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ORIGINAL ARTICLE

Extraction, characterization and antioxidant properties of phenolic compounds in açaí juçara (*Euterpe edulis* Mart.) from Atlantic Forest

*Extração, caracterização e propriedades antioxidantes de compostos fenólicos em açaí de juçara (*Euterpe edulis *Mart.) da Floresta Atlântica*

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Abstract

The açaí is a popular Brazilian fruit, however, already part of the world's healthy eating habits owing to its antioxidant properties. The study aimed to determine the effect of solvent in extracting phenolic compounds with antioxidant potential in açaí juçara (*Euterpe edulis* Mart.) using a Completely Randomized Design (CRD). The phenolic compound profile was quantified by High-Performance Liquid Chromatography (HPLC), and the data set was analyzed by Principal Component Analysis (PCA). The PCA was applied to evidence the relationships between the concentration of phenolic compounds and the solvents. Furthermore, the antioxidant activity was also determined by 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Ferric Reducing Antioxidant Power (FRAP) methods. The solvent ethanol: water 70% was more efficient in extracting phenolic compounds with high antioxidant activity. In this extract, salicylic acid was found in high concentrations as well as catechin, epicatechin, and coumaric acid. Based on a consensus that phenolic compounds are associated with the most powerful antioxidant activities of fruits, the "açaí juçara" from the Atlantic Forest is a potential source of polyphenols. They could be used as natural antioxidants for application in the food and pharmaceutical industry in order to substitute the synthetic antioxidants.

Keywords: Natural antioxidants; Phenolic profile; HPLC; Experimental design; Optimized conditions; Principal component analysis.

Resumo

O açaí é uma fruta popular brasileira, porém já faz parte dos hábitos alimentares saudáveis da população mundial, devido às suas propriedades antioxidantes. O objetivo deste estudo foi determinar o efeito do solvente na extração de compostos fenólicos com potencial antioxidante em açaí juçara (*Euterpe edulis* Mart.), utilizando um Delineamento Inteiramente Casualizado (DIC). O perfil dos compostos fenólicos foi quantificado por Cromatografia Líquida de Alta Eficiência (CLAE) e o conjunto de dados foi analisado por Análise de Componentes Principais (PCA). O PCA foi aplicado para evidenciar as relações entre a concentração de compostos fenólicos e os solventes. Além disso, a atividade antioxidante também foi determinada pelos métodos DPPH, ABTS e FRAP. O solvente etanol:água a 70% foi mais eficiente na extração de compostos fenólicos com alta atividade antioxidante. Neste extrato, o ácido salicílico foi encontrado em alta concentração, além de catequina, epicatequina e ácido cumárico. Com base no consenso de que os compostos fenólicos são os principais compostos relacionados à atividade antioxidante das frutas, o açaí juçara da Mata Atlântica é uma fonte potencial de compostos fenólicos e poderia ser usado como antioxidante natural para aplicação nas indústrias alimentícia e farmacêutica, em substituição aos antioxidantes sintéticos.

Palavras-chave: Antioxidante natural; Perfil fenólico; CLAE; Desenho experimental; Otimização de condições; Análise dos componentes principais.

1 Introduction

Brazil has a large number of açaí fruits, many of them are native and exotic. Among them, there are several açaí species that are palm tree and belong to the Arecaceae family. *Euterpe precatoria* Mart. and *E. oleraceae* Mart. are species more socially and economically important that grow in various areas of the Amazon Forest (Oliveira & Schwartz, 2018). On the other hand, *E. edulis* Mart. is an endemic palm tree from the Atlantic Forest and is distributed in the coast region of the South and Southeast of Brazil (Souza & Prevedello, 2019; Lopes et al., 2021). The three species differ in growth and phytochemical composition of their fruits. The açaí from Amazon Forest is widely exploited due to their relevant agro-industrial interest and significant income for the local population. However, the açaí from Atlantic Forest and other species are still far from being able to ensure the sustainable exploitation (Oliveira & Schwartz, 2018).

In fact, *E. edulis* has a wide geographical distribution in the Atlantic Forest, mainly in the states of São Paulo (SP), Paraná (PR), Santa Catarina (SC), and Rio Grande do Sul (RS) (Schulz et al., 2016). In the general survey of açaí production in Brazil, açaí juçara production was not included, due to the failure to fully establish the production chain for this fruit in the processing industries. However, it is estimated that the state of Santa Catarina is one of the largest producers, with an average value of 200 thousand tons of fruit per year (Companhia Nacional de Abastecimento, 2020). Due to the illegal extraction of heart-of-palm, the *E. edulis* palm tree is one of the endangered species. However, government agencies have encouraged the exploitation of its fruit called by “açaí de juçara”. Thus, the preservation of species and the possible financial income for the natives come from the non-harmful and conservative use of palm trees (Schulz et al., 2016).

However, according to Schulz et al. (2021), the açaí juçara has gained worldwide attention, mainly because their nutritional value, in general, resembles that of açaí of *E. oleracea* and *E. precatoria* Mart. from Amazon Forest. Açaí juçara contains different macro and micronutrients, beyond a good source of vitamins C and E (Cardoso et al., 2018). Several phytochemicals with antioxidant and anti-inflammatory activities in the açaí fruit were reported (Seraglio et al., 2018; Schulz et al., 2016). The gallic, caffeic, p-coumaric, p-hydroxybenzoic, vanillic, chlorogenic and ferulic acids were found by many authors (Schulz et al., 2016; Rocha et al., 2018).

