activities of fifteen species of plants from the Amazon with the total content of phenols was also observed.

The seeds of non-conventional Brazilian fruits constituted relevant sources of lipids, suggesting the extraction of oil from them as alternatives to the use of commercial waste of the fruit seeds. They also presented significant percentages of protein and carbohydrates. Moreover, the ethanol extracts of non-conventional fruit seeds showed relevant antioxidant activity, as well as high amount of phenolic compounds being, thus, able to be used in food and feed.

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