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Prospecção química e potencial biológico de extratos de resíduos de frutas tropicais

Chemical prospection and biological potential of tropical fruit waste extracts

Prospección química y potencial biológico de extractos de residuos de frutas tropicales

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Resumo

Os resíduos agroindustriais de frutas podem apresentar uma fonte promissora para os setores industriais, com a extração de princípios ativos com diversas atividades biológicas. Diante do exposto o presente trabalho teve como objetivo avaliar a potencialidade biológica dos extratos de frutos tropicais frente as atividades antioxidantes e amnmicrobianas. O estudo avaliou diferentes métodos de atividades antioxidantes (pelos métodos DPPH e FRAP), caracterização e quantificação de compostos fenólicos por cromatografia líquida de alta eficiência (HPLC), atividades antibacteriana e antifúngica de quatro extratos de resíduos de frutas tropicais. Nos extratos foram identificados vários compostos fenólicos, os valores encontrados variaram de 0,21 a 5,73 mg/L em ácido gálico, observando a predominância de ácido gálico, catequina, clorogênico e ácido p-cumárico. A atividade antibacteriana foi avaliada pelo método de difusão em ágar usando os microrganismos *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa e Salmonella cholerasuis*. A atividade antifúngica foi determinada pela técnica de difusão em disco utilizando *Aspergillus carbonarius, Aspergillus niger, Aspergillus flavus, Penicillium commune* e *Penicillium cladosporoides*; os extratos apresentaram potencial atividade antioxidante, antibacteriana e antifúngica nos dois testes. O extrato de limão não mostrou inibição para os fungos. .

Palavras-chave: Antixiodante; Cromatografia; Compostos fenólicos; Antimicrobianos.

Abstract

Agro-industries waste from fruits can present a promising source for industrial sectors with the extraction of active principles that have several biological activities. In view of the above, this study aimed to evaluate the biological potential of tropical fruit extracts against antioxidant and amnmicrobial activities. The study evaluated different methods of antioxidant activities (by DPPH and FRAP method), characterization and quantification of phenolic compounds by High Performance Liquid Chromatography (HPLC), antibacterial and antifungal activities of four tropical fruit waste extract. In the extracts were identified several phenolic compounds, the values found were varied from 0.21 to 5.73 mg/L in gallic acid, observing the predominance of gallic acid, catechin, chlorogenic and acid p-coumaric. The antibacterial activity was evaluated by the agar diffusion method using the microorganism *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella cholerasuis*. The antifungal activity was determined by the disc diffusion technique using *Aspergillus carbonarius, Aspergillus niger, Aspergillus flavus, Penicillium*

commune and *Penicillium cladosporoide*s, the extracts showed potential antioxidant activity, antibacterial and antifungal in both tests. The lemon extract showed no inhibition for the fungi.

Keywords: Antioxidant; Chromatography; Phenolic compounds; Antimicrobials.

Resumen

Los residuos agroindustriales de las frutas pueden presentar una fuente prometedora para los sectores industriales, con la extracción de ingredientes activos con diferentes actividades biológicas. En vista de lo anterior, el presente estudio tuvo como objetivo evaluar el potencial biológico de los extractos de frutas tropicales contra las actividades antioxidantes y amnmicrobianas. El estudio evaluó diferentes métodos de actividades antioxidantes (mediante los métodos DPPH y FRAP), caracterización y cuantificación de compuestos fenólicos por cromatografía líquida de alta resolución (HPLC), actividades antibacterianas y antifúngicas de cuatro extractos de residuos de frutas tropicales. En los extractos se identificaron varios compuestos fenólicos, los valores encontrados variaron de 0.21 a 5.73 mg/L en ácido gálico, observando el predominio de ácido gálico, catequina, ácido clorogénico y ácido p-cumárico. La actividad antibacteriana se evaluó mediante el método de difusión en agar utilizando los microorganismos *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* y *Salmonella cholerasuis*. La actividad antifúngica se determinó mediante la técnica de difusión en disco utilizando *Aspergillus carbonarius, Aspergillus niger, Aspergillus flavus, Penicillium commune* y *Penicillium cladosporoides*; los extractos mostraron actividad antioxidante, antibacteriana y antifúngica potencial en ambas pruebas. El extracto de limón no mostró inhibición para hongos.

Palabras clave: Antioxidante; Cromatografía; Compuestos fenólicos; Antimicrobianos.

1. Introduction

Brazil has a great plant collection with extensive and diversified flora because it has a varied climate and also because it allows the adaptation of plant species from almost all regions of the planet. Brazil is the third largest producer of fruit with an annual production of over 43.5 million tons, per year (Anuário, 2018). About 53% of Brazilian production is destined to the processed fruit market and 47% to the fresh fruit market (Silva et al., 2015).

The processing of these fruits, generate high volumes of by-products and waste that are potentially rich in substances of high nutritional and functional value, including

carbohydrates, proteins, lipids and, in particular, bioactive compounds (Esparza et al., 2020). Among the bioactive substances present in fruits in their wastes, as in their residues, especially phenolic compounds (Tavares et al., 2017; Tavares et al., 2019). These compounds have been extensively investigated and gaining great prominence for their functional characteristics, i.e., antioxidant and antimicrobial, activities (Banerjee et al., 2017; Maqsood et al., 2020; Tavares et al., 2017).

A food industry strategy has been the incorporation of these compounds in processed foods, in the form of extracts (Esparza et al., 2020; Banerjee et al., 2017; Maqsood et al., 2020; Tavares et al., 2019). Thus, the functional properties of these compounds are transferred to food, enriching them. However, it is necessary to know the composition of the phenolic compounds and their antioxidant and antimicrobial potential of the extracts obtained from these residues to evaluate possible applications, especially of tropical fruits.

In this context the use of fruit wastes presents very promising for extraction of active principle with different beneficial biological activities, being able to be used as antioxidants and antibacterial due to the expressive quantity of phenolic compounds that however these compounds have not yet been identified, mainly waste that are discarded. The objective of the present work was to evaluate the antioxidant capacities using different methods, to evaluate the antibacterial and antifungal activities of different tropical fruit extracts from the Southwest region of Bahia (cajá, tamarind, jenipapo and lemon Citrus), to determine and quantify the phenolic compounds by high performance liquid chromatography (HPLC) find a possible alternative to the food industry as well as add value to the fruit.

2. Material and Methods

The fruits were obtained at the market in the city of Itapetinga, in the state of Bahia - BA, in July 2017, with Spondias mombin cajá in bark and seed, Tamarindus indica in bark and seed, American genipap genipap in bagasse and lemon Citrus latifolia using the peel of the fruit, these residues were discarded by the industry or by the population, therefore the fruits had a large amount of biotive compounds in the pulp itself, being, therefore, the need to study their residues for a possible application in food in order to guarantee the food safety and quality.

Obtaining the crude extract

The fruits were hygienized and peeled, pulp and seed separated, and dehydrated at 50 ° C in a forced air circulation oven. Then shells, seeds and bagasse were pulverized in a knife mill (Wiley type). The chemical compounds were extracted with ethanol (3:1) for 72 h at room temperature (25ºC). The mixture was filtered and the solvent was evaporated at a controlled temperature of 60°C with the aid of a rotary evaporator.

Evaluation of antioxidant activity

DPPH (1,1-Diphenyl-2-picrylhydrazyl)

The antioxidant activity was evaluated by the sequestering ability of the extracts to capture the 1,1-diphenyl-2-picrylhydrazine radicals (DPPH) according to the methodology described by Sousa et al. (2007), modified by Guimarães et al. (2011). A methanolic solution of DPPH was prepared at the concentration of 40 μg.mL^{-1}. In a test tube 2,7 mL of DPPH stock solution was added, followed by the addition of 0,3 mL of each dilution of the extract in ethanol (750; 500; 250; 200; 150; 100; 50 μg.mL^{-1}). In parallel, the control was prepared containing all the reagents except the extract. Afterwards a 60 minute reading was performed on a spectrophotometer at a wavelength of 515 nm, and the percentage of antioxidant activity was calculated using equation 1:

$$\%AA = [1- (A_{am}/A_{cont})] \times 100$$

On what;

A_{am}: absorbance of the analyzed sample;

A_{cont}: absorbance of the control.

For comparison purposes the BHT standard was tested. The analytical curve was constructed using the same concentrations of the extract.

Determination of antioxidant activity by iron reduction method (FRAP)

The methodology of reducing iron ions was performed as described by Rufino et al. (2006). The antioxidant assay for iron ion reduction (FRAP) determination is based on Fe^{2+} ion production from the reduction of the Fe^{3+} ion present in the 2,4,6-tripyridyl-s-triazine

complex (TPTZ). When the reduction occurs there is a change in the hue of the reaction mixture from light purple to intense purple whose absorbance can be measured at the wavelength of 595 nm. The higher the absorbance or the intensity of the staining the greater the antioxidant potential. In the dark environment, a 90 µL aliquot of each dilution of the extracts was added to the test tubes, 270 µL of distilled water was added, mixed with 2.7 mL of the FRAP reagent, homogenized on a tube shaker and kept in a water bath at 37°C. The reading (595 nm) was performed after 30 minutes of the prepared mixture and the FRAP reagent was used as blank to calibrate the spectrophotometer. The standard curve was constructed with ferrous sulfate solutions at concentrations of (750; 500; 250; 200; 150; 100; 50 $\mu g.mL^{-1}$). From the equation of the line ($y = 0.000637x + 0.029333$, $R^2 = 0.999104$) obtained by the standard curve the calculation was performed and the results were expressed as mg Fe^{2+}/g extract.