Açaí juçara has been reported as a potent natural antioxidant and can be consumed directly or used as a natural alternative ingredient to synthetic antioxidants in the food and pharmaceutical industry. The açaí

purple pigmentation can be used as a novel natural colourant in isotonic soft drink (Albarici & Pessoa, 2012; Gironés-Vilaplana et al., 2013; Castro et al., 2016), gelatine (Bernardes et al., 2019) and yogurt (Geraldi et al., 2018). The açaí pulp has recently been used as an ingredient or additive in animal feed (Fortuoso et al., 2020; Santos et al., 2020). Additionally, the açaí agro-industrial wastes (fibers) have been used to increase the durability of mortars used in building construction (Azevedo et al., 2021; Marvila et al., 2020).

However, the presence and quantity of phenolic compounds vary depending on maturation, genetic and edaphoclimatic factors (Schulz et al., 2021; Cardoso et al., 2018; Geraldi et al., 2018). On the other hand, the standardization of methods and the conditions of extraction of these bioactive compounds are significant factors that must be taken into consideration. Due to this fruit's nutritional importance and considering that the extraction of bioactive compounds with antioxidant potential involves several factors, an experimental design is necessary. Thus, this work aimed to investigate the influence of different solvents in the phenolic compound profile and antioxidant properties of açaí juçara from the Atlantic Forest in the Paraná state.

2 Material and methods

2.1 Chemicals

Gallic acid (GA), catechin (CAT), vanillic acid (VA), caffeic acid (CAA), syringic acid (SA), epicatechin (EPI), coumaric acid (COA), ferulic acid (FA), salicylic acid (SAA), trans-cinnamic acid (TCA), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Trolox, potassium persulphate, 2,4,6-Tris (2-pyridyl)-1,3,5-triazine (TPTZ), and Folin Ciocalteu reagents, were all of analytical High-Performance Liquid Chromatography (HPLC) grade Merck (Darmstadt, Germany).

2.2 Sample

The açaí juçara pulp (*E. edulis* Mart.) was kindly donated by the Agronomic Institute of Paraná (*Instituto Agronômico do Paraná* (IAPAR)) in the city of Morretes/ PR. The pulp was lyophilized (Liotop - L1019, São Paulo, Brazil) and stored at -12 °C.

2.3 Experimental design and preparation of the extract

A Completely Randomized Design (CRD) was used with only a factor and three levels, totalling three runs in quadruplicate. The effect of the solvent variable (water, ethanol 99.9%, and ethanol: water 70% v/v) at 60 °C during 30 min on the extraction of phenolic compounds with antioxidant activity was determined. The dependent variables used were the Total Phenolic Compounds (TPC) by the Folin-Ciocateau spectrophotometric method and the evaluation of antioxidant activity through DPPH, ABTS and Ferric Reducing Antioxidant Power (FRAP) methods.

The solvents used for extraction were water, ethanol pure and ethanol: water 70% v/v. The lyophilized pulp of açaí juçara (3 g) was added to 30 mL of solvent and subjected to stirring in a shaker (SL 222, Piracicaba, Brazil) at 120 rpm for 15 min. After this time, the extracts were centrifuged at 5000 rpm (Hermle Z 200 A) for 15 min. The supernatant was filtered and stored at -12 °C. All the determinations were performed in triplicate.

2.4 Total Phenolic Compounds (TPC)

The TPC was analyzed by the Folin-Ciocalteu technique reported in Singleton et al. (1999). The standard used was GA, and the values of TPC were expressed in mg GAE/g açaí (GAE: gallic acid equivalent).

2.5 Antioxidant activities

2.5.1 DPPH (2,2 Diphenyl-1-picryl-hydrazyl) radical scavenging assay

The determination of free radical scavenging activity was described on a spectrophotometer at 517 nm as according to Brand-Williams et al. (1995), using Trolox as standard.

2.5.2 Ferric Reducing Antioxidant Power (FRAP) assay

The antioxidant power of iron reduction was determined according to Pulido et al. (2000), based on the antioxidant ability to reduce the Fe^{+3} to Fe^{+2}, in the presence of TPTZ and the absorbance of the samples was measured on a spectrophotometer (Femto UV 2000, Brazil) at 595 nm. The results were expressed as µmol Fe^{2+}/g of açaí juçara.

2.5.3 ABTS (2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid)) assay

The ABTS method for determination of antioxidant activity was performed according to the methodology described by Re et al. (1999), and the extracts were measured at 734 nm on a spectrophotometer (Femto UV 2000, Brazil). The results were expressed in µmol of TEAC/g of açaí juçara (TEAC: antioxidant capacity equivalent to Trolox).

2.6 Identification of phenolic compounds using HPLC/DAD

In this analysis, it was used the same equipment, column, Limit of Quantification (LQ) and Limit of Detection (LD) as described by Oldoni et al. (2015). The mobile phase (A) consisted of ultrapure water and the mobile phase (B) acetonitrile, both containing acetic acid at 0.2 mL/L. A flow rate was 1mL/min and a total run time of 45 min (started with 5% B to 95% B in 36 min and returned to the initial condition) at 30 °C. The phenolic compounds in the extracts were identified based on the comparison of the spectra of 280 and 320 nm with available external standards (CAA, CAT, COA, EPI, FA, SAA, SA, TCA and VA). The results were expressed in µg/g of sample. All analyses were performed in the three analytical replicates.

2.7 Statistical analysis

Statistical analyses were performed using the computer STATISTICA program 8.0 version (StatSoft, USA). The data were subjected to Analysis of Variance (ANOVA) ