Study of jambolan pulp bioactive compounds

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Abstract

Jambolan fruit is small and the taste, although a little astringent, is pleasant to the palate, especially when ripe; the color presents great visual impact, but with no characteristic aroma. It is an excellent source of antioxidants, thus could be included in the ranking of fruits with excellent nutraceutical potential. These natural compounds have aroused interest, due to their nutritional and therapeutic effects, due to their antioxidant action. The objective of this work was to characterize the jambolan fruit as to the bioactive compounds present in its pulp. Analyzes of total phenolic compounds, anthocyanins, tannins and, antioxidant capacity were carried out by the DPPH free radical scavenging method. The jambolan presented total phenolic compounds content of 309.03 mg GAE 100 mL^{-1} of pulp; anthocyanins of 220.87 mg L^{-1} of pulp, tannins of 16.82 mg TA 100 mL^{-1} of pulp, EC_{50} of 245.61 mg L^{-1} of pulp, and 96.03% of free radical sequestration. Jambolan is a fruit that has high antioxidant capacity and its processing in the form of jelly, sweets and beverages would be a way to add value to the fruit and provide a food with nutraceutical properties for the consumers.

Keywords: Antioxidants. Anthocyanins. Polyphenols. Tannins. *Syzygium cumini.*

Estudo de compostos bioativos presentes na polpa de jambolão

Resumo

O fruto jambolão é pequeno e o sabor, apesar de um pouco adstringente, é agradável ao paladar, especialmente quando maduro, a cor apresenta grande impacto visual, porém sem aroma característico forte. Excelente fonte de antioxidantes, pode ser incluído no *ranking* dos frutos com ótimo potencial nutracêutico. Estes compostos naturais têm despertado interesse, devido aos seus efeitos nutricionais e terapêuticos, pela ação antioxidante. O objetivo deste trabalho foi caracterizar o jambolão quanto aos compostos bioativos presentes em sua polpa. Foram realizadas as análises de compostos fenólicos totais, antocianinas, taninos e capacidade antioxidante, pelo método do sequestro de radicais livres do DPPH. O jambolão apresentou médias do teor de compostos fenólicos totais de 309.03 mg ác. gálico 100 mL^{-1} de polpa; antocianinas de 220.87 mg L^{-1} de polpa, taninos de 16.82 mg ác. tânico 100 mL^{-1} de polpa, EC_{50} de 245.61 mg L^{-1} de polpa e 96.03% de sequestro de radicais livres. O jambolão é uma fruta

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que apresenta alta capacidade antioxidante e o seu processamento em forma de geleia, doces e bebidas seria uma maneira de agregar valor à fruta e propiciar um alimento com propriedades nutracêuticas para o consumidor.

Palavras-chave: Antioxidantes. Antocianinas. Polifenois. Taninos. *Syzygium cumini.*

Introduction

Jambolan fruit (*Syzygium cumini*) originated in India was widely spread by cultivation in the tropical regions, including the banks of watercourse, since they are much appreciated by fishes. The jambolan adapted very well to the soil and climate conditions of Brazil, becoming, at first, subspontaneous species in the Northeast region. Soon after, it spread throughout the country, from north to south (SILVA, 2008).

The fruit is small of ovoid shape, resembling an olive, which turns dark purple when completely ripe. The skin is thin, lustrous and adherent. Its pulp, almost translucent white, is fleshy and involves a single core. The taste, although a little astringent, is pleasant to the palate, especially when mature, the color presents great visual impact, but without characteristic strong aroma (MORTON, 2004; SILVA, 2008).

The purple coloration of the fruits has a great visual impact due to the high content of anthocyanin pigments, which are hydrophilic antioxidant pigments also found in fruits such as grape (*Vitis* sp.), Blueberry (*Vaccinium myrtillus*) and jabuticaba (*Myrciaria cauliflora*), which present the great advantage of being highly soluble in aqueous mixtures (VEIGAS *et al.*, 2007; SILVA, 2008). These natural compounds have aroused interest, due to their nutritional and therapeutic effects, by the antioxidant action. The importance of free radicals in the manifestation of several pathologies such as cancer and atherosclerosis has led to a growing search for sources not yet exploited that can act as antioxidants and thus reduce the imbalance between the system of antioxidative defense of the human body and free radicals (BRAVO, 1998).

The astringency of the jambolan pulp is due to the presence of tannins, phenolic compounds of high molecular weight, which are also present in fruits such as cashew (*Anacardium* sp.) And green banana (*Musa* sp.). As fruits mature, a reduction in astringency usually occurs, which is attributed to the loss of solubility of the tannin. However, in small proportions or in combination with other components of the food, astringency can contribute to a desirable flavor, as in wines made with pigmented grapes cultivars (SILVA, 2008).

