Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil

Maria Mozarina Beserra Almeida a,b,⁎, Paulo Henrique Machado de Sousa c, Ângela Martha Campos Arriaga b, Giovana Matias do Prado c, Carlos Emanuel de Carvalho Magalhães d, Geraldo Arraes Maia c, Telma Leda Gomes de Lemos b

⁎ Corresponding author at: Departamento de Química Analítica e Físico-Química, Departament of Organic and Inorganic Chemical, Federal University of Ceará, CP 12200, 60451, Fortaleza, CE, Brazil. Tel.: +55 85 33669982. E-mail addresses: mzxabufc.br, mozarinagmail.com (M.M.B. Almeida), pheuiriqhemacho@gmail.com (P.H.M. de Sousa), angufc.br (Â.M.C. Arriaga), vanaprado@yahoo.com.br (G.M. do Prado), cemmag@yahoo.com.br (C.E.C. Magalhães), gmaia@secrel.com.br (G.A. Maia), tlemos@ufc.br (T.L.G. de Lemos).

a Department of Analytical Chemical and Physicochemical, Federal University of Ceará, CP 12200, 60451–970, Fortaleza, CE, Brazil
b Departament of Food Technology, Federal University of Ceará, CP 12200, 60451–970, Fortaleza, CE, Brazil
c Departament of Organic and Inorganic Chemical, Federal University of Ceará, CP 12200, 60451–970, Fortaleza, CE, Brazil
d Center of Science and Technology, State University of Ceará, Campus do Itaperi, Av. Paranjana, 1700, CEP.: 60740–903, Fortaleza, CE, Brazil

1. Introduction

The uncontrolled production of oxygen-derived from free radicals, is involved in the onset of many diseases such as cancer, rheumatoid arthritis, as well as in the degenerative process associated with aging, including Parkinson’s and Alzheimer’s diseases (Ali et al., 2008; Di Matteo & Esposito, 2003). Recently, many epidemiological studies suggested that the consumption of natural antioxidant such as polyphenol-rich food, fresh fruits, vegetables or teas have protective effects against the aforesaid diseases and its protection has partly been ascribed due to the presence of several components as vitamins, flavonoids, anthocyanins and other phenolics compounds (Klimczak, Malecka, Szlachta, & Gliszcynska-Swiglo, 2007; Serrano, Goni, & Matteo & Esposito, 2003). Vitamin C is considered as a major, naturally occurring nutrient of physiological functions, which could occur in the coronary heart diseases and cancer (Scarffotti, Fabris, Cestaro, & Galianni, 1997).

Apart from their biological properties, the natural antioxidants are also of interest in the cosmetic, pharmaceutical and especially in the food industries, since they can be also used as substitutes for synthetic antioxidants (Moure et al., 2001), providing protection against oxidative degradation from free radicals.

The fruits are a source of antioxidant compounds, such as phenolics, vitamins, carotenoids and minerals, which contribute to their chemo-preventive potential (Kusoski, Asero, Troncoso, Mancini-Filho, & Pett, 2005; Mahattanatawee et al., 2006). The phenolic compounds are comprised of over 8000 identified substances, and these can be divided into groups, according to their chemical structure, such as phenolic acids, stilbenes, coumarins, lignins and flavonoids (Ross & Kasum, 2002). Vitamin C is considered as a major, naturally occurring nutrient and antioxidant in our daily diet. It has an anticarcinogenic effect (Kim, Lee, Lee, & Lee, 2002) and it reduces tocopherol radicals back to their active form in cellular membranes (Klimczak et al., 2007).

The world trade of fresh fruits today, is esteemed to earn approximately 20 billions of U.S. dollars every year, and Brazil has a great importance to this particular market, since the country is the...
third largest producer of tropical fruits worldwide (Maia, Sousa, Lima, Carvalho, & Figueiredo, 2009). Brazil boasts a large number of underexploited native and exotic fruit species which are of a potential interest in the agroindustry and a possible future source of income for the local population. Fruits have been subjected to several studies around the world, reporting in their nutritional values, especially regarding the evaluation of antioxidant activities (Hassimotto, Genovese, & Lajolo, 2005; Kuskoski et al., 2005; Roesler, Malta, Carrasco, & Pastore, 2006; Silva, Souza, Rogez, Rees, & Larondelle, 2007).

Several methods are used to measure the antioxidant activity of biological material. The most commonly used are those involving chromogen compounds of a radical nature which stimulates reductive oxygen species, because to their ease, speed and sensitivity, and the presence of antioxidants leads to the disappearance of the radical chromogens. The most widely used methods being the ABTS and DPPH (Ali et al., 2008).