Determination of phenolic compounds

The spectrophotometric determination of the phenolic compounds was performed according to (Lin et al, 2005) using the Folin-Ciocalteu reagent the calibration curve was obtained using five dilutions of gallic acid (100-50-25- 12,5 - 6,25 mg/L) The samples under analysis were subjected to the same procedure. 1 mL was taken in the test tube for each dilution and 5 mL of the Folin Ciocalteau reagent (10%) was added. The solution was homogenized and, after 8 min, 4 mL of saturated sodium carbonate solution (Na_2CO_3) was added. After 1 hour of rest, the spectrophotometer absorbance readings were taken in triplicate at 773 nm. The blank was run under the same conditions, replacing the sample with distilled water. From the equation of the line ($y = 0.010578x + 0.003586$, $R^2 = 0.999891$) obtained by the calibration curve, the total phenolic content, expressed as mg of gallic acid / 100g of sample.

Chromatographic analysis of fruit extracts

The phenolic compounds were quantified by high performance liquid chromatography by the external standardization method. The analytical curves were performed by dilutions of stock solution ($1x10^{-3}$ mol L^{-1}) containing a mixture of all standards, as described in Table 1.

Table 1- High Efficiency Liquid Chromatograph (HPLC) conditions for the analysis of phenolic compounds.

Time (min)	Solvent A (% v/v)	Solvent B (% v/v)	Wavelength (nm)
0,01	100	0	280
25,00	60	40	280
40,00	45	55	280
43,00	40	60	280
50,00	0	100	280
55,00	100	0	280

Solvent A: water: acetic acid, 2% in water (v/v).
Solvent B: methanol: water: acetic acid, 70:28:2% (v/v).
Source: Authors.

Each standard was diluted in 50% ethanol solution. The analytical curves were obtained by linear regression and the coefficient of determination (R2) was 0.9900 (Aquino et al., 2006).

Samples and standards were filtered on a 0.45 μm polyethylene membrane and directly injected into the chromatographic system. Injections were performed in duplicate and the identity of the analytes was confirmed by the retention times of the samples, compared to the standards. The elution solvents used were: 2% acetic acid solution in water (step A), solvent A and methanol: water: acetic acid (70:28:2% v/v) (step B) solvent B. The wavelength used was 280 nm, the flow of 1.25 mL/ min^{-1}, injection volume of 20 μL and run time of 60 min, performed at a temperature of 40°C (Aquino et al. 2006). Identification of the compounds was performed by comparing the retention time of the samples against the standards. For the construction of the analytical curve, a mixture of all the standards was made by dilutions previously prepared, obtaining the following concentrations: gallic acid (6,80 mg/L), catechin (11,61 mg/L), ferulic acid (5,33 mg/L), vanillic acid (6,73 mg/L), phenol (3,76 mg/L), 2-chlorophenol (4,70 mg/L), syringic acid (7,93 mg/L), chlorogenic (7,60 mg/L), hydroquinone (6,93 mg/L), p-hydroxybenzoic acid (7,43 mg/L), trans-cinnamic acid (6,50 mg/L), malic acid (7,80 mg/L), cinnamic acid (5,20 mg/L), rosmarinic acid (6,75 mg/L), synapic acid (8,97 mg/L), caffeic acid (8,90 mg/L), 4-ethyl-catechol (6,74 mg/L), resorcinol (8,10 mg/L), eugenol (8,23 mg/L), vanillin (6,08 mg/L), syringaldehyde (7,29 mg/L), 4-methylubeliferone (7,05 mg/L), p-coumaric acid (6,56 mg/L), p-coumaric acid (6,56 mg/L), coumarin (5,85 mg/L), 4-methylumbelliferone (7,05 mg/L) and o-coumaric acid (6,56 mg/L). (Aquino et al., 2006,).

Analysis of the phenolic compounds were performed by chromatography using a Shimadzu HPLC high performance liquid chromatograph containing two (LC-6AD model) high pressure pumps, with a SPD-M20A diode array detector (DAD), a DGU-20°C degasser, a CBM-20A model interface and an automatic injector with an self-sampler (model SIL-10AF). Separations were performed using an Agilent-Zorbax Eclipse XDB C18 (4.6 x 250 mm, 5 µm with 5 µm spherical particles) column connected to a pre-column: Agilent-Zorbax Eclipse XDB-C18 4-Pack (12, 5 x 4.6 mm, 5µm).

Biological activity phenolic extracts of fruit.

