Brazilian fruit pulps as functional foods and additives: Evaluation of bioactive compounds

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A B S T R A C T

Eight tropical fruit pulps from Brazil were simultaneously characterised in terms of their antioxidant and antimicrobial properties. Antioxidant activity was screened by DPPH radical scavenging activity (126–3987 mg TE/100 g DW) and ferric reduction activity power (368–20819 mg AAE/100 g DW), and complemented with total phenolic content (329–12466 mg GAE/100 g DW) and total flavonoid content measurements (46–672 mg EE /100 g DW), whereas antimicrobial activity was tested against the most frequently found food pathogens.

Acerola and açaí presented the highest values for the antioxidant-related measurements. Direct correlations between these measurements could be observed for some of the fruits. Tamarind exhibited the broadest antimicrobial potential, having revealed growth inhibition of *Pseudomonas aeruginosa*. *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp. and *Staphylococcus aureus*.

Açaí and tamarind extracts presented an inverse relationship between antibacterial and antioxidant activities, and therefore, the antibacterial activity cannot be attributed (only) to phenolic compounds.

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Flavonoid content
Functional foods
Functional ingredients
FRAP
Phenolic content
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1. Introduction

Tropical fruits were widely cultivated at subsistence and local commercial purposes until the 1970's, when trade volumes expanded, as these fruits started to be perceived as economically interesting options from traditional export crops. Nowadays, since the market for tropical fruit has evolved significantly, price premiums based on novelty have virtually disappeared, being replaced by quality-based premiums. World production of tropical fruits handled around 82 million ton in 2009 (FAO, 2011), with a total value of USD 5.4 billion and mango as the dominant variety.

Asia is the largest producing region for tropical fruits, followed by Latin America, Caribbean, Africa and Oceania (FAO, 2011). Brazil possesses a geographical region with suitable climatic conditions for a large number of native fruits that may possess an excellent agro-industrial potential, thus representing an interesting economic income for local growers. The evaluation of their bioactive properties will thus strengthen its position in the market, reaching either specific markets created by consumers' demand for new products able to maintain health and preventing diseases, as well as the growing market of functional ingredients.

Research on bioactive ingredients for human consumption has increased in the recent past due to consumer awareness of their associated benefits with health maintenance and well-being. Among these compounds, those related with antioxidant activity are the subject of various research studies, due to their enormous importance in human health.

Antioxidant activity is usually related to the presence of phenolic compounds; these exhibit specific common structures that allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers, among other reaction mechanisms (Pietta, 2000). At a cellular level, various antioxidant compounds are known to be able to stabilise or deactivate free radicals, thus preventing damage to cell structures. Their significance within human health has been extensively described, playing such diverse roles as protection against cardiovascular diseases (by reducing chronic inflammation and improving endothelium function), certain forms of cancer and

cytotoxic effects, among others (Proestos, Boziaris, Nychas, & Komaitis, 2006; Robles-Sánchez et al., 2011).

Although the human body relies on endogenous defence mechanisms against oxidative stress, exogenous (dietary) sources of antioxidants are also necessary. Several naturally occurring compounds found in food products have been shown to present strong antioxidant activity, therefore placing these foods as candidates to serve as functional foods or as functional ingredients, when only parts of the food are extracted and incorporated into other different food products. Plants or their parts are commonly excellent sources of antioxidant compounds, and their direct consumption or incorporation in processed foods is gathering importance, as an alternative to synthetic antioxidants, less preferred by consumers due to their stronger side effects and higher toxicity (Jain, Bhuiyan, Hossain, & Bachar, 2011; Madsen & Bertelsen, 1995).

Besides their antioxidant capacity, phenolic compounds present in plants may also possess antimicrobial properties (Chakraborty & Mitra, 2008; Rauha et al., 2000). Although there are several studies on the antioxidant capacity of fruits, their antimicrobial properties are scarcely screened. The antimicrobial capacity of a fruit (or its extracts) is of utmost importance, because despite the large amount of preservation techniques available nowadays, the spoilage and deterioration of food products by microorganisms is still a problem that has not yet been completely controlled. Most food-borne illnesses are caused by microbial pathogens present in the food due to a contamination during the process from farm to fork, or caused by toxins produced by those contaminants. Certain species, including specific strains of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*, can even cause fatal infections in humans (Šiler et al., 2014). Therefore, many food products need to be protected through addition of preservatives, but the growing tendency to avoid chemical compounds in foods is leading to the search and development of alternative natural substances able to simultaneously increase shelf life of foods and provide a high degree of safety regarding foodborne pathogens, as well as present reduced hypersensitivity reactions.