The present paper evaluated in vitro antioxidant activity, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, and its correlation with phenolics, vitamin C and anthocyanins from a series of exotic fruits produced and consumed in the Northeastern part of Brazil. Furthermore, this paper compared the DPPH and ABTS assays.

2. Materials and methods

2.1. Samples

Specimen of fruits (minimum of 500 g fresh weigh for each species), was collected from their natural habitats, mainly in the city of Fortaleza, Ceará, Brazil, from January to March 2008 and properly authenticated by Prof. Dr. Edson Paula Nunes (Department of Biology, Federal University of Ceará, Brazil), with their vouchers specimens deposited at the Herbarium Prisco Bezerra (EAC), at the Federal University of Ceará, Brazil. Eleven exotic fruits were investigated: Ananas comosus (L.) Merr. (pineapple), Annona squamosa L. (soursop), Annona squamosa L. (sugar apple or sweetsop), Arctocarpus integrifolia L. (jackfruit), Bynsonima crassifolia (L.) Kunth. (muciri), Carica papaya L. (papaya), Hancornia speciosa Gomes (mangaba), Manilkara zapota (L.) P. Rayen (sapodilla), Spondias purpurea L. (ciruela), Spondias tuberosa Arruda Camara (umbu) and Tamarindus indica L. (tamarind).

2.2. Chemicals

Ascorbic acid, gallic acid, ABTS, DPPH, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). All the other chemicals used were in an analytical grade.

2.3. Methods of sample preparation

At least 10 fruits were combined for each of the three replicated samples. The fruits except S. purpurea and S. tuberosa were peeled. The edible portions of the fruits were homogenized using a blender and centrifuged at 15000 rpm during 15 min. The supernatant (juice fraction) was recovered, filtered and antioxidant activity was evaluated; fresh pulps were used for the analysis of total phenolics, anthocyanin and ascorbic acids.

2.4. Phytochemical analyses

Total phenolics were determined according to the adapted Folin-Ciocalteu method (Zielinski & Kozlowska, 2000). The samples (5 g) were homogenized with 10 mL of 60% methanol containing 0.1% HCl, and placed in a water bath during 2 h, at 85 °C for the elimination of vitamin C, in accordance with Georgé, Brat, Alter, and Amiot (2005). After cooling they were added to a 100 mL volumetric flask, completing the volume with distilled water. The extracts were filtered under reduced pressure through paper filter (Whatman No. 1). An aliquot of 5 mL of extracts was added to 15 mL distilled water, 5 mL of Folin-Ciocalteu reagent and 10 mL of saturated solution with sodium carbonate and completing the volume to 100 mL with distilled water. After standing for 30 min at room temperature, the absorbance was measured at 750 nm using a UV–vis spectrophotometer (Micronal, Model B582, São Paulo, Brazil). All determinations were made in triplicate and values were calculated from calibration curves obtained with a minimum of five gallic acid concentrations. Linearity was obtained between 0 and 5 mg/mL, therefore corresponding to absorbance values between 0.0 and 0.5 (R² = 0.99). The total phenolics were expressed as milligram of gallic acid equivalents (GAE) per 100 g of fresh weight.

The anthocyanins were extracted from 1 g of fresh pulp fruits, with 30 mL of 95% ethanol/1.5 M HCl (85:15, v:v). The extract was transferred to a 50 mL volumetric flask, completing the volume with ethanol–HCl (1.5 M) and stored for 12 h at 4 °C (Francis, 1982). After filtration, the absorbance was measured in a UV–vis spectrophotometer at 535 nm. The total anthocyanin content was determined applying the Lambert–Beer law, calculated as mg/100 g of fresh weight, through the following formula: \( A_{535} \times \text{dilution factor} = \frac{E_{1}^{	ext{1 cm}, 535}}{\text{weight}} \), where \( A_{535} \) is the absorbance in the diluted sample and \( E_{1}^{	ext{1 cm}, 535} \) is the value factor (98.2) of molar absorptivity for the acid-ethanol solvent and it refers to the absorption of a mixture of cranberry anthocyanins in acid-ethanol, measured in a 1-cm cell at 535 nm, at a concentration of 1% (w/v).