In India, besides being consumed *in natura*, the pulp of the jambolão is also used in the production of sweets. Tea from leaves and seeds of the species is also well known in Indian folk medicine, mainly due to hypoglycemic effects. Different studies suggest that the treatment with the jambolan seed extract reduces the glycemia of diabetic rats (AGOSTINI-COSTA; SILVA, 2008). In Brazil, a minority of its production is used by the local populations. However, a large part of its fruit is wasted during the harvest, due to the high production per tree, the short shelf life of the fruit in natura and, mainly, Lack of processed utilization (LAGO; GOMES; SILVA, 2006). The objective of this work was to characterize the jambolão as to the bioactive compounds present in its pulp.

Material and methods

Material

Jambolan (*Syzygium cumini*) fruits were manually harvested in the city of Goiânia and Bela Vista de Goiás in the period from December 2014 to March 2015.

Once the fruits were harvested, they were stored in polyethylene boxes (previously sanitized) and transported to the vegetable processing plant (Agronomy School, Federal University of Goiás). After the removal of the calyces, it was carried out the selection and classification of fruits according to the presence of mechanical injuries, sanity and maturation by visual analysis (dark purple fruits) in order to obtain homogenous batches. The selected fruits were washed in tap water to remove coarse soil residues and then sanitized in sodium hypochlorite solution (200 ppm/15 min). The seeds were removed from the pulp with the aid of an industrial sweeper (NPC Equipamentos, Bonina 0.25 df, Itabuna, Brazil). The pulp was then placed in polyethylene bags (1 L) and stored in a freezer (-18°C) until the preparing of extracts to the analyzes.

Extracts

In order to carry out the analyzes of total phenolic compounds and antioxidant activity, an extract was prepared from 0.5 g of sample (jambolan pulp) in triplicates. In a beaker, the sample was weighed and 20 mL of 50% methanol was added, which remained standing for 60 min in the dark at room temperature. This content was centrifuged at 15,000 rpm for 15 min and the supernatant was filtered through a cellulose qualitative paper filter (Whatman 1) and stored in a 50 mL volumetric flask. The operation was repeated, however, with 70% acetone intstead of 50% methanol. Both filtrates were stored in the same flask and the volume was made up to 50 mL with distilled water.

Total phenolic compounds (TPC)

The TPC were determined by the method proposed by Larrauri, Rupérez, and Saura-Calixto (1997), with modification. An aliquot of 0.5 mL of the sample (extract), 2.5 mL of 10% Folin-Ciocalteau solution, and 2 mL of 4% sodium carbonate solution were pipetted into a test tube, respectivelly. The tube was stirred (speed 5) in a tube agitator (Phoenix, AP 56, Araraquara, Brazil) and kept at room temperature for 120 min in a dark environment. The reading was then performed at 750 nm on a Cary 50 Probe UV-Vis spectrophotometer (Varian, California, United States), with computerized system. The results were expressed as mg of gallic acid equivalente (GAE) in a 100 g of fresh pulp, using the linear regression equation obtained from the standard curve.

Antioxidant activity

The antioxidant activity was evaluated trhough the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method, according with Brand-Williams, Cuvelier, and Berset (1995), with adaptation. Three dilutions (1:1, 1:4, and 1:5) of the extract were performed in triplicate, and from each one an aliquot of 100 µL was taken and 3.9 mL of the methanolic solution of DPPH (2.4 mg 100 mL^{-1}) was added to it; the final volume was homogenized (speed 5) (Phoenix, AP 56, Araraquara, Brazil). The tubes were kept at room temperature in the absence of light for 60 min (time to stabilize the sample). The absorbance readings were carried out at 515 nm in a Cary 50 Probe UV-Vis spectrophotometer (Varian, California, United States). The blank corresponding to the DPPH assay, standards, and samples were prepared. A control solution was prepared by adding 40 mL of 50% methyl alcohol solution to 40 mL of 70% acetone solution, and the volume was made up to 100 mL with distilled water. An aliquot of 100 µL of this control solution was taken and 3.9 mL of the DPPH solution was added to it in triplicate. A blank should also be prepared with pure methanol. The same steps performed to the extract samples should be carried out to the control. The control solution for EC_{50} calculation was read in triplicate after reading the blank, and before reading the samples. A control curve was also prepared with seven different concentrations (10µM to 60µM) of the DPPH solution (2.4 mg 100 mL^{-1}).

Anthocyanins

Anthocyanins were determined by the pH differential method (WROLSTAD, 1993). An aliquot of 10 mL of the sample was taken and diluted in 50 mL of buffer solution pH = 1 and another 10 mL in 50 mL of buffer solution pH = 4.5. This dilution has a dilution factor of 5. After dilution in the buffer solutions the samples became clear, without the presence of sediments. To confirm the absence of turbidity, the absorbance of the solution was measured at 700 nm and the absorbance value found was zero. The absorbance of the samples, diluted in buffer solution pH = 1 and 4.5, was read in a Cary 50 Probe UV-Vis spectrophotometer (Varian, California, United States) at 510 nm and at both pH. To measure the absorbance both solutions, the calculation was performed according to equation 1.

$$Absorbance = A = \left(\left(A_{510nm;pH\ 1}\right) - \left(A_{700nm;pH\ 1}\right)\right) - \left(\left(A_{510nm;pH\ 4.5}\right) - \left(A_{700nm;pH\ 4.5}\right)\right) \quad (1)$$

In order to determine the concentration of anthocyanins in the samples, it was first found which anthocyanin is present in the highest quantity in the product being analyzed, in this case, the jambolan pulp. According to Faria, Marques, and Mercadante (2011), the anthocyanin present in the highest quantity in jamboln fruit is cyanidin-3-glucoside, which has a molecular weight (MW) of 449. The calculation was then performed using equation 2.

$$Anthocyanin\ (mg\ L^{-1}) = \frac{A}{E \times L} \times 1000 \times MW \times Fc \quad (2)$$

Where, A = Absorbance; E = cuvete thickness (cm); L = Molar absorbance; Fc = Diluction factor.

Tannins

The tannins were extracted according to the technique of Goldstein and Swain (1963), with modification. Three successive extractions were performed with 80% methanol. For the determination, the method of Folin-Denis (method 955.25) (AOAC, 2010) was used. A sample of 5 g was weighed in a flask, and 50 mL of 80% methanol was added. This flask containing the solution was subjected to heating in an electric plate (Novatécnica, NT-339, Piracicaba, Brazil) for 15 min (the time was set from the moment that the boiling began). The solution was then filtered, and the residue placed in the same flask which was previously used. Another 50 mL of 80% methanol was added, and the same procedure was repeated twice more, resulting in three extractions. The content from the three extractions was pooled in a single flask, which was subjected to heating on an electric plate to the volume of 5 mL, followed by an addition of 25 mL of distilled water. From this volume of 30 mL, an aliquot of 0.1 mL was taken, 8.4 mL of distilled water, 0.5 mL of the Folin-Denis reagent, and 1 mL of sodium carbonate were added to it in a tube. This mixture was kept for 30 min at room temperature and away from the light before the reading, which was performed on a Cary 50 Probe UV-Vis spectrophotometer (Varian, California, United States) with a computerized system at a wavelength of 760 nm. The results were expressed as mg of tannic acid (TA) in 100 mL of fresh pulp.

Results and discussion

The values found for TPC, antioxidant activity, anthocyanin, and tannin present in the pulp of jambolan fruit are available in Table 1.

Table 1 – Total phenolic compounds (TPC) (mg GAE 100 mL^{-1} of pulp), anthocyanin (mg L^{-1} of pulp), tannin (mg TA 100 mL^{-1} of pulp), EC_{50} (mg L^{-1} of pulp), and scavenging activity (%) of jambolan pulp

Bioactive compounds	Average (n=6)
TPC	309.03 ± 1.01
Anthocyanin	220.87 ± 2.89
Tannin	16.82 ± 0.39
EC_{50}	245.61 ± 2.76
Scavenging activity	96.03 ± 1.64

Fonte: Elaborada pelos autores, 2017.

Phenolic compounds belong to the class of compounds that includes diversity of simple and complex structures, with at least one aromatic ring in which at least one hydrogen is replaced by a hydroxyl group (ESCARPA; GONZALES, 2001).

The quantification of TPC is an estimate of the content of all compounds belonging to the subclasses of phenolic compounds present in a sample. The content found in this study are superior to those reported by Kuskoski *et al.* (2003) in jambolan fruit (229.6 mg 100 g^{-1}). Barcia (2009) also determined the content of phenolic compounds in jambolan fruits from several regions of the Brazilian territory and found values varying from 279 to 574 mg GAE 100 g^{-1}. The average values found in the jambolan pulp in this study are within this range.

Jacques, Pertuzatti, and Barcia (2009), analyzed several fruits, including blueberry, blackberry, and loquat, and reported TPC content of 816.9 mg GAE 100 g^{-1} for blueberry cultivar (cv.) Powderblue, 750.5 mg GAE 100 g^{-1} for blueberry cv. Delite, and 645.5 mg GAE 100 g^{-1} for blackberry cv. Tupy. Only the loquat showed inferior content to that of the jambolan pulp (55.8 mg GAE 100 g^{-1}).

The difference of phenolic compounds in diverse fruit types or even fruit samples can be explained by the chemical nature of the bioactive compounds, ranging from simple substances to highly polymerized compounds, which include different proportions of phenolic acids, anthocyanins, tannins, and others (CÓTÊ; CAILLET; DOYON, 2010).

The antioxidant activity of the jambolan pulp extracts were evaluated by the free radical DPPH scavenging method and then quantified by the EC_{50}, which corresponds to the amount of extract necessary to reduce the DPPH radical by 50%. The average result obtained for the jambolan

pulp was 245.61 mg L^{-1}. The lower the EC_{50}, the better the antioxidant activity of the extract. The jambolan pulp presented high antioxidant activity, which can be proven not only by the low EC_{50} values, but also by the high percentage of free radical scavenging (Table 1). Hassimoto, Genovese, and Lajolo (2005) by evaluating the antioxidant activity of fruits, vegetables, and fruit pulps, observed a greater antioxidant activity in samples containing higher anthocyanin content. The same correlation was observed by Munóz-Espada *et al.* (2004), who evaluated the cv. *Vitis vinifera* and *Vitis labrusca*, in which they observed a positive association between anthocyanin content and antioxidant activity by the DPPH free radical scavenging method. This relationship could also assumed in this study, once the jambolon pulp has an anthocyanin content considered high when compared to the same content of traditionally rich fruits, as well as the antioxidant activity obtained for the jambolan pulp. Chim (2008) reported an inhibition of free radicals in blueberries cv. Brazos of 88.14%, followed by cv. Tupy (87.6%) and cv. Guarani (86.93%). These values were lower than the average observed in this study for the jambolan pulp. The highest value found by the author was 8% lower than that found in this work.

Studies by Veigas *et al.* (2007) confirm the presence of three major anthocyanins identified as delphinidin glucosides, petunidine, and malvidin. Also, the high levels of anthocyanins found in jambolan (220.87 mg L^{-1} or 22.087 mg 100 g^{-1}) are equivalent to the levels found in blueberries, recently classified as the first nutraceutical commodity of great commercial value. The results presented in this study suggest that the high antioxidant activity of the jambolan extract, together with its strong dyeing potential, with desirable characteristics of solubility and stability, could stimulate the incorporation of the extract as a natural additive to be used in food and pharmaceutical formulations (VEIGAS *et al.*, 2007; SILVA, 2008). Barcia (2009) reported anthocyanins levels in jambolan ranging from 7.43 to 16.95 mg cyanidin 3-glycoside 100 g^{-1}. The average anthocyanin content in this work was 5 times lower than that reported by Kuskoski *et al.* (2003) for jambolan fruits from the Southern region of Brazil (108 mg $100g^{-1}$), and seventeen times lower than that found by Moyer et. al. (2002) for blueberry cv. CVAC (381.34 mg 100 g^{-1}). The anthocyanin content may be a consequence of the soil type of the region, or it may also be related to the genetic variation and environmental condition during the harvest period (BARCIA, 2009), and also to the growth and maturation phases of the fruit, as in mature fruits there is na increase in the degradation of chlorophyll and the detachment (synthesis) of anthocyanins (FASAWANG; ANPRUNG, 2014). Thus, the great variation of anthocyanin content among fruits of the same species can be justified.

In plants, tannin can be found in roots, flowers, fruits, leaves, bark, and on the stem. These compounds contribute to the astringent taste in food and beverages, such as that sense when consuming red wines, teas, and green fruits (ABE *et al.*, 2007). Barcia (2009) investigated jambolan fruits from various Brazilian cities and found an average of 23.77% for jambolan fruits from Capão do Leão, 29.65% for fruits from Pelotas, and 17.06% for fruits from Santa Vitória do Palmar. The latter indicates that there is variation in the tannin content between different cities, however, the values found for the fruits of Santa Vitória do Palmar are similar to those obtained in this study. Soares (2014) analyzed jambolan fruits from different regions of the city of Goiânia (Brazil) and found values ranging from 17.88 to 33.79 g catechin 100 g^{-1}, values higher than that found in this study for jambolan pulp from the same city, although the lower limit found by the author was similar to that found in this work.

Conclusion

Jambolan is a fruit that has high antioxidant activity, evidenced by the great content of phenolic compounds and the low value of EC_{50}, but also by the high free radical scavenging activity. Thus, the processing of jambolan in the form of jelly, sweets, and beverages would be a way to add value to the fruit, benefiting the small producer, and the use of the fruit, which in Brazil, is consumed only in natura, besides providing a food product with nutraceuticals properties to the consumer.

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