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Jabuticaba skin extracts: phenolic compounds and antibacterial activity

Extratos de casca de jabuticaba: compostos fenólicos e atividade antibacteriana

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Abstract

The phenolic compounds from various extracts of jabuticaba skin powder (JSP) were characterized in this study, and the antibacterial activity assessed. The phenolic compounds were extracted from the JSP using four methods: a) acetone extraction - 1 g JSP: 10 mL 70% acetone, resting for 2 hours; b) aqueous extract - 1 g JSP: 15 mL water, under agitation; c) ethanolic extract - 1 g JSP: 15 mL acidified ethanol, under agitation; and d) methanolic extract - 1 g JSP: 50 mL 50% methanol, under reflux. The antibacterial activity was evaluated by the agar diffusion assay, using *Escherichia coli* ATCC 11229, *Salmonella choleraesuis* ATCC 6539, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 19117. The ethanolic and methanolic extracts showed the highest levels of phenolic compounds, especially of cyanidin chloride, catechin and epicatechin. The extracts did not inhibit the growth of *Escherichia coli* and *Salmonella choleraesuis*, but inhibited 30% of the growth of *Pseudomonas aeruginosa* with an extract concentration of 250 μg mL^{-1}. Against *Staphylococcus aureus* and *Listeria monocytogenes* the highest inhibitory effect observed was 41.8% for the ethanolic extract, followed by 36% inhibition by the methanolic extract, thus revealing the potential of these extracts as possible alternatives for use in the food and/or pharmaceutical industries.

Keywords: *Plinia jaboticaba; Bioactive compounds; Bactericidal; Fruit residue; Microorganisms; Natural product.*

Resumo

Neste estudo, caracterizaram-se os compostos fenólicos e avaliou-se a atividade antibacteriana de extratos obtidos da farinha da casca de jabuticaba (FCJ). Os compostos fenólicos da FCJ foram extraídos de quatro formas: a) extrato acetônico - 1 g FCJ: 10 mL acetona 70%, duas horas em repouso; b) extrato aquoso - 1 g FCJ: 15 mL água, sob agitação; c) extrato etanólico - 1 g FCJ: 15 mL etanol acidificado, sob agitação; e d) extrato metanólico - 1 g FCJ: 50 mL metanol 50%, sob refluxo. A atividade antibacteriana foi avaliada pela técnica de difusão cavidade em Ágar, utilizando-se os microrganismos *Escherichia coli* ATCC 11229, *Salmonella cholerasuis* ATCC 6539, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538 e *Listeria monocytogenes* ATCC 19117. Os extratos etanólico e metanólico apresentaram os teores mais elevados de compostos fenólicos, sobretudo o cloreto de cianidina, catequina e epicatequina. Os extratos não inibiram o crescimento de *Escherichia coli* e *Salmonella choleraesuis*, mas inibiu em 30% o crescimento de *Pseudomonas aeruginosa* na concentração do extrato de 250 μg mL^{-1}. A maior inibição de crescimento registrada foi de 41,8% pelo extrato etanólico, seguida pela inibição de 36% pelo extrato metanólico, contra as bactérias *Staphylococcus aureus e Listeria monocytogenes*, revelando assim a potencialidade destes extratos como possível alternativa para utilização na indústria de alimentos e/ou farmacêutica.

Palavras-chave: *Plinia jaboticaba; Compostos bioativos; Bactericida; Resíduo de fruta; Microrganismos; Produto natural.*

1 Introduction

One of the ways of controlling undesirable microorganisms in foods is by using synthetic chemical preservatives. However, the use of these agents is not compatible with a "natural product" image, which is of great commercial appeal. Currently, there is a strong debate regarding the safety of synthetic preservatives, since they are associated with carcinogenic and teratogenic processes, besides their residual toxicity when consumed for long periods (ORTEGA-RAMIREZ et al., 2014). Moreover, the task of developing and approving new synthetic preservatives, aiming to improve the safety and increase the shelf life and expiration time, takes time and considerable investment (TAJKARIMI et al., 2010).

Lately, synthetic food additives have been facing serious consumption resistance by the public throughout the world and increased restrictions for their regulation and usage. The need to ensure safety and to meet the demands for preserving food nutritional and quality attributes, has resulted in a growing search for natural preservatives with potential applications in food products, which could be used alone or in combination with other technologies (ORTEGA-RAMIREZ et al., 2014).

As the interests of people in feeding habits and health increases, so does the interests of industry in the search for natural products with potential application in foods, in order to preserve their sensory properties and extend their shelf life. For this reason, careful research is needed to substitute the usual synthetic additives by natural and nontoxic ones. For the consumer, using natural food additives of plant based makes a given product much more attractive. Amongst the natural substances with this potential, phenolic compounds are an interesting alternative, since some possess significant antimicrobial potential, and may be used to prolong the shelf life of foods.

Phenolic compounds are bioactive substances that can be used as natural antimicrobial agents in foods. This antimicrobial action occurs on different cell structures, causing cell membrane disruption, complexation with the cell wall, substrate deprivation, interaction with genetic material and inactivation of ATP synthase (essential for microbial metabolism). Phenolic compounds also alter the cell membrane structure and function, impairing the flux of protons and electrons and active transport (BURT, 2004; AHMAD et al., 2012). Many bacteria commonly involved in foodborne illness outbreaks are resistant not only to pre-established antimicrobial agents, but also to the latest generation ones, posing an increasing worldwide health problem (SILVA et al., 2010). These include *Escherichia coli* ATCC 11229 (gram-negative), *Salmonella choleraesuis* ATCC 6539 (gram-negative), *Pseudomonas aeruginosa* ATCC 15442 (gram-negative), *Staphylococcus aureus* ATCC 6538 (gram-positive) and *Listeria monocytogenes* ATCC 19117 (gram-positive).

Recently, a number of research studies have reported the presence of bioactive compounds in different agro-industrial residues, including phenolic compounds, with great potential for use in many industrial sectors. Many residues, such as grape seeds and bagasse, pomegranate peel, lemon peel, mango peel and seeds, and citrus processing residues are known to possess antimicrobial activities (KATALINIC et al., 2010; DELGADO ADÁMEZ et al., 2012; GERHARDT et al., 2012; ARBOS et al., 2013; OLIVEIRA et al., 2013).

However, no reports were found in the literature concerning the antimicrobial activity of jabuticaba skin. The jabuticaba skin is rich in phenolic compounds, with a phenolic compounds content of 11.99 g 100 g^{-1} dry matter (LIMA et al., 2011a).

The use of jabuticaba skin extracts represents a viable alternative in products susceptible to physical, chemical and microbiological alterations. It is known that different extraction methods (i.e. the use of different solvents and apparatuses) lead to the obtaining of different compounds and yields, and, as a consequence, to different properties. Using residues as a source of phenolic compounds may contribute to a reduction in the use of synthetic chemical conservatives, providing benefit to consumer health, besides aggregating commercial value to the fruit.

Considering the above, the objective of this study was to characterize the phenolic compounds present in different jabuticaba skin powder (JSP) extracts, as well as determining their antimicrobial potential, aiming at a possible use in the food and drugs industries.

2 Material and methods

2.1 Fruit harvest and preparation of jabuticaba skin powder

A total of 21.4 kg of jabuticaba fruits (*Plinia jaboticaba* (Vell.) Berg, Sabará genotype), were hand-picked in the municipality of Coqueiral, MG, Brazil.

The fruits were selected, washed in running water and sanitised with a sodium hypochlorite solution (200 mg kg^{-1}) by immersion for 10 minutes. The fruits were then squeezed through a sieve, obtaining 5.15 kg of skins. The skins were placed in wire baskets and dehydrated in a kiln at 45 °C to constant weight. After drying, the jabuticaba skins were ground in a knife mill (TE 631 Tecnal) for 3 minutes, obtaining 1.07 kg of jabuticaba skin powder (JSP).

The JSP was sieved through 35, 60, 80 and 100 mesh sieves, in order to determine the granulometry. Most of the particles were retained on the 60 and 80 mesh sieves, and according to Zanotto and Bellaver (1996), the powder was classified as a fine grain powder. The JSP was conditioned in hermetically sealed flasks, and stored in the absence of light at room temperature until used.

2.2 Obtaining the jabuticaba skin extracts

The following extraction processes were each carried out three times, and after freeze-drying, were weighed to determine the extraction yields.

a) **Acetone extract:** To 1 g of JSP 10 mL of an acetone: water (7:3, v/v) solution was added. This mixture was kept at room temperature for 2 hours, applying vortex agitation for 3 minutes after 0 h, 1 h and 2 h. The solution so obtained was filtered through glass wool and washed with 10 mL of the solvent (AGOSTINI-COSTA et al., 2003). The filtered solution was separated and placed in a rocta-evaporator at 45 °C to completely evaporate the acetone off. The residue was removed with water and freeze-dried;

b) **Aqueous extract:** To 1 g of JSP 10 mL of distilled water were added, followed by 15 minutes of agitation in a horizontal shaker at room temperature. The solution was then filtered through organza. The previous steps were carried out twice and the final filtered solution freeze-dried;

c) **Ethanolic extract:** To 1 g of JSP, 15 mL of acidified ethanol (85% ethanol and 15% 1.5 mol L^{-1} HCl) were added, followed by 2 minutes homogenization in a benchtop homogenizer and maceration for 12 hours at 4 °C in the absence of light (LIMA et al., 2011b). The solution was filtered through Whatman nº 1 paper in a Buchner funnel under pressure. The residue was washed with the same solvent to a total volume of 100 mL and the filtered solution placed in a rocta-evaporator at 45 °C to eliminate any remaining solvent. The residue was collected and freeze-dried;

d) **Methanolic extract:** In a 250 mL Erlenmeyer, 1 g of JSP was mixed with 50 mL of 50% (v/v) methanol, and sealed with a reflux stopper. The Erlenmeyer was placed on a hotplate at 80 °C and after boiling for 15 minutes, the extract was filtered through filter paper and collected in a 250 mL beaker. The residue was submitted to this process twice (LATIMER JUNIOR, 2012). The remaining filtered solution was heated once again to 80 °C on the hotplate until elimination of the methanol and then freeze-dried.

2.3 Chromatographic analysis of the phenolic compounds

The chromatographic analysis was carried out in a Shimadzu UFLC, equipped with two model LC-20AT pumps, a model SPD-M20A UV-vis detector, a model CTO-20AC column oven, a model CBM-20A interface and a model SIL-20A automated injector with auto sampler. The separations were carried out used a Shim-pack VP-ODS-C18 (250 mm × 4.6 mm) column connected to a pre-column in a Shim-pack Column Holder (10 mm × 4.6 mm).

The freeze-dried extracts were dissolved in water (1:16, m/v), and the standards filtered through a 0.45 µm nylon membrane (Millipore®) and injected directly into the chromatograph. The phenolic compounds present in the four extracts were identified by comparing the retention times of the samples with those of the standards. Analytical curves obtained by linear regression were constructed for quantification, considering a coefficient of determination (R^2) of 0.99.

a) **Identification of the flavonoids, tannins and phenolic acids:** The mobile phase was composed of 2% acetic acid in water (A) and methanol: water: acetic acid (70:28:2, v/v/v) (B). The analyses took 65 minutes at 40 °C, with a flow rate of 1.0 mL min^{-1}, wavelength of 280 nm and an injection volume of 20 µL in a gradient system (100% solvent A from 0.01 to 5.0 minutes; 70% solvent A from 5.0 to 25.0 minutes; 60% solvent A from 25.0 to 43.0 minutes; 55% solvent A from 43.0 to 50 minutes; and 0% solvent A for 10 minutes) up to the end of the run. Solvent A was increased to 100% to maintain column equilibrium (MARQUES et al., 2016). The standards used were: ferulic acid, salicylic acid, vanillic acid, siringic acid, gallic acid, *o*- and *p*-cumaric acids, epicatechin, catechin, epicatechin gallate, resveratrol and quercetin;

b) **Identification of the anthocyanins:** The mobile phase was composed of an acetonitrile solution (A) and water: acetic acid (80: 20, v/v). The analyses took 30 minutes at 40 °C, with a flow rate of 1.0 mL min^{-1}, wavelength of 545 nm and an injection volume of 20 µL in a gradient system varying from 0 to 30% (PRATA, 2005). The standards used were: malvidin chloride, cyanidin chloride and delphinidin chloride.

2.4 Evaluation of the antibacterial activity

To assess the antibacterial activity the following bacteria were used: *Escherichia coli* ATCC 11229 (gram negative), *Salmonella choleraesuis* ATCC 6539 (gram negative), *Pseudomonas aeruginosa* ATCC 15442 (gram negative), *Staphylococcus aureus* ATCC 6538 (gram positive) and *Listeria monocytogenes* ATCC 19117 (gram positive). The agar diffusion assay was used, in which a thin layer of agar (Mueller-Hinton) was added to Petri dishes and the bacterial culture deposited on the layer. The JSP

extract was added to wells, which were made with the aid of glass balls.

The wells were filled with 10 µL aliquots of the extracts at concentrations of 250; 125; 62.5; 31.25; 15.62; 7.81 and 3.90 µg mL^{-1}. Three replicates were used for each treatment, a negative control with the application of 10 µL of water and as a standard for comparison, a solution containing 100 µg mL^{-1} of the antibiotic chloramphenicol was used (NCCLS, 2003; PEREIRA et al., 2008). The plates were incubated in a BOD chamber at 37 °C for 24 hours and the halos formed were measured, to evaluate the sensitivity profile of each bacterium when using different JSP extract concentrations. The minimum inhibitory concentration (MIC) for each extract was measured, and defined as the smallest JSP extract concentration which resulted in a significant inhibitory halo.

2.5 Experimental design and statistical analysis

The phenolic compounds were evaluated in all four extracts. The evaluation process was carried out using a completely randomized design, with 4 treatments (JSP extracts) and 3 replicates. Twelve phenolic compounds were also evaluated in each of the extracts also with 3 replicates. The antibacterial activity was evaluated using a 1 × 5 completely randomized factorial design (JSP extract × 5 concentrations), with 3 replicates for each bacterium tested.

The data were submitted to a one-way ANOVA on SISVAR (variance analysis system for balanced data) according to Ferreira (2011), and when significant, the Tukey's test was applied at 5% of probability, in order to compare the means.

3 Results and discussion

3.1 Phenolic compounds yield and identification

The dry weight yields of the acetone, aqueous, ethanolic and methanolic JSP extracts were, respectively, 47.99 ± 2.34%; 71.06 ± 2.01%; 43.16 ± 1.24% and 53.76 ± 4.60%. As can be seen, the aqueous extract showed the highest dry weight yield and the ethanolic extract the lowest. However, the latter displayed the highest levels of total phenolic compounds in comparison with the other extracts (Table 1).

Figures 1, 2, 3 and 4 show the chromatographic profiles with the phenolic compounds identified in each of the four JSP extracts.

In order of the yields in total phenolic compounds, the highest was the ethanolic extract (Figure 3; Table 1), followed by the methanolic extract (Figure 4; Table 1), the aqueous extract (Figure 2; Table 1) and finally the acetone extract (Figure 1; Table 1). It can be seen that each extract had a different phenolic compounds composition. For instance, malvidin chloride was not detected in the acetone, aqueous or ethanolic extracts, whereas *o*-cumaric acid and delphinidin chloride were not found in the methanolic extract. Ferulic acid, *o*-cumaric acid, cyanidin chloride and malvidin chloride were not identified in the aqueous extract; whilst *o*-cumaric acid, siringic acid, delphinidin chloride, malvidin chloride and epicatechin gallate were not found in the acetone extract. The compound *o*-cumaric acid was only found in the ethanolic extract, whereas malvidin chloride was only found in the methanolic extract.

The differences between the phenolic compounds contents observed in each of the extracts are related to many factors. For example, the most prominent factor,

Table 1. Phenolic compound contents in mg 100 g^{-1} found in the different freeze dried extracts obtained from jabuticaba skin powder.

Phenolic compounds	Acetone extract	Aqueous extract	Ethanolic extract	Methanolic extract
Ferulic acid	0.82 ± 0.06 ^{aE}	0.00 ± 0.00 ^{bD}	0.83 ± 0.02 ^{aE}	0.89 ± 0.19 ^{aE}
Gallic acid	2.72 ± 0.21 ^{aD}	1.88 ± 0.07 ^{bcD}	2.29 ± 0.07 ^{bE}	1.83 ± 0.00 ^{cDE}
o-cumaric acid	0.00 ± 0.00 ^{bE}	0.00 ± 0.00 ^{bD}	1.52 ± 0.04 ^{aE}	0.00 ± 0.00 ^{bE}
p-cumaric acid	1.23 ± 0.07 ^{cE}	0.66 ± 0.04 ^{cD}	8.33 ± 0.33 ^{aE}	4.74 ± 0.62 ^{bD}
Siringic acid	0.00 ± 0.00 ^{dE}	1.92 ± 0.09 ^{aD}	0.80 ± 0.06 ^{cE}	1.58 ± 0.08 ^{bE}
Cyanidin chloride	9.77 ± 0.09 ^{cC}	0.00 ± 0.00 ^{dD}	29.14 ± 1.82 ^{bD}	61.26 ± 2.3 ^{aA}
Delphinidin chloride	0.00 ± 0.00 ^{cE}	45.19 ± 2.77 ^{aA}	35.89 ± 0.81 ^{bC}	0.00 ± 0.00 ^{cE}
Malvidin chloride	0.00 ± 0.00 ^{bE}	0.00 ± 0.00 ^{bC}	0.00 ± 0.00 ^{bE}	39.52 ± 1.74 ^{aB}
Catechin	42.01 ± 1.62 ^{cA}	10.54 ± 0.06 ^{dD}	117.5 ± 8.83 ^{aA}	58.33 ± 1.66 ^{bA}
Epicatechin	35.87 ± 0.55 ^{aB}	8.24 ± 0.23 ^{bD}	33.38 ± 3.94 ^{aC}	10.98 ± 0.94 ^{bC}
Epicatechin gallate	0.00 ± 0.00 ^{cE}	28.57 ± 0.07 ^{bB}	84.62 ± 5.44 ^{aB}	1.09 ± 0.20 ^{cE}
Total	92.42	97.00	314.3	180.26
VC (%)	6.44	10.52	13.82	6.77

Data obtained from three replicates plus the standard deviation. Small case letters in the same line compare the yields of a particular phenolic compound, if present. Upper case letters in the same column compare the phenolic compound contents of a particular extract. Means followed by the same letter do not differ by Tukey's test at 5% probability.

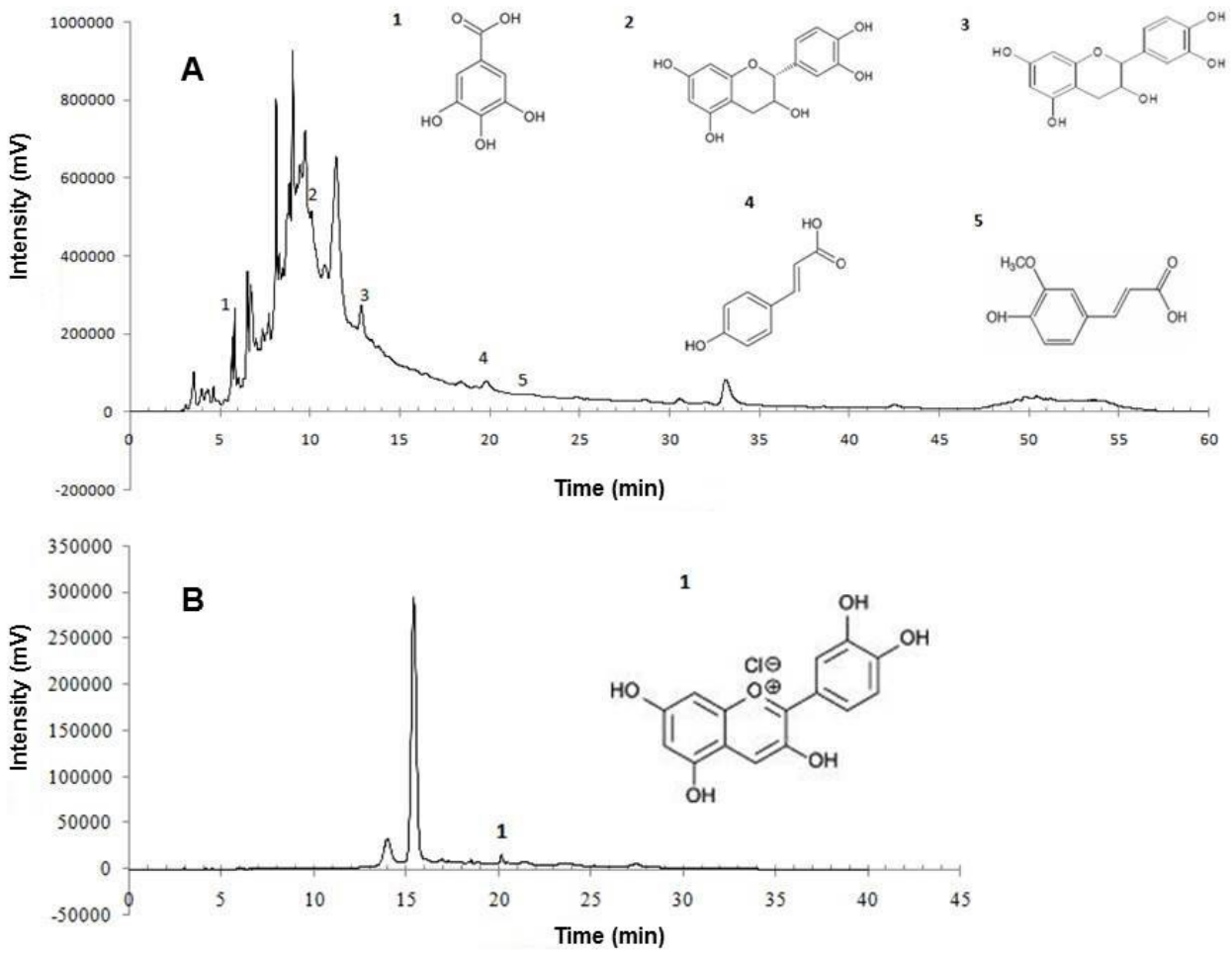

Figure 1. (A) Chromatogram of the acetone jabuticaba skin extract, with the peaks identified and the corresponding phenolic compound: 1 - gallic acid (time = 6.541); 2 - catechin (time = 10.419); 3 - epicatechin (time = 13.750); 4 - *p*-cumaric acid (time = 19.889); 5 - ferulic acid (time = 22.609). (B) Chromatogram of the anthocyanins in the acetone jabuticaba skin extract, with one peak identified 1 - cyanidin chloride (time = 21.318).

the polarity of the solvent, as well as the temperature and time of extraction, are all critical points in the extraction of phenolic compounds. Other factors, such as agitation, also play a role in the extraction yield. It is important to mention that there are a great number of phenolic compounds, with different levels of complexity, all susceptible to the extraction conditions, and therefore their presence in the final extract is bound to the extraction methodology adopted (YILMAZ; TOLEDO, 2006; ROCKENBACH et al., 2008).

There are plenty of studies trying to find a single extraction method capable of obtaining a better yield of phenolic compounds, but it is no easy task. There is a disparity of results obtained when using different solvents at different concentrations, which can be observed in the next few lines. Deng et al. (2014) observed that the extractions made with 70% acetone and with 70% methanol yielded more phenolic compounds from blueberry than 95% ethanol. Tomsone et al. (2012) when extracting the phenolic compounds present in horseradish (*Armoracia rusticana*), verified that 95% ethanol showed a higher yield than 100% acetone, whilst Zhao and Hall III (2008) obtained better results using ethanol to extract the phenolic compounds from raisins than when using acetone and Rusak et al. (2008) obtained a higher phenolic compound content from green tea when using an aqueous extraction.

Other authors have previously quantified the phenolic compounds present in different JSP extracts. Lage et al. (2014) identified, in increasing order of concentration, the following compounds in a methanolic JSP extract from the genotype Sabará: epicatechin, salicylic acid, ellagic acid, gallic acid and gallocatechin. Alves et al. (2014), working under the same conditions as this work (including genotype and methodologies), identified the following phenolic compounds in an acetone JSP extract

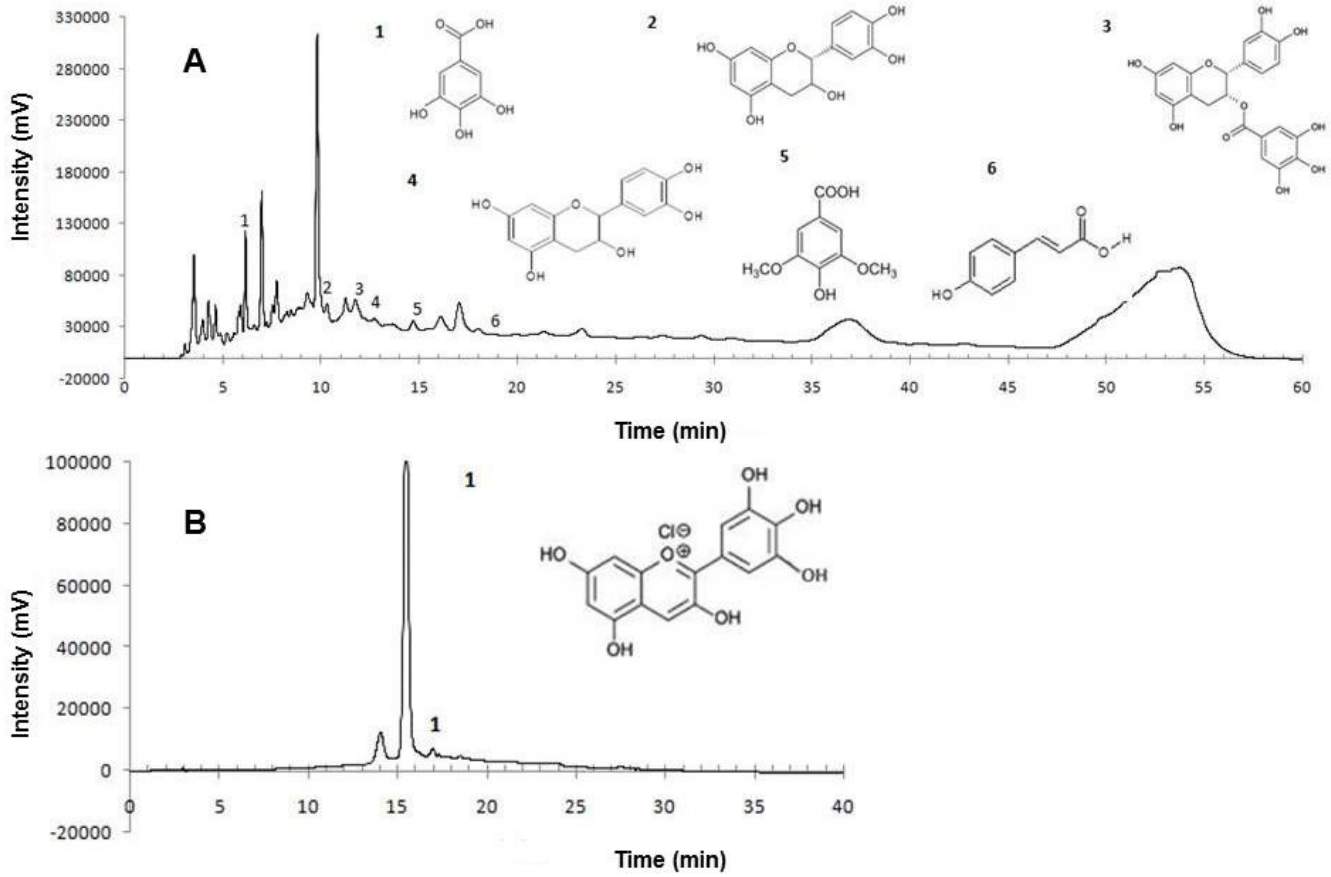

Figure 2. (A) Chromatogram of the aqueous jabuticaba skin extract, with the peaks identified and the corresponding phenolic compounds: 1 - gallic acid (time = 6.541); 2 - catechin (time = 10.419); 3 - epicatechin gallate (time = 12.154); 4 - epicatechin (time = 13.750); 5 - siringic acid (time = 15.034); 6 - *o*-cumaric acid (time = 16.048). (B) Chromatogram of the anthocyanins in the aqueous jabuticaba skin extract, with one peak identified: 1 - delphinidin chloride (time = 17.345).

(in mg 100 g^{-1} of extract): gallocatechin (27.20), ellagic acid (35.44), catechin (50.46), gallic acid (52.00), salicylic acid (133.44) and epicatechin (145.47). However, neither Alves et al. (2014) nor Lage et al. (2014) use the standards delphinidin chloride, cyanidin chloride or malvidin chloride. In addition, in the present work, the contents observed were significantly different, probably due to the harvest season and environmental conditions, besides the standards used in identification.

As explained above and from the data in the literature, the quantification of phenolic compounds is bound to the nature of the material (i.e. roots, leaves, bark or flowers), environmental conditions, postharvest processing and extraction methods (GURJAR et al., 2012; MOULEHI et al., 2012). Thus, the difficulty of proposing a single extraction method for all kinds of material, with a good yield, becomes clear.

The results showed that JSP contains phenolic compounds of interest that may offer *in vivo* protection against oxidative stress, DNA damage and cancer, and also be effective against metabolic diseases, such as obesity-induced oxidative stress (LEITE-LEGATTI et al., 2012; PLAZA et al., 2016). According to Plaza et al. (2016) several phenolic compounds, such as anthocyanins, tannins and flavonoids, were identified in jabuticaba skins, supporting the results of the present work.

Therefore the chemical characterization of such by-products is of great economic interest, adding value to the wastes and targeting their possible applications, especially, in the food and pharmaceutical industries.

3.2 Antibacterial activity

The minimum concentration necessary to induce an inhibitory (MIC) effect on bacterial growth was first determined, and then the inhibitory halos observed for each concentration of each extract were measured. The highest dose used was 250 μg mL^{-1}. Higher doses were not considered feasible due to the difficulty of dissolving

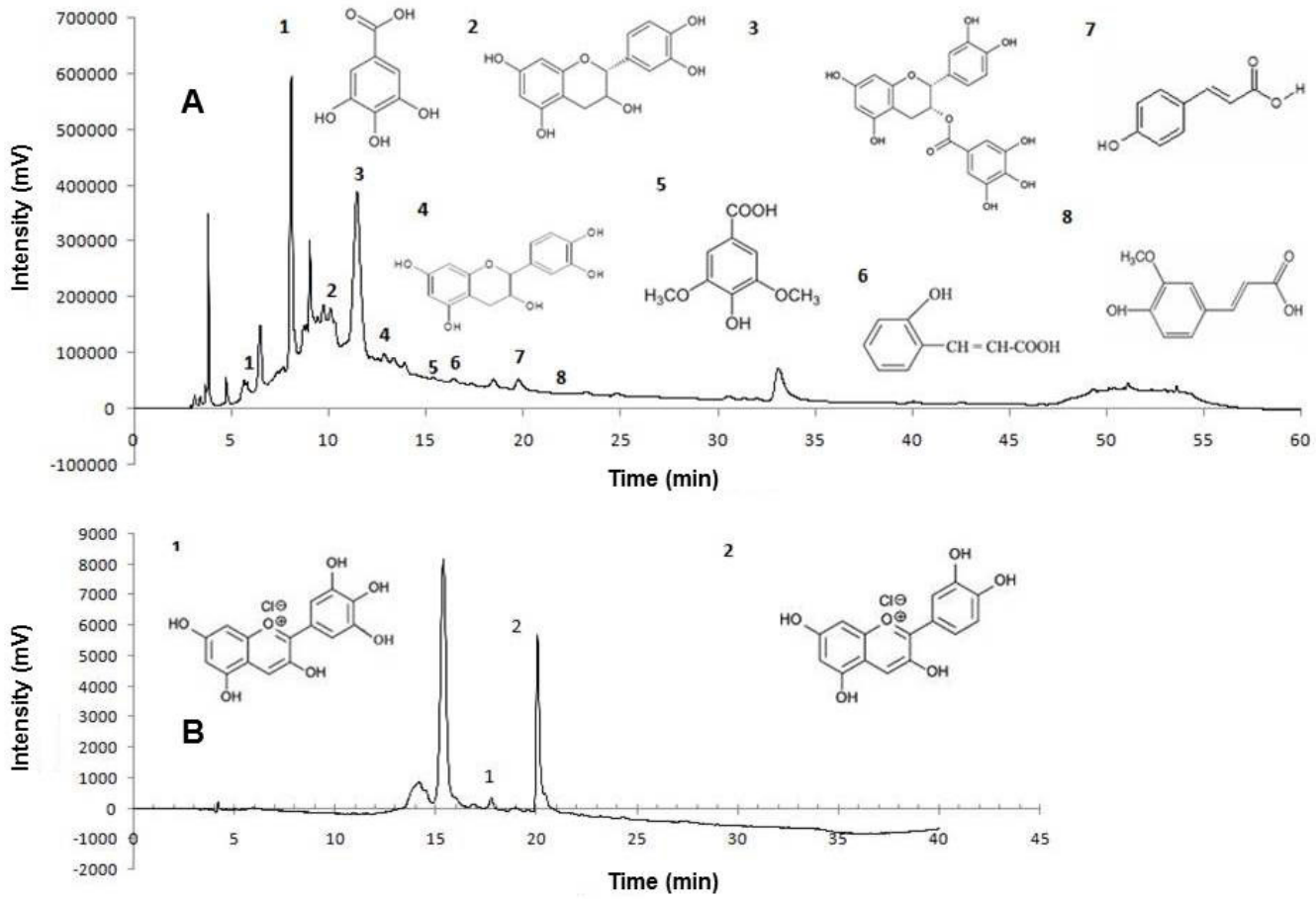

Figure 3. (A) Chromatogram of the ethanolic jabuticaba skin extract, with the peaks identified and the corresponding phenolic compounds: 1 - gallic acid (time = 6.541); 2 - catechin (time = 10.419); 3 - epicatechin gallate (time = 12.154); 4 - epicatechin (time = 13.750); 5 - siringic acid (time = 15.034); 6 - *o*-cumaric acid (time = 16.048); 7 - *p*-cumaric acid (time = 19.889); 8 - ferulic acid (time = 22.609). (B) Chromatogram of the anthocyanins in the ethanolic jabuticaba skin extract, with the peaks identified: 1 - delphinidin chloride (time = 17.345); 2 - cyanidin chloride (time = 21.318).

the material and the intense colour they would display, which could impair the reading of the results.

None of the extracts tested were effective in inhibiting the growth of the gram negative microorganisms *Escherichia coli* (ATCC 11229) and *Salmonella choleraesuis* (ATCC 6539). However, for *Pseudomonas aeruginosa* ATCC 15442 (gram negative), the MICs for all extracts were 250 μg mL^{-1} with inhibitory halos of 6.3 mm, representing an antimicrobial effects of only 30% when compared to chloramphenicol, for which the inhibitory halos were 21 mm.

The acetone extract only inhibited the growth of *S. aureus* (Table 2) with the highest concentration (250 μg mL^{-1}). However, the aqueous and methanolic extracts were able to inhibit growth of this microorganism in doses of 62.5 μg mL^{-1} and above, while the ethanolic extract was effective in doses of 31.25 μg mL^{-1} and above. As expected, the ethanolic extract showed the greatest inhibitory effect, inhibiting 41.8% of the bacterial growth, when compared to chloramphenicol. The second on the list was the methanolic extract, inhibiting 36%, followed by the aqueous extract with 26.7% inhibition and in last place the acetone extract, with 25.2%. Only the smallest dose of 15.62 μg mL^{-1} was ineffective against bacterial growth.