This study aims to simultaneously characterise the antioxidant and antimicrobial properties of eight tropical fruit pulps from Brazil (açaí, acerola, caja, guava, soursop, mango, pineapple and tamarind), some of which to our best of knowledge have not been studied in terms of such properties, in order to ascertain their potential as functional foods and functional ingredients. Antioxidant activity was screened with two different methods (DPPH-RSA and FRAP), and complemented with TPC and TFC measurements, whereas antimicrobial activity was tested against some of the most frequently found food pathogens, both Gram positive and Gram negative.

2. Materials and methods

2.1. Samples

The fruits açaí (*Euterpe oleracea*), acerola (*Malpighia emarginata* D.C.), caja (*Spondias mombin* L.), guava (*Psidium guajava* L.), soursop (*Annona muricata* L.), mango (*Mangifera indica* L.), pineapple (*Ananas comosus* L.) and tamarind (*Tamarindus indica* L.), were randomly collected in local markets of Fortaleza, Northeastern Brazil; subsequently, their pulp was separated and freeze-dried, transported to Portugal and stored at −15 °C until used.

2.2. Chemicals and equipment

Ascorbic acid, gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid) and (-)-epicatechin standards were obtained from Sigma Aldrich (Madrid, Spain). DPPH (2,2-diphenyl-1-picrylhydrazyl) and Folin–Ciocalteu reagent were also from Sigma Aldrich. The remaining reagents were of analytical grade and obtained from Merck (Darmstadt, Germany). Mueller-Hinton broth was from Sigma Aldrich, UK.

All the spectrophotometric assays were performed in a Synergy HT W/TRF Multi Mode Microplate Reader with Gen5 2.0 software (BioTek Instruments, USA).

2.3. Preparation of crude extracts

Extracts were prepared in triplicate by adding ca. 2.5 g of each freeze dried pulp to 100 ml of water/ethanol (1:1, v/v), and mixing at 100 rpm for 1 h in a shaker (Rotabit, Selecta) placed inside a controlled chamber (Thermostat cabinet Lovibond) at 25 °C. Extracts were subsequently centrifuged at 4000 rpm for 30 min (Sartorius 2-16), filtered and concentrated in a rotary evaporator (Rotavapor R-210, Buchi) at 40 °C and 40 mbar. Subsequently, extracts were redissolved in DMSO so as to obtain a concentration of 400 mg ml^{-1} and stored at −18° C until used.

2.4. Total phenolics content (TPC) determination

TPC values were determined by a colorimetric assay based on a modified procedure initially described by Singleton and Rossi (1965). The reaction mixture consisted of 25 μl of sample or standard solution, 75 μl of deionised water and 25 μl of Folin–Ciocalteu reagent. After 6 min, 100 μl of Na_2CO_3 7.5% (w/v) were added. Absorbance was measured at 765 nm in the microplate reader, after 90 min. Calibration curves were done using gallic acid (GA) as standard antioxidant and results were expressed as gallic acid equivalents (GAE) on a dry weight (DW) basis.

2.5. Total flavonoids content (TFC) determination

The assay consisted in adding 100 μl of deionised water followed by 10 μl of sodium nitrite solution (50 g l^{-1}) and 25 μl of standard, sample or deionised water (blank) into a microplate. After 5 min, 15 μl of aluminium chloride (100 g l^{-1}) were added and, after 1 min of reaction, 50 μl of a sodium hydroxide (1 mol l^{-1}) was also added. Finally, the microplate was introduced in the microplate reader, submitted to smooth stirring for 10 min, and the absorbance was measured at 510 nm. (-)-Epicatechin was used as standard antioxidant.

2.6. Antioxidant capacity assays

2.6.1. DPPH radical scavenging activity (DPPH-RSA)

DPPH-RSA of samples was determined spectrophotometrically at 517 nm, against the stable nitrogen radical DPPH·. Briefly, 25 μl of sample was mixed with 200 μl ethanolic solution of DPPH· (0.04 mg ml^{-1}). The mixture, vigorously shaken, was left to stand for 30 min in the dark (until stable absorption values). Lower absorbance values of the reactive mixture indicated higher free radical scavenging activity. The calibration curve was prepared with Trolox.