The ascorbic acid (AA) content was determined using the 2,6-dichlorophenol-indophenol titration method, described by Zenebon, Pascuet, and Tiglea (2008). An overestimation of the AA content in all the extracts was tested for interferences, such as basic substances (using pH indicator thymol blue) and reducing ions Fe(II), Sn(II) and Cu(II) using methylene blue indicators and indigo carmine, before AA determination, in accordance with AOAC (1990). Fresh pulps (5 g) were diluted with 40 mL of a 4% oxalic acid aqueous solution and mixed during 30 min. in a dark room. The solution was titrated by adding the 2,6-dichlorophenol-indophenol solution until a distinct pink–rose color persisted. Several precautions were taken to avoid loss AA such as the use of reduced light and temperature of 4 °C. It was used L-ascorbic acid to prepare a standard solution (0.5 mg/mL) and the concentration was calculated by comparison to the standard and expressed as mg/100 g fresh mass.

2.5. Antioxidant activity determinations

For ABTS assay, the procedure followed the method of Re et al. (1999) with few modifications. The ABTS radical cation (ABTS+) was generated by reaction of 5 mL of aqueous ABTS solution (7 mM) and 88 μL of 140 mM (2.45 mM final concentration) of a potassium persulfate solution. The mixture was held in dark at 29 °C for 14 h before being used, and then it was diluted with ethanol in order to obtain an absorbance of 0.7 ± 0.02 units at 734 nm using a UV–vis spectrophotometer (Micronal, Model B582, São Paulo, Brazil). Fruit extracts (30 μL) or reference substances (Trolox and Vitamin C), were allowed to react with 3 mL of the resulting blue-green ABTS radical solution in a dark condition. The decrease of absorbance at 734 nm was measured at the end point of 6 min. The standard curve was linear between 0–15 μM Trolox (final concentration) and 0–20 mg of ascorbic acid/100 mL. The results were expressed as TEAC (Trolox Equivalent Antioxidant Capacity) and VCEAC (Vitamin C Equivalent Antioxidant Capacity) values, the latter is more suitable for food. Absorbance response (y) of Trolox \( y = -0.0004x + 0.751, \) \( r^2 = 0.979 \) and Vitamin C \( y = -0.0152x + 0.676, \) \( r^2 = 0.986, \) were with linear concentrations. The activity of extracts was estimated at a
minimum of three different concentrations. All the tests were performed in triplicate.

The DPPH assay was carried out according to the procedure described by Brand-Williams, Cuvelier, and Berset (1995) with some modifications. The assay procedure was similar to the ABTS method described above. The solution of DPPH (600 μM) was diluted with ethanol in order to obtain an absorbance of 0.7 ± 0.02 units at 517 nm. Fruit extracts (30 μL) or controls (Trolox, Vitamin C) were allowed to react with 3 mL of DPPH radical solution for 30 min in dark and the decrease in absorbance from the resulting solution was monitored. The standard curve was linear between 0–20 μM Trolox (final concentration) and 0–20 μg of ascorbic acid/100 mL. These results were expressed as TEAC (μM/g fresh mass) and VCEAC (mg/100 g) values. Absorbance response (y) of Trolox (y = −0.0003x + 0.701, \( r^2 = 0.999 \)) and vitamin C (y = −0.0192x + 0.785, \( r^2 = 0.984 \)) concentrations was linear. The activity of extracts was estimated at a minimum of three different concentrations. All tests were performed in triplicate.

2.6. Statistical analysis

All data presented are means of three replicates along with standard deviations. Correlations between antioxidant capacity and phenolics, anthocyanins and vitamin C constituents, were determined using Pearson’s Correlation Coefficient Test.

3. Results and discussion

3.1. Phytochemical analyses

The results for the determination of total phenolics, total anthocyanins and ascorbic acid are in Table 1. Total phenolics content varied from 13.5 ± 1.1 to 159.9 ± 5.6 mg of GAE/100 g of fresh weight. Among the 11 tropical fruits studied, low values were found in sapodilla (13.5 ± 1.1 mg of GAE/100 g), jackfruit (29.0 ± 6.3 mg of GAE/100 g) and pineapple (38.1 ± 0.7 mg of GAE/100 g), whereas murici, mangaba, tamarind and sweetsop contained relatively high amounts of phenolics (81.7 ± 4.0–159.9 ± 5.6 mg of GAE/100 g). Moderate values were also found in ciruela, soursop, papaya and umbu (55.0 ± 2.1–44.6 ± 2.7 mg of GAE/100 g). The obtained data on the total phenolic values of these fruits are scarce in the available literature. The phenolics concentration of fruits evaluated in this work was lower than that found by other authors. Hassimotto et al. (2005) reported the following data for phenolics (mg of GAE/100 g of fresh weight): pineapple (67.2 ± 0.6 mg/100 g), soursop (120.0 ± 8.0) and murici (67.0 ± 3.0). Kulkarni, Policegoudra, and Aradhya (2007) reported the following data for phenolics (mg of GAE/100 g of fresh weight): pineapple (67.2 ± 0.6 mg/100 g), soursop (120.0 ± 8.0) and murici (67.0 ± 3.0). Kulkarni, Policegoudra, and Aradhya (2007) showed a value of 134.6 ± 4.5 mg of total phenolics/100 g in sapodilla. Tamarind presented the highest total anthocyanins content (3.16 ± 0.40 mg/100 g), followed by ciruela (1.35 ± 0.04 mg/100 g) and murici (1.02 ± 0.00 mg/100 g). These results for total anthocyanins contents are compatible with others already registered for pulps of fruits (Kuskoski et al., 2005).