The inhibitions were significantly higher for *L. monocytogenes* (Table 2) than for *S. aureus*. The acetone extract was effective at a concentration of 125 μg mL^{-1} and above, while the aqueous and ethanolic extracts were effective in doses equal to or above 62.5 μg mL^{-1}. The methanolic extract was able to inhibit bacterial growth at doses equal to or above 31.25 μg mL^{-1}. At its highest dose (250 μg mL^{-1}) the methanolic extract was able to inhibit 64.8% of the bacterial growth when compared to chloramphenicol, followed by the ethanolic extract with 57.4% of inhibition. The highest inhibition observed by the aqueous extract was 38.8%, and finally in last place, the acetone extract, with 37.0% of inhibition.

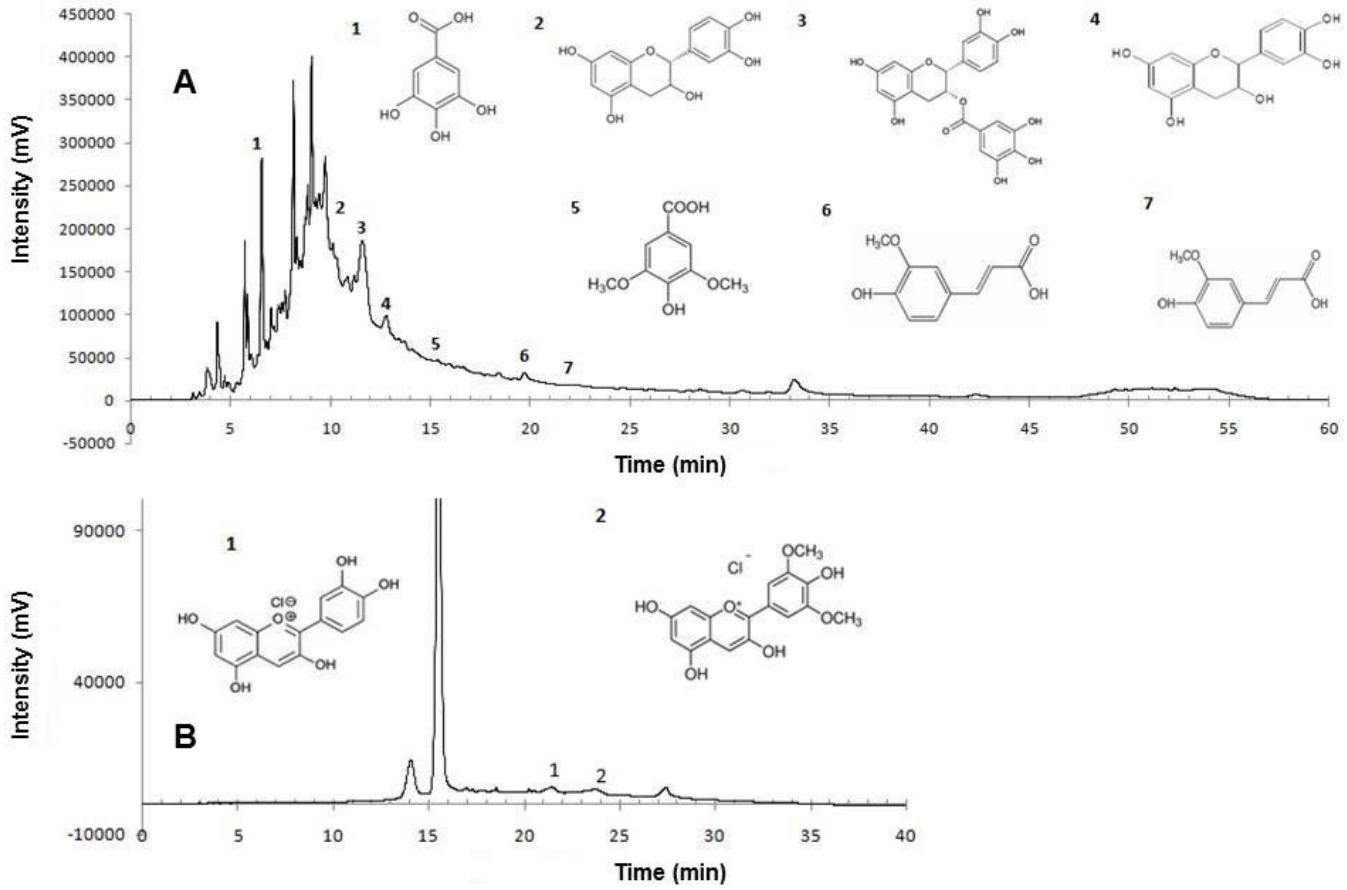

Figure 4. (A) Chromatogram of the methanolic jabuticaba skin extract, with the peaks identified and the corresponding phenolic compounds: 1 - gallic acid (time = 6.541); 2 - catechin (time = 10.419); 3 - epicatechin gallate (time = 12.154); 4 - epicatechin (time = 13.750); 5 - siringic acid (time = 15.034); 6 - *p*-cumaric acid (time = 19.889); 7 - ferulic acid (time = 22.609). (B) Chromatogram of the anthocyanins in the methanolic jabuticaba skin extract, with the peaks identified: 1 - cyanidin chloride (time = 21.318); 2 - malvidin chloride (time = 24.344).

Table 2. Inhibition halo diameter for *Staphylococcus aureus* and *Listeria monocytogenes* (mm)* using different jabuticaba skin extracts.