2.6.2. Ferric reduction activity power (FRAP)

The FRAP assay, originally developed by Benzie and Strain (1996) was performed with some modifications. In short, FRAP reagent (10 ml of 300 mmol l^{-1} acetate buffer (pH 3.6), 1 mL of 10 mmol l^{-1} TPTZ in 40 mmol l^{-1} HCl, and 1 ml of 20 mmol l^{-1} $FeCl_3$) was diluted to one-third with acetate buffer. 180 μl of this solution was added to each well, along with 20 μl of sample. The control assay was performed using 180 μl of FRAP reagent and 20 μl of ethanol. Absorbance was measured at 593 nm and 37 °C. The calibration curve was prepared with ascorbic acid (AA).

2.7. Antimicrobial activity

2.7.1. Microorganisms and culture

The antibacterial properties of the extracts were tested against the following bacterial strains: *L. monocytogenes* (isolated from cured goat cheese, accession number 3375, collection from Centro de Inovação e Apoio Empresarial – CINATE), *S. aureus* (isolated from food sample, accession number 18N, collection from CINATE), *Pseudomonas aeruginosa*, *E. coli* and *Salmonella* sp. (all isolated from food sources, internal collection from CINATE).

Strains were stored in cryovials with glycerol at 15% (v/v) and maintained at −80 °C before use. Active cultures for experiments were grown in sterile Mueller-Hinton broth (MHB) at 37 °C for 6 h, and then an aliquot from each culture was transferred to fresh MHB and cultured overnight at 37 °C. Overnight cultures were properly diluted for diffusion assays. Purity and cell numbers were checked by plate observation and counting.

2.7.2. Agar diffusion assay

Screening of the antimicrobial activity of the extracts was performed by the agar diffusion method. Briefly, each isolate was seeded on Mueller-Hinton agar plates with sterilised cotton swabs. The plates were previously divided in three quadrants, and 20 µl of each test extract (400 mg ml^{-1}), DMSO (negative control) and lactic acid at 40% (v/v) (positive control) were placed in each quadrant. Plates were incubated at 37 °C for 48 h. Growth inhibition was quantified as inhibition circular zones, and their diameters were measured. Triplicate plates were tested for each microorganism. Those extracts that revealed growth inhibition were further tested as follows.

2.7.3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

MIC was assayed in the microplate reader, using sterile 96 wells trays. Each well was filled with a total volume of 300 µl containing *ca.* 10^6 colony forming units (CFU)/ml of test bacteria, fresh MHB and diluted extract sample. Tested extract concentrations ranged from 200 to 10 mg ml^{-1}. Negative controls contained non-inoculated medium with extract samples and positive control wells were prepared with inoculated medium without extract samples.

Bacterial growth was followed during 24 h at 37 °C through absorbance measurements at 630 nm each min. Wells were examined and the lowest extract concentration that completely inhibited bacterial growth (indicated by clear wells) was determined as the MIC value.

To determine MBC values, solutions with extract concentrations equal or higher to the MIC values were used, assuming the maximum concentration of 400 mg ml^{-1}. Briefly, 50 µl were taken from each well of MIC assays were no significant variations in absorbance were observed, and spread on plate count agar medium. After incubation at 37 °C for 24 h, MBC was defined as the lowest concentration of extract that resulted in no bacterial growth on agar plates.

All tests were performed in triplicate. An example of the growth curves obtained after 24 h growth in microplates is presented in Fig. 1.

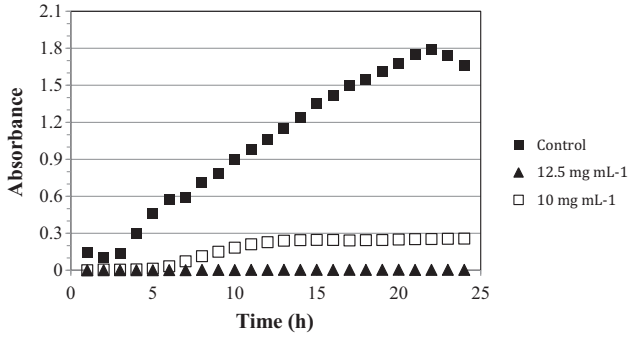

Fig. 1. Growth curve of *P. aeruginosa* in microplates, with the following concentrations of tamarind extract: ■ control (without extract), □ 10 mg ml^{-1}, Δ 12.5 mg ml^{-1}.