Since none of the extracts contained interfering substances the titrimetric method was used to determine AA in all the fruits examined. Ascorbic acid content ranged from 1.2 ± 0.0 to 96.3 ± 1.7 mg/100 g of fresh weight. Among the 11 fruits studied, only the mangaba presented higher ascorbic acid content (96.3 ± 1.7 mg/100 g) than that of fresh orange (71.0 mg/100 g), which is one of the most consumed sources of vitamin C (USDA, 2010). Ciruela, sweetsop, umbu and murici had moderate amounts of ascorbic acid. The remaining fruits had AA less than 10 mg/100 g. The concentration of ascorbic acid in the tropical fruits evaluated in this study was in accordance with other authors. Hassimotto et al. (2005) reported the following values for ascorbic acid: pineapple (22.4 ± 0.9 mg/100 g) and no values were found for soursop and murici, whereas Kulkarni et al. (2007) reported 10.52 ± 1.2 mg/100 g for sapodilla.

3.2. Antioxidant activity assays

Since the antioxidant capacity of food is determined by a mixture of different antioxidants with different action mechanisms, among which synergistic interactions, it is necessary to combine more than one method in order to determine in vitro, the antioxidant capacity of foodstuffs (Frankel & Meyer, 2000; Pérez-Jiménez et al., 2008). Therefore, two oxidant systems were selected in the present work, which involved the measurement of color disappearance with free radicals DPPH or ABTS. These assays are typically based on the scavenging of radical (ABTS, DPPH), converting it to a colorless product. The degree of this discoloration affects the amount of ABTS or DPPH that has been scavenged. The methods using ABTS or DPPH scavenging are among the most popular spectrophotometer methods for determining the antioxidant capacity in foods and chemical compounds (Kim et al., 2002; Kuskoski et al., 2005).

Results of antioxidant activity assays using ABTS and DPPH scavenging (expressed as TEAC and VCEAC), are summarized in Table 2.

The range of TEAC and VCEAC values obtained in the present work agrees with other results already registered in a study with frozen fruit pulps from the southern part of Brazil (Kuskoski et al., 2005). The TEAC values (μM/g) found in the present work are similar to or higher than those of other plants, which are rich in antioxidants, such as strawberry (25.9), raspberry (18.5), red cabbage (13.8), broccoli (6.5) and spinach (7.6), (Proteggente et al., 2002). Pacheco-palencia, Hawken, and Talcott (2007) found high antioxidant contents in açaí

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**Table 1**

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Common Names</th>
<th>Scientific names</th>
<th>Ascorbic acid (mg AA/100 g)</th>
<th>Total anthocyanin (mg TA/100 g)</th>
<th>Total phenolics (mg GAE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciruela</td>
<td>Spondias purpurea L.</td>
<td>29.6 ± 0.9</td>
<td>1.35 ± 0.04</td>
<td>55.0 ± 2.1</td>
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<tr>
<td>Jackfruit</td>
<td>Artocarpus integefolia L.</td>
<td>1.2 ± 0.0</td>
<td>0.46 ± 0.00</td>
<td>29.0 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Mangaba</td>
<td>Hancornia speciosa Gomes</td>
<td>96.3 ± 1.7</td>
<td>0.79 ± 0.04</td>
<td>98.8 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>Murici</td>
<td>Byrsinoma crassifolia (L.) Kunth</td>
<td>11.8 ± 0.0</td>
<td>1.02 ± 0.00</td>
<td>159.9 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>Papaya</td>
<td>Carica papaya L.</td>
<td>8.6 ± 0.0</td>
<td>0.69 ± 0.04</td>
<td>53.2 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>Ananas comosus (L.) Merr.</td>
<td>13.0 ± 0.0</td>
<td>0.32 ± 0.15</td>
<td>38.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Sapodilla</td>
<td>Manilkara zapota (L.) P. Royen</td>
<td>3.9 ± 0.0</td>
<td>0.46 ± 0.07</td>
<td>135 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Soursop</td>
<td>Annona muricata L.</td>
<td>3.3 ± 0.9</td>
<td>0.19 ± 0.03</td>
<td>54.8 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Sweetssop</td>
<td>Annona squamosa L.</td>
<td>29.6 ± 0.9</td>
<td>0.73 ± 0.18</td>
<td>81.7 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>Tamarind</td>
<td>Tamarindus indica L.</td>
<td>3.1 ± 0.9</td>
<td>3.16 ± 0.40</td>
<td>83.8 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Umbu</td>
<td>Spondias tuberosa Arruda Câmara</td>
<td>12.1 ± 0.4</td>
<td>0.46 ± 0.00</td>
<td>44.6 ± 2.7</td>
<td></td>
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</tbody>
</table>