The evaluation of the antibacterial activity of the phenolic extracts was carried out with the following strains of bacteria, *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 13565, *Salmonella choleraesuis* ATCC 6539, *Listeria monocytogenes* ATCC 19117 and *Pseudomonas aeruginosa* ATCC 15442. During the experiment the microorganisms were kept in eppendorfs containing freezing medium under refrigeration (4°C). For culture activation the strains were picked in broth-infusion brain and heart (BHI) and incubated at 37°C for 24 hours. After activation, the plating was carried out in media specific to each species.

Cultures of *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 13565, *Salmonella cholerasuis* ATCC 6539, *Listeria monocytogenes* ATCC 19117 and *Pseudomonas aeruginosa* ATCC 15442 were standardized using the McFarland scale of 0.5 BaSO4. The active culture was peaked into brain infusion broth (BHI) and incubated at 37°C for 24 hours. Subsequently, a 300 µL aliquot was transferred to Triptic Soy broth (TSB) and incubated again under the same conditions, being monitored every half-hour increase in inoculum number by spectrophotometer with optical density at 625 nm, according to the McFarland scale of 0.5 $BaSO_4$, until the concentration of 108 CFU mL^{-1}. This was diluted until it reached a concentration of 10^6 UFU mL^{-1}. Afterwards, the plating was performed in TSA Agar (Tryptic Soy Agar) for the species Listeria monocytogenes, Pseudomonas aeruginosa and for the other species was used Müller-Hinton Agar, for confirmation of inoculum concentration (National commitee for clinical laboratory stantards - NCCLS, 2003).

The inhibitory effect of extracts was determined using agar diffusion and diffusion by vapor methodologies. In this analysis, the methodology used was the cavity diffusion in Agar, using TSA Agar (Triptic Soy Agar) for the species *Listeria monocytogenes* and *Pseudomonas aeruginosa* and for the other species, Müeller-Hinton Agar (Pereira et al., 2008).

Initially, a thin layer of agar was added to Petri dishes (140 mm diameter). After solidification, sterile glass beads 4 mm in diameter were positioned on the solid medium. Standardized aliquots of *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 13565, *Salmonella cholerasuis* ATCC 6539, *Listeria monocytogenes* ATCC 19117 and *Pseudomonas aeruginosa* ATCC 15442, were transferred to erlenmeyers containing 200 mL of Müeller-Hinton or TSA agar, obtaining a concentration of 106 UFU mL^{-1}. The still liquid medium was poured over the anterior layer. After solidification of the agar the glass pearls were removed with sterile tweezers, and then the extracts were deposited in the wells formed. Dilutions were carried out in dimethyl sulfoxide solution (DMSO) using the following proportions: pure extract, 1:1; 1:2; 1:4; 1:8; 1:16, equivalent to the respective concentrations 1000 (pure extract) 500; 250; 125; 62,5; 31,25µL mL^{-1}. 10 µl of the diluted extracts were applied to the wells. For each extract used the control consisted of 1% DMSO solution. The plates were incubated in BOD 37°C for 24 hours and the diameters of the inhibition halos formed were measured. Three replicates were performed for each treatment, one relative control with the application of 10 µL of DMSO; as a standard of comparison, was used a solution of 100 µg mL^{-1} of the antibiotic chloramphenicol (CL) (Ogunwande et al., 2005).

From the diameters formed by the action of the extracts that showed the sensitivity of the microorganism, the sensitivity profile of the bacteria in different concentrations of the extracts can be evaluated. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of extract in which the presence of inhibition halo occurred.

Antifungal Activity

The inhibitory effect of filamentous fungi was verified using the disc diffusion test, accepted by the Food and Drug Administration (FDA) and established by the National Committee for Clinical Laboratory Standards (NCCLS) (2013). Initially, the fungi were identified for their species and were picked in Agar Extract Malt (MEA). For this, an inoculum at the concentration of 10^7 spores/mL was used, with count Newbauer's chambers. The inoculum was transferred to the plate containing Extract Malt Agar (MEA) medium by the surface scattering technique. Filter paper discs of 5 mm diameter soaked with 10 µL of extracts or essential oil at the concentrations of 1000 (pure extract) (extract only), 500, 250, 125, 62,5, 31,25, 15,62, 7,81 µg/mL^{-1} were placed on the culture medium, as suggested by Karaman et al. (2003). The negative control was performed by means of disks containing 10

µL of DMSO (dimethylsulfoxide) and a control fungicide (sodium hypochlorite 2%) was used for the positive control. The plates were incubated in BOD at 25°C for a period of 72 hours. The evaluation was comparative following a reference biological standard (positive control) and the zone or halo of growth inhibition was measured starting from the circumference of the disc, to the margin where the fungus was growing, orthogonal measurements were made of the diameter, each measurement corresponding to the mean of two diametrically opposite measurements. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of extract in which the presence of inhibition halo was identified. (Zacaroni et al., 2011).