2.8. Statistical analysis

Analysis of variance and Tukey (HSD) or Games-Howell post hoc tests were employed to statistically analyse the results, using software SPSS version 20 (IBM). Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Antioxidant activity

There is a general consensus that antioxidant activity should be evaluated with several different methods, as they respond to and quantify different reaction mechanisms (Prior, Wu, & Schaich 2005); therefore, this study included four different protocols, aiming at measuring the total reducing capacity of the extracts (Folin–Ciocalteu method), total flavonoids, and antioxidant activity through DPPH scavenging radical activity and ferric reduction activity power of the extracts.

Phenolic compounds, with their ability to donate hydrogen or electrons beyond their capacity to form stable radical intermediates, are considered as major active antioxidant metabolites from plants (Koolen, Silva, Gozzo, Souza, & Souza, 2013). TPC, determined by Folin–Ciocalteu method, is not an absolute measurement of the amounts of phenolic compounds, but instead, a measurement of the chemical reducing capacity of compounds present in the extract relative to gallic acid, and therefore expressed as gallic acid equivalents (GAE). According to the classification suggested by Vasco, Ruales, and Kamal-Eldin (2008), TPC levels can be divided into three categories: (i) low, when polyphenol contents are below 500 mg GAE/100 g DW; (ii) intermediate, when they range between 500 and 2500 mg GAE/100 g DW; and (iii) high, when they are higher than 2500 mg GAE/100 g DW. By applying the proposed classification to the results from this study, presented in Table 1, pineapple and tamarind (329 and 474 mg GAE/100 g, respectively) presented low polyphenol levels, açaí, caja, guava, mango and soursop (1808, 744, 1152, 721 and 817 mg GAE/100 g, respectively) presented medium polyphenol levels, and the highest amount was found for acerola (12,466 mg GAE/100 g).

Silva et al. (2014) also found high TPC levels in acerola pulp, thus confirming that this fruit is an excellent source of polyphenolic compounds, presenting also high level of anthocyanins and β-carotene. They reported 29,093 and 2886 mg GAE/100 g DW for acerola and soursop, respectively, whereas guava, pineapple, tamarind and mango reached intermediate levels (1723, 990, 923 and 652 mg GAE/100 g, respectively); these values are generally higher than those found in this work, except for mango, which was lower. Vasco et al. (2008) found lower values for guava and mango (462 and 60 mg GAE/100 g, respectively), as well as Rufino et al. (2011) (1500 mg GAE/100 g on açaí pulp). As the extraction procedure was similar in these studies, the differences found in results could be associated to agronomic (agricultural practices, soil composition, climatological conditions) and physiological (ripening stage) factors (Morales-Soto et al., 2014). Although TPC determination is simple and sensitive, it must be kept in mind that this method may suffer from interferences

Table 1
Average concentration and standard deviation of total phenolic, flavonoids content and antioxidant activity assays.

	TPC (mg GAE/100 g DW)	TFC (mg EE/100 g DW)	DPPH-RSA (mg TE/100 g DW)	FRAP (mg AAE/100 g DW)
Açaí	1808 ± 28^{b}	672 ± 40^{a}	1574 ± 101^{b}	1367 ± 118^{b}
Acerola	12,466 ± 1256^{a}	158 ± 23^{c}	3987 ± 15^{a}	20,819 ± 550^{a}
Caja	744 ± 84^{d}	184 ± 31^{c}	1199 ± 60^{c}	872 ± 53^{d}
Guava	1152 ± 52^{c}	217 ± 27^{b,c}	1507 ± 40^{b,c}	1104 ± 58^{c}
Mango	721 ± 37^{d}	70 ± 18^{d}	1764 ± 227^{b}	914 ± 107^{c,d}
Pineapple	329 ± 53^{e}	46 ± 6^{d}	126 ± 11^{f}	368 ± 18^{f}
Soursop	817 ± 41^{d}	252 ± 21^{b}	645 ± 69^{d}	648 ± 59^{e}
Tamarind	474 ± 47^{e}	178 ± 32^{c}	331 ± 48^{e}	615 ± 42^{e}

All values are mean ± standard deviation of triplicates.
Values in the same column that are not followed by the same letter are significantly different ($p < 0.05$).

caused by the presence of reducing sugars and some amino acids), thus overestimating the results (Barroso, Noronha, Delerue-Matos, & Oliveira, 2011).

TFC was measured by $AlCl_3$ method and expressed as epicatechin equivalents (EE). TFC values obtained ranged from 46 to 672 mg EE/100 g DW. Açaí pulp presented the highest level of total flavonoids compounds, followed by soursop, guava, caja, tamarind, acerola, mango and pineapple. Since flavonoids are mainly present in plants as colouring pigments (Hossain & Rahman, 2011), and açaí was indeed the pulp with the strongest colour, it is not surprising that it is also the extract sample with the highest TFC.

Although Silva et al. (2014) did not measure the TFC, but only a subclass of flavonoids, the anthocyanins, they indicated for acerola, pineapple, guava, soursop, mango and tamarind anthocyanins values ranging from 2.92 to 144 mg anthocyanins/100 g DW; these values are indicative that these fruits have anthocyanins and other flavonoids compounds in their composition.

Antioxidant activity was measured using DPPH-RSA and FRAP assays. DPPH-RSA is a technique based on the reduction of the DPPH· radical in the presence of a hydrogen-donating antioxidant, being expressed in mg trolox equivalent (TE).

From Table 1, three groups of samples can be distinguished as well. The first one, acerola, containing the highest DPPH· antiradical level (3987 mg trolox E/100 g DW), correlates with the highest TPC value. The second group (values ranging from 500 to 2000 mg trolox E/100 g DW) includes mango, açaí, guava, caja and soursop, and the third group (values less than 500 mg trolox E/100 g DW), with pineapple and tamarind. According to Vasco et al. (2008), low antiradical efficiency of the fruit extracts (group 3) may be associated to the fact that the phenolic compounds are bound to other molecules, for example carbohydrates, which considerably reduce the antioxidant activity.

The presence of high antioxidant activity through DPPH assay in açaí had already been reported by Gonçalves, Santos, and Srebernich (2011) and Hogan et al. (2010).

FRAP assay also assesses the antioxidant activity, although by measuring the potential to reduce the yellow ferric-TPTZ complex to a blue ferrous-TPTZ complex by electrodonating substances under acidic conditions, and is expressed as ascorbic acid equivalents (AAE).

As occurred in DPPH-RSA assay, it is also possible to create three distinct groups within FRAP assay results: (i) acerola in the highest group (20,819 mg AAE/100 g DW), which coincide with the highest TPC and DPPH-RSA values; (ii) açaí, guava, mango, caja, soursop and tamarind in the second group, with FRAP values ranging from 615 to 1375 mg AAE/100 g; and (iii) pineapple in the third group, with a FRAP value of 368 mg AAE/100 g.

The results obtained in the four methodologies used showed a wide range of variation in what concerns TPC, TFC and antioxidant activity, being possible to catalogue the data into three clear groups of tropical fruit extracts with high, medium and low polyphenolic compounds and antioxidant capacity. Furthermore, some of the extracts exhibit direct correlations between the measurements (e.g. pineapple presents the lowest values in the various measurements, whereas acerola has the highest levels for all but TFC assay); however, such correlation is not straightforward for all the fruit extracts.

Oh, Jo, Cho, Kim, and Han (2013) related significant correlations among TPC and DPPH, ABTS, and reducing power assays in herbal teas, with higher correlations in ethanol extracts when compared to those in water. Therefore, they concluded that the presence of phenolic compounds in herbal tea extracts contributes significantly to their antioxidant potential. Several other reports described direct relationships between TPC and antioxidant activity (Deighton, Brennan, Finn, & Davies, 2000; Silva, Souza, Rogez, Rees, & Larondelle, 2007), but synergistic or antagonistic effects between the different phenolics present within the extracts cannot be ruled out, and therefore such relationships may be not so close (Imeh & Khokhar, 2002; Ismail, Marjan, & Foong, 2004). In fact, several studies have demonstrated that the individual antioxidant activity of phenolic compounds is different whether they are assayed individually or together, due to interactions among them, which can be either synergistic or antagonistic, depending on the compounds and conditions under study within the model system (Almeida et al., 2011; Hidalgo, Sánchez-Morano, & Pascual-Teresa, 2010; Terpinc & Abramovic, 2010).

3.2. Antibacterial activity

The agar diffusion assay was used as a preliminary assessment for the antibacterial activity of fruit extracts. The microorganisms were selected by taking into account the most frequent microorganisms that affect several foods, considering future use of the crude extract as a possible food additive (Barbosa-Pereira et al., 2014).

From the results, presented in Table 2, extracts from tamarind pulp exhibited the broadest antimicrobial spectrum, as they inhibited growth of all the microorganisms tested, both Gram positive and Gram negative. The demonstration of antibacterial activity against both Gram types of bacteria suggests the presence of a several potential antibiotic compounds within tamarind extract that may interplay an additive or synergistic role. These results are in agreement with previous studies with stem bark and leaves of the same fruit (Doughari, 2006). None of the bacteria tested were sensitive to açaí pulp extract under the concentration tested (400 mg ml^{-1}), although higher values could eventually exert inhibitory effect. These results are in agreement with those reported by Gonçalves et al. (2011), where aqueous açaí extracts at concentrations of 8.9% (v/v) showed no activity against *S. aureus* and *P. aeruginosa*.

Table 2
Average diameter of inhibition zone ± standard deviation (mm) of fruit pulp extracts, positive and negative controls.

	Gram (−)			Gram (+)	
	E. coli	*P. aeruginosa*	*Salmonella* sp.	*S. aureus*	*L. monocytogenes*
Açaí	–	–	–	–	–
Acerola	–	10.0 ± 0.4	–	14.0 ± 0.8	13.0 ± 0.8
Caja	–	–	–	14.0 ± 1.0	12.0 ± 0.8
Guava	–	–	–	–	10.0 ± 1.0
Mango	–	10.0 ± 0.5	–	10.0 ± 0.4	15.0 ± 1.0
Pineapple	–	–	–	10.0 ± 0.3	10.0 ± 0.5
Soursop	–	–	–	–	10.0 ± 0.5
Tamarind	9.0 ± 0.3	10.0 ± 0.3	10.0 ± 0.4	10.0 ± 0.4	10.0 ± 0.3
DMSO (+)	–	–	–	–	–
Lactic acid (−)	13.0 ± 1.0	18.0 ± 1.0	17.0 ± 1.0	19.0 ± 0.7	23.0 ± 2.0

(−) – Without inhibition zone.

Table 3
Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) (mg ml^{-1}) of fruit pulp extracts.

	Gram (−)						Gram (+)			
	E. coli		*P. aeruginosa*		*Salmonella* sp.		*S. aureus*		*L. monocytogenes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Açaí	>400	–	>400	–	>400	–	>400	–	>400	–
Acerola	>400	–	12.5	18.75	>400	–	25	50	10	12.5
Caja	>400	–	>400	–	>400	–	18.75	37.5	18.75	25
Guava	>400	–	>400	–	>400	–	>400	–	25	50
Mango	>400	–	37.5	50	>400	–	37.5	50	25	37.5
Pineapple	>400	–	>400	–	>400	–	18.75	25	37.5	50
Soursop	>400	–	>400	–	>400	–	>400	–	37.5	75
Tamarind	25	37.5	10	12.5	12.5	25	25	37.5	25	37.5

Results from MIC and MBC are presented in Table 3. For those strains with no inhibition zone, MIC was identified as above 400 mg ml^{-1} and MBC was not determined. The extracts with lowest values were from tamarind and acerola against *P. aeruginosa* and *L. monocytogenes*, respectively. *L. monocytogenes* was the most sensitive microorganism to the extracts examined in this study, whereas almost no effect was noticed against *E.coli* and *Salmonella* sp. (only tamarind extracts were active against these strains).

The majority of studies concerning both antimicrobial and antioxidant activities attribute the antimicrobial activity to the phenolic content of extracts, and therefore establish a direct relationship between both activities (Chakraborty & Mitra, 2008; Proestos et al., 2006). It has been suggested that such compounds act as natural protecting agents against microbial pathogens and insect predators in plants. However, in the present study, the reasoning for the high antibacterial activity found in tamarind extracts could not be exclusively attributed to the presence of antioxidant compounds, as tamarind extracts were those that revealed some of the lowest antioxidant results among the fruits tested.

Açaí extracts also presented an inverse relationship between antibacterial and antioxidant activities, i.e., one of the lowest results (among the fruits tested) regarding antibacterial activity and one of the highest for antioxidant capacity. This fact may be tentatively explained by the presence of growth-promoting compounds in the açaí extract, which can interfere in the antimicrobial potential of antioxidant compounds and even reverse its effect; the fact that these fruits present a high amount of dietary fibre with possible prebiotic effect may tentatively justify the results, although more studies are obviously necessary. Another possible explanation was proposed by Doughari (2006), that reported higher antimicrobial activity of stem bark than leaf extracts of tamarind, and suggested that this could be due to the fact that stem bark contained less pigments and other phenolics that could interfere with the antimicrobial activity; interestingly, açaí extracts presented the darkest colours, a result from the high pigment content of the fruit. Finally, the absence of correlation between phenolics/flavonoids/antioxidant activity against antimicrobial activity could be assigned to the specific type of phenolic content and not on their presence *per se* (Koolen et al., 2013).

The antibacterial activity of the extracts tested was higher against Gram positive bacteria when compared with their Gram negative counterparts. Such effect, already reported by several authors (Gonçalves et al., 2011; Oh et al., 2013) is attributed to the membrane structure, as the Gram negative present a multilayered structure with an outer lipopolysaccharide layer and higher fat content on the cell wall, thus allowing less interaction with the extracts (Gonçalves et al., 2011). Besides, as most of the extracts are of hydrophilic nature, due to the nature of the solvents usually employed, their interaction with bacterial cell wall would probably be more difficult.

Phenolic compounds measured through Folin–Ciocalteu procedure are essentially simple soluble phenolics. Soluble phenolics are believed to exert their antimicrobial effect by generating a hyperacidification at the plasma membrane interface of the microorganism, thus promoting disruption of the H+-ATPase activity of the bacterial cell membrane (required for ATP synthesis). The efficacy of such localised protonation effects on microorganisms depends on the nature of their cell wall membrane structure (Vattem, Lin, Labbe, & Shetty, 2004). As Gram negative bacteria present an external lipopolysaccharide layer around the cell, compounds able to stack on the membrane and destabilise it, resulting in membrane disruption or destabilisation, must present, at least, partial hydrophobicity. This may tentatively explain the antimicrobial activity found for acerola and mango extracts, which also present high and medium TPC, respectively. These two extracts revealed antimicrobial activity for both Gram positive bacteria and also for *P. aeruginosa*. Apart from them, only tamarind extract revealed antimicrobial activity for these two bacteria, but its

amounts of TPC are quite low, and therefore the mechanism responsible for the bactericidal effect should be another.

In conclusion, for a phenolic compound to be able to function as antimicrobial against Gram positive and Gram negative bacteria, it should present at least partial hydrophobicity, so as to be able to act at the Gram negative membrane interface. Such statement is supported by the research of Koolen et al. (2013), which hypothesised a relationship between the absence of antimicrobial activity in buriti phenolic extracts and the concomitant absence (or short concentration) of low polarity compounds, such as unsaturated fatty acids, proven to possess antimicrobial activity (Nazif, 2002). The nature of phenolic compounds present in the extract depends on its polarity; polarity of the extracts is strongly dependent on the conditions prevailing during their preparations, especially the polarity of the solvent selected.

4. Conclusions

Fruit consumption is no longer merely a result of taste and personal preference, becoming a health concern due to their vital nutrient content (minerals, fibres, vitamins, phenolic compounds and antioxidants). In particular, tropical fruit consumption is increasing both in domestic and international markets, due to growing recognition of its nutritional and therapeutic value, apart from the raising interest in new natural sources of antimicrobial and antioxidant compounds for incorporation in food products.

Acerola and açaí presented the highest values for TPC, TFC and antioxidant activity, whereas tamarind extracts may be further studied in the prevention or adjuvant treatment of diseases in animals and humans caused by *P. aeruginosa*, *E. coli*, *L. monocytogenes*, *Salmonella* sp. and *S. aureus*. Furthermore, in terms of food safety and preservation, it can also be used to naturally preserve foods and beverages from microbial growth, thus enhancing its shelf life.

Açaí and tamarind extracts present an inverse relationship between antibacterial and antioxidant activities, and therefore, the antibacterial activity cannot be attributed (only) to phenolic compounds. Besides, the antioxidant capacities measured within the extracts of the fruits tested cannot be solely attributed to their phenolic contents, but also to other compounds with antioxidant capacity, which may contribute to the final antioxidant amount isolated, or acting synergistically or antagonistically.

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