* Data are expressed as means ± standard deviation (n = 3); GAE: gallic acid equivalents.
pulp (54.4 ± 1.7 μM TEAC/mL). These results indicate that the fruits murici and mangaba are good sources of antioxidants.

The Pearson’s correlation coefficients, between antioxidant activity (expressed on the basis of TEAC using ABTS and DPPH), total phenolics, ascorbic acid and total anthocyanins, are presented in Table 3. The total antioxidant activity from ABTS (R = 0.94, P ≤ 0.001) and DPPH (R = 0.88, P ≤ 0.001) assays, was highly correlated with phenolics contents. No correlation was obtained with any of the other antioxidant constituents, such as vitamin C and anthocyanins. The results suggest that the phenolic compounds (other than anthocyanin), such as phenolic acids, tannic acid and proanthocyanidin, may be the most important contributors to the antioxidant activity in the fruits studied in this research.

Several studies reported the relationships between phenolic content and antioxidant activity; some authors found a high correlation between the phenolic content and the antioxidant activity (Kuskoski et al., 2005; Mahattanatawee et al., 2006; Reddy, Sereramu, & Raghunath, 2010; Silva et al., 2007; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006), while others found no relationship (Imeh & Khokhar, 2002; Ismail, Marjan, & Foong, 2004).

Several lines of evidence suggest that vitamin C is a powerful antioxidant in fruits and vegetables. People with high intakes of dietary ascorbic acid have been repeatedly associated with a lower risk of developing cancer (Leong & Shui, 2002). However, the present work shows that the contributions of vitamin C to the total antioxidant activities of the 11 fruits tested, differ extensively from one fruit to the other (varying over 26-fold to the lowest value), suggesting that contributions of other compounds to the VCEAC from these fruits must not be neglected. Other authors have previously reported a low contribution of vitamin C (0.35–8.6% of the total antioxidant activity) in the following 11 fruits (cranberry, apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pear, and grapefruit) (Sun, Chu, Wu, & Liu, 2002), and less than 5% for apple and pineapple juices (Gardner, White, McPhail, & Duthie, 2000). Gil, Tomás-Barberán, Hess-Pierce, and Kader (2002) demonstrated that for nectarines, peaches and plums, there was no correlation between ascorbic acid and antioxidant activity, as determined by DPPH assay.

Therefore, small molecules that have a better access to the radical site and of other researchers (Pérez-Jiménez et al., 2008; Pérez-Jiménez & Saura-Calixto, 2006), it is suggested that the antioxidant capacity values, ought to be compared when the measurements have been made by the same method and with the same solvent.

Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient (R)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ABTS</td>
</tr>
<tr>
<td>DPPH</td>
<td>0.92**</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.38*</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>0.36*</td>
</tr>
<tr>
<td>Total phenolic</td>
<td>0.94**</td>
</tr>
</tbody>
</table>

** Significance level at P ≤ 0.001.
* Not significant at P ≤ 0.05 level.

** Significant level at P ≤ 0.05.
4. Conclusions

This study reveals in overall that the consumption of exotic fruits from the Northeastern part of Brazil, may deliver greater health benefits through the supply of natural antioxidants, mainly the fruits such as mangaba (H. speciosa Gomes) and murici (B. crassifolia L. Kunth), for their high antioxidant potential. Phenolics contents showed positive correlation with the investigated antioxidant capacity. However, this correlation was not observed when examining vitamin C and anthocyanins contents. The 11 fruits studied had a comparable antioxidant activity in both, ABTS and DPPH assays. These methods are recommended as useful tools for the evaluation of the total antioxidant capacity in fruits. The results indicate promising perspectives for the exploitation of the fruits species, studied that showed a considerable levels of antioxidants capacity. This study will also be useful to consumers, planning rich antioxidant diets and to nutritionists in estimating the daily intakes of phenolic antioxidants and their impact on health.

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