Extracts	*Staphylococcus aureus*				
	Concentration in μg mL^{-1}				
	250	125	62.5	31.25	15.62
Acetone	6.8 ± 0.57 ^{aC}	0.0 ± 0.00 ^{bC}	0.0 ± 0.00 ^{bC}	0.0 ± 0.0 ^{bB}	0.0 ± 0.0 ^{bA}
Aqueous	7.2 ± 0.7 ^{aC}	5.5 ± 0.86 ^{bB}	4.8 ± 0.58 ^{bB}	0.0 ± 0.0 ^{cB}	0.0 ± 0.0 ^{cA}
Ethanolic	11.2 ± 0.7 ^{aA}	8.3 ± 1.52 ^{bA}	6.7 ± 0.57 ^{cA}	5.0 ± 0.0 ^{dA}	0.0 ± 0.0 ^{eA}
Methanolic	9.7 ± 0.29 ^{aB}	7.3 ± 0.29 ^{bA}	6.2 ± 0.58 ^{bA}	0.0 ± 0.0 ^{cB}	0.0 ± 0.0 ^{cA}
Extracts	***Listeria monocytogenes***				
	Concentration in μg mL^{-1}				
	250	125	62.5	31.25	15.62
Acetone	10.0 ± 0.5 ^{aC}	7.0 ± 0.5 ^{bD}	0.0 ± 0.0 ^{cC}	0.0 ± 0.0 ^{cB}	0.0 ± 0.0 ^{c}
Aqueous	10.5 ± 0.5 ^{aC}	8.5 ± 0.5 ^{bC}	7.2 ± 0.3 ^{cB}	0.0 ± 0.0 ^{dB}	0.0 ± 0.0 ^{d}
Ethanolic	15.5 ± 0.5 ^{aB}	11.5 ± 1.0 ^{bB}	8.0 ± 0.0 ^{cAB}	0.0 ± 0.0 ^{dB}	0.0 ± 0.0 ^{d}
Methanolic	17.5 ± 0.5 ^{aA}	13.8 ± 1.6 ^{bA}	9.2 ± 0.3 ^{cA}	7.2 ± 0.3 ^{dA}	0.0 ± 0.0 ^{e}

*Data from three replicates ± standard deviation. Small case letters in the same line compare the inhibitory effect of an extract in different doses. Upper case letters in the same column compare the inhibitory effect of different extracts with the same dose. Means followed by the same letter do not differ by Tukey's test at 5% of probability. Positive control: Chloramphenicol (100 μg mL^{-1}), inhibitory halo = 21 ± 1.1 mm.

No data was found in the literature about a single criterion to evaluate the antimicrobial efficiency of vegetable extracts. However, according to Mothana and Lindequist (2005), extracts producing inhibition halos ranging from 8 to 13 mm are considered moderately inhibitory, while halos ranging from 14 and above are considered highly inhibitory. Based on this classification, the ethanolic extract was considered moderately active against *S. aureus* at concentrations of 250 μg mL^{-1} and 125 μg mL^{-1}, while the methanolic extract was only considered moderately active at the highest dose of 250 μg mL^{-1}. For *L. monocytogenes*, both the methanolic and ethanolic extracts were considered highly active at 250 μg mL^{-1} and moderately active at 125 and 62.5 μg mL^{-1}. The aqueous extract was considered moderately active at concentrations of 250 and 125 μg mL^{-1} while the acetone extract was considered moderately active at 250 μg mL^{-1}.

Another view concerning the antibacterial potential of these compounds was proposed by Holetz et al. (2002). They classified the antibacterial activity according to the presence of an inhibition halo. When present with concentrations below 100 μg mL^{-1}, the extract was considered highly active; at concentrations between 100 and 500 μg mL^{-1}, it was considered moderately active; and between 500 and 1000 μg mL^{-1} the extracts was considered weakly active; concentrations above this limit being considered inactive. Thus considering this classification, the aqueous, methanolic and ethanolic extracts were highly active for *S. aureus* and *L. monocytogenes*, and moderately active for *P. aeruginosa*.

Gram-negative bacteria are notably more resistant against antimicrobial agents than gram-positive ones. This fact is justified by the more complex nature of the cell wall, due to the presence of a lipid barrier, which makes it difficult for many antimicrobial agents, including the plant extracts used in this work, to enter and act (GUIMARÃES et al., 2010). Due to the lack of this important barrier, gram-positive bacteria are more susceptible to the mechanisms of action displayed by the different JSP extracts (RABÊLO et al., 2014).

Many studies relate the antimicrobial action found in vegetable extracts to their phenolic compound contents, as well as to their composition (AL-HABIB et al., 2010; KUMAR et al., 2011; MARTINS, 2011). Phenolic compounds can act in many ways, impairing the functioning of bacterial cells. For example, phenolic compounds can impair enzyme action, either by complexing with their substrates or bonding directly with the enzyme; another mechanism is complexation with the metallic ions essential for many metabolic processes in the cell; and also the modification of metabolic routes by intercepting or donating electrons and modifying or inactivating metabolic intermediates (HAVSTEEN, 2002). Tannins and flavonoids possess similar properties to phenolic compounds, and can both inactivate enzymes and form complexes with extracellular soluble proteins and with the bacterial cell wall, which are probably the action mechanisms that occurred in the present work (MENDES et al., 2011). Together, such information permits us to suggest that phenolic compounds are the agents responsible for the inhibitions observed, since the ethanolic and methanolic extracts showed the greatest inhibition of bacterial growth, and in turn, these same extracts are those with the highest phenolic compound contents.

It is worth mentioning that the phenolic compound content in 10 μL of the ethanolic extract at 250 μg mL^{-1}, used to inhibit bacterial activity, was 7.87×10^{-6} μg, which is very low in comparison with the quantity of chloramphenicol (100 μg mL^{-1}) in 10 μL, which was 1 μg. Therefore, it could be suggested that at higher concentrations, the phenolic compounds found in the extracts might exhibit greater antibacterial action.

4 Conclusion

The ethanolic and methanolic extracts of JSP had the highest contents of phenolic compounds, especially cyanidin chloride, catechin and epicatechin. They also possessed good antibacterial activity, being more effective against *Staphylococcus aureus* and *Listeria monocytogenes*. Taking into account the need for natural conservatives for the food and drugs industries, and the growing trend to aggregate value to residues, research into the use of jabuticaba skin extracts is an interesting work material. Besides, in the future, the use of conservatives from natural sources, such as phenolic compounds from JSP, could attract the attention of consumers searching for healthier products. However, more research is necessary to assess its safety for the food and drugs industries.

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