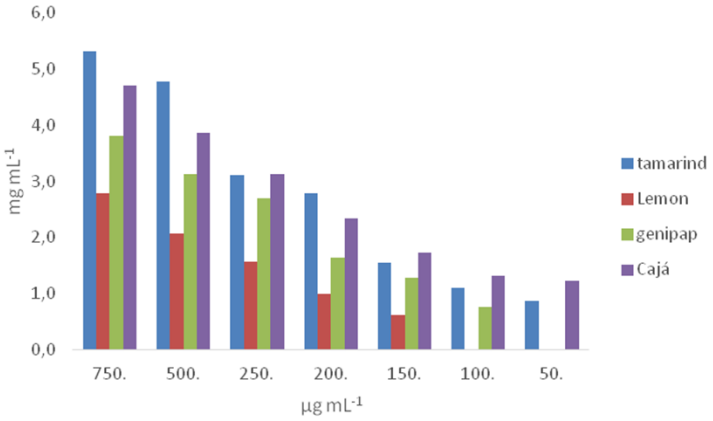
Statistical analyzes

The data obtained from the physico-chemical analyzes were submitted to analysis of variance, and the means were compared by the Scoot-Knott test at the 95% confidence level used by the SISVAR statistical program (Ferreira, 2013). Then, through the analysis of the main components (PCA) the averages of the phenolic compounds were distributed in a biplot plot.

3. Results and Discussions

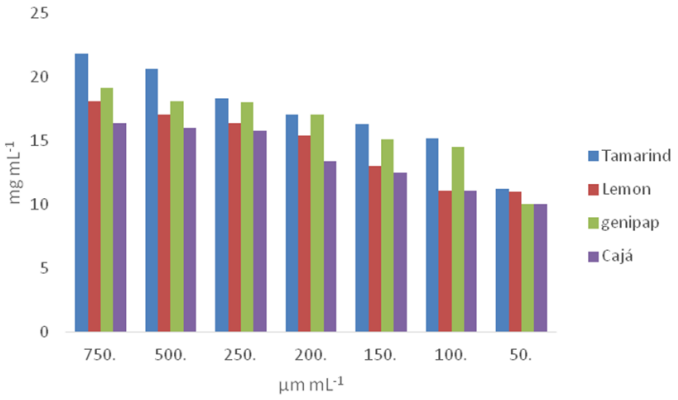
The results of antioxidant activity (%AA) by the method of inhibiting DPPH and FRAP radicals of phenolic extracts at concentrations 750; 500; 250, 200, 150, 100, 50 µg/mL^{-1} are shown in Figures 1 and 2. We can observe that in higher concentrations, the anti-toxicity activity of the two methods used is greater. In Figure 1, the tamarind extract showed good activity compared to the DPPH method tested even at low concentrations, and the other extracts also showed significant values. only lemon extract did not show antioxidant activity in the last two concentrations. In Figure 2, for all extracts tested even at low and high concentrations, given the method used, they showed good activity, being a possible application in food to guarantee their quality.

Figure 1- Antioxidant activity DPPH of bark of tamarind, lemon, genipap and cajá extracts.



Source: Autor (2020).

Figure 2- Antioxidant activity FRAP of bark extracts of tamarind, lemon, genipap and cajá.



Source: Autor (2020).

According to the presented results, it is observed that the extracts of the fruits provided a good antioxidant activity for both tests. It is observed that the extracts provided a high antioxidant activity with considerable capacity to reduce the DPPH radical, except the lemon extract that presented a low activity even in high concentrations. The fact that the lemon extract did not present satisfactory antioxidant activity for the DPPH test can be justified due to the non-ease of donation of a hydrogen atom by the constituents present in the extracts under study to neutralize the DPPH radical.

Evaluating the antioxidant capacity of the extracts under study by the FRAP can be observed by the results presented in Figure 2 that there was a significant difference for the extracts tested in the concentrations 750; 500; 250, 200, 150, 100, 50 µg/mL^{-1} It was verified by analysis of variance that there was a significant difference between the extracts in concentrations of 250 and 150 µg/L^{-1}, except for the tamarind extract that did not present a variation for this test.

Considering that the tamarind extract presents a high amount of phenolic compounds in its chemical composition, it is observed that probably was what influenced the inhibition response to DPPH.

The antioxidant activity of phenolic compounds is attributed to the reducing power of the aromatic hydroxyl group, which reduces reactive free radicals and produces the radical phenoxylated stabilized by resonance.

Antioxidant capacity of phenolic structures is influenced by the number and position of hydroxyl groups, as well as by glycosylation positions that are particularly capable of binding iron and copper (Jung et al., 2003).

The difficulty in establishing a correlation between the antioxidant activity and its components in the case of alcoholic extracts is great due to its complex composition, besides the possible occurrence of synergism. In addition, there is a possible influence of other factors, such as concentration, storage conditions temperature, light, unit and the antioxidant assay used (Viuda-Martos et al., 2009).

According Silva et al., (2015) Plant extracts can be produced from all parts of the plant, such as root, stem, leaves, flowers, fruits or the whole plant., these byproducts were studied by some researchers who demonstrated the presence of several bioactive compounds such as flavonoids, tannins and aromatic compounds.

Several researches indicate that the antioxidant potential of several extracts obtained from fruit wastes from Brazil shows the possibility of a better use of agro-industrial wastes, adding value to the wastes and their products due to the importance of the antioxidants it is hoped that the studies carried out will serve as an incentive for the food industry to use natural antioxidants instead of synthetic ones (Infante et al., 2013, Melo et al., 2011; Sousa, Vieira e Lima, 2011). Therefore, certain knowledge should be taken with the application of more in-depth quantification and certification analyzes on the various compounds found in the vejetal kingdom. Because many can have great biological potential as antioxidant and antimicrobial activities, among others, and some can be toxic to the organimino when they are ingested. The correct thing would be a thorough characterization of the organic components before a possible application.

Quantification of phenolic compounds

In all the analyzed samples it is possible to observe the predominance of different phenolic compounds. Table 2 shows the values of the phenolic compounds of all fruit wastes

Table 2- Quantification of the phenolic compounds by High Performance Liquid Chromatography (HPLC) of the extracts of the fruits of the Brazilian cerrado: tamarind, lemon, genipap and cajá.

Phenolic Compounds	**Tamarind mg mL^{-1}**	Standard deviation	**Lemon mg mL^{-1}**	Standard deviation	**genipap mg mL^{-1}**	Standard deviation	**Cajá mg mL^{-1}**	Standard deviation
Catechin	5.734	±0,032	<LQ	±0,053	3,542	±0,053	1,233	±0,053
Ferulic acid	2.761	±0,012	<LQ	±0,015	0.765	±0,001	1.721	±0,001
Vanillin	1.007	±0,086	<LQ	±0,081	0.217	±0,008	<LQ	±0,008
Gallic acid	2.348	±0,027	5.223	±0,023	3.308	±0,023	5.567	±0,023
Chlorogenic	3.531	±0,043	<LQ	±0,001	<LQ	±0,000	0,876	±0,006
Hydroquinone	1.357	±0,052	0,654	±0,050	3.308	±0,050	1,098	±0,050
P-coumaric acid	3,702	±0,021	3,219	±0,022	3,702	±0,022	0,702	±0,022
Synaptic acid	1,021	±0,039	<LQ	±0,003	1,021	±0,003	<LQ	±0,003
P-hydroxybenzoic acid	0,746	±0,014	<LQ	±0,019	<LQ	±0,019	0,876	±0,019
M-coumaric acid	<LQ	±0,023	1,732	±0,032	<LQ	±0,032	<LQ	±0,032
Trans-cinnamic	<LQ	±0,095	<LQ	±0,008	1,079	±0,008	1,125	±0,008

Source: Autor (2020).

In the tamarind extract, the catechin (5.73 mg/L) and chlorogenic (3.53 mg/L) predominate; in lemon extract, gallic acid (5.22 mg/L) and p-coumaric acid (3.21 mg/L); in extract of genipap, catechin (3.54 mg/L) and gallic acid (3.30 mg/L); in the cajá, gallic acid (5.56 mg/L) and catechin (1.23 mg/L). These results are in agreement with those obtained by Souza et al, (2010). Analyzing different fruits of the cerrado were found differences as to the presence and concentration of phenolic compounds, observed that the main compounds found were syringaldehyde, catechin and gallic acid. The major compounds found in fruit pulps for juice processing were syringaldehyde, citric acid and gallic acid, which are probably present in several citrus fruits and also have antioxidant activity, results that corroborate with those found in this study.

One of the factors that interferes in the extraction of the phenolic compounds is the type of waste of the fruit used to the shell because it is a material of complex structure formed by different cell wall materials (cellulose, polioses and lignin), being able to present an extraction of the compounds of form varied (Mendes et al., 2011). Other methods that also interfere in the extraction of these bioactive compounds are the extraction method and also how is the state of conservation of the material, since some compounds may lose their activity during the extraction or at the time of extraction due to handling and storage conditions and obtaining.

Total phenolic compounds

The results obtained for the total phenolics concentration are shown in Table 3.

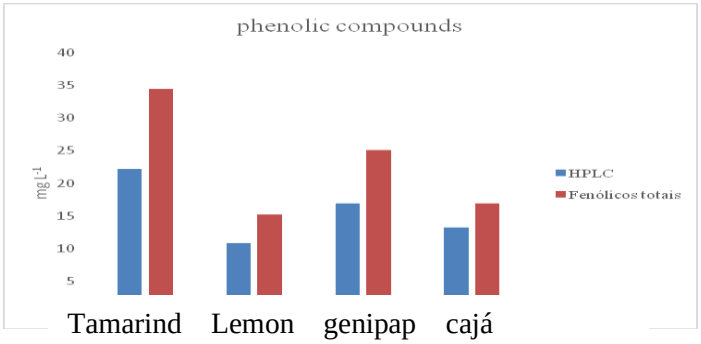
Table 3- Concentration of total phenolics of bark of Brazilian cerrado fruits: tamarind, lemon, genipap and cajá.

Total Phenolic Compounds	**Tamarind**	**Lemon**	**genipap**	**Cajá**
(mg/L^{-1})	34,492±0,053	15,280±0,043	25,111±0,023	16,957±0,039

Source: Autor (2020).

From the results obtained it can be verified that the concentrations of total phenolic compounds presented a great variation among the analyzed extracts. Tamarind extracts showed a higher extraction of phenolic compounds compared to the lemon extract that had a lower phenolic concentration (15.28 mg/L^{-1}). According to Lima (2001), phenolic compounds may vary according to fruit type, as well as environmental conditions, moisture content and temperature (Mendes et al., 2011). When evaluating the phenolic composition of Brazilian tropical fruits observed that the content of these compounds varied according to the climatic seasons of the year. Figure 3 shows the total phenolic compounds and the sum of phenolic compounds quantified in high performance liquid chromatography (HPLC) of the extracts.

Figure 3 Total phenolic compounds and the sum of phenolic compounds by HPLC of wastes from fruit extracts.



Source: Autor (2020).

Considering the results presented above, it is observed that the concentration of total phenolic compounds was higher in relation to the concentration of phenolic compounds

analyzed by HPLC. Such data can be explained by the presence of other compounds which are also present in the wastes as for example some aromatic esters are formed by the interconversion of the phenolic compounds such as ethyl syringate and ethyl vanillate and others, these compounds being also quantified by the analysis of total phenolic compounds yielding higher values (Vichi et al., 2007). In this case, other compounds can also be quantified by the analysis of total phenolic compounds, which are the stresses, aldehydes and others, which can have a synergistic function with the other compounds quantified by HPLC and can promote greater antioxidant and antimicrobial activity.

Inhibitory effect of extracts of fruit wastes on fungi Aspergillus flavus, Aspergillus carbonarius , Aspergillus niger, Penicillium comune e Penicillium cladosporoides.

The results of the inhibitory effects are shown in Table 4. The minimum inhibitory concentration (MIC) found when using the extracts of tamarind and jackfruit for the fungus *Penicillium comune* and *Aspergillus flauos* was 250 μg/mL^{-1} and for the lemon extract no inhibition was observed for fungi *Aspergillus flavus, Aspergillus carbonarius , Aspergillus niger Penicillium comune e Penicillium cladosporoides.*

Table 4- Minimum inhibitory concentration of tamarind, lemon, genipap and cajá extract for fungi *Aspergillus flauos, Aspergillus carbonarius , Aspergillus niger, Penicillium comune e Penicillium cladosporoides.*

	MIC (μg mL^{-1})		Inhibition halo (mm)		
Fungi	**Tamarind**	**Lemon**	**genipap**	**Jackfruit**	**CL**
A. flavus	250	NI	1000	500	100
A. carbonarius	500	NI	1000	NI	100
A.niger	NI	NI	NI	NI	100
P.cumune	100	NI	500	250	100
p. cladosporoides	50	NI	NI	500	100

*NI: no inhibition occurred, HP: Sodium hypochlorite. Source: Authors.

It was observed that the lemon extracts did not inhibit fungal growth, even at concentrations higher than 1000 mg/mL^{-1}, with the tamarind extract, an inhibition with 250 μg/mL^{-1} was observed for the fungus *Aspergillus flauos* and for the minimal inhibitory inhibition of. was observed at 1000 μg/mL^{-1} and *P. cladosporoides* at 500 μg/mL^{-1}. Probably the phenolic compounds, catechin, chlorogenic, gallic acid, which are present in the extracts, were responsible for this inhibition. According to Cerezo et al. (2010), in several species of plants and several fruits are found these phenolics that also act as antibiotics, and promote

antifungal activity (Johann et al., 2009). Quantified alcoholic extracts of various types of fruit pulps and verified the presence of phenolic compounds such as vanillic acid, vanillin, gallic acid and coumarins. They observed, by disc diffusion method, that these compounds showed an inhibitory effect on the growth of fungi, *Rhyzopus oryzae*, *Aspergillus flavus, Aspergillus carbonarius* e *Aspergillus fumigatus*;

Ergosterol is a steroid that is present in the fungal membrane. It acts as a modulator of the fungal membrane fluidity and any action of some chemical elements triggers an imbalance in the fungal plasma membrane fluidity, promoting changes in intracellular homeostasis.In general, any alteration that interferes in the composition of the cellular microbial plasma membrane will be rendering it devoid of an essential organelle to the homeostatic equilibrium, a fundamental condition for its optimal physiological functioning. A change in this cellular structure may render the intracellular microbial environment incompatible with its survival (Souza et al., 2003).

According to Perrone et al. (2009) the class of *Aspergillus* presents some morphological characteristics, such as the presence of conidia black or brown and dark, also has rough and rigid cell walls, long and broad stipes, and may be resistant to some phenolic compounds used. Other species of filamentous fungi may be resistant and do not promote inhibition against quantified compounds due to the amount and bioavailability that are present in the fungi or the small amount of the major compound that promotes inhibition.

Inhibitory effect of extracts of fruit wastes on bacteria Staphylococcuaureus, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa and Salmonella choleraesuis.

The results of the inhibitory effects are shown in Table 5. The minimum inhibitory concentration (MIC) found when extracts of tamarind and jackfruit were used for the bacteria *L. monocytogene*, was 125 $\mu g/mL^{-1}$ and for the lemon extract no inhibition occurred for the *E. coli, P.aeruginosa e S.choleraesuis*

Table 5- Minimum inhibitory concentration of tamarind, lemon, genipap and cajá extract found for bacteria *S. aureus*, *L. monocytogenes E. coli*, *P.aeruginosa* and *S.Choleraesuis.*

	MIC (μg mL^{-1})		Inhibition halo (mm)		
Bacterium	**Tamarind**	**Lemon**	**genipap**	**Jackfruit**	**CL**
S. aureus ATCC 13565	250	1000	1000	500	100
L. monocytogenes ATCC 19117	125	500	125	125	100
E. coli ATCC 11229	1000	NI	NI	NI	100
P.aeruginosa ATCC 15442	NI	NI	NI	NI	100
S.Choleraesuis ATCC 6539	250	NI	NI	500	100

*NI: no inhibition occurred, CL: Chloramphenicol. Source: Autors.

It is observed that the lemon extract inhibited only the growth of two bacteria *S. aureus*, and *L. monocytogenes*, even at concentrations greater than 1000 μg/mL^{-1}, and 500 μg/ mL^{-1} with the tamarind extract, inhibition occurred with 125 μg/mL^{-1} for *L. monocytogenes* and for the jackfruit extract.

According to the results obtained, an inhibition of the extracts of the bacteria *Staphylococcus aureus, Listeria monocytogenes,* and *Salmonella choleraesuis was observed*. Probably this inhibition occurred by the presence of the phenolic compounds, gallic acid, catechin, chlorogenic, which presented in expressive amounts in these extracts. These data corroborate with those found by Vaquero et al. (2007), who studied the inhibition of microorganisms by p-coumaric acids, catechin, gallic acid and syringaldehyde extracted from vegetables.

It was observed that the tamarind extract showed higher antimicrobial activity than the other extracts, suggesting that a higher concentration of the extracts probably increase the inhibition against the bacteria tested. It is known that, in most cases, gram-negative bacteria are less sensitive to antibacterial action than Gram-positive bacteria because they present polysaccharides in their structure that inhibits the penetration of antimicrobial substances (Burt, 2004). Other types of bacteria can promote greater inhibition compared to those that were tested or not in the present study, however the tested bacteria are the most common ones found as contaminants in food. Some other species may have greater or lesser inhibition which will depend on their physiological characteristics.

4. Conclusion

The major compounds found in the extracts were catechin (tamarind), gallic acid (lemon), catechin (Jenipapo) and gallic acid (cajá).

The antioxidant activity was favorable due to two methodologies used DPPH and FRAP for all extracts, but the tamarind extract presented a better antioxidant activity in both tests when compared to the others and could have a significant potential to be explored as an antioxidant and antibacterial agent, in the food and pharmaceutical industry.

The extracts presented a considerable biological activity on the fungi, *Penicillium comune and Penicillium cladosporoides* and on bacteria *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli,* and *Salmonella choleraesuis*; but was not efficient in the control of *Pseudomonas aeruginosa*. Therefore, it can be concluded that the extracts can be used in some foods as antioxidants and natural antimicrobials but not discarding the other analyzes to certify that the compounds found are beneficial to consumers' health. It would also be a possible use of these natural compounds in the development of packaging, promoting a direct conservation in the food, guaranteeing its safety and quality.

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Conflict of Interest

The authors declare no conflicts of interest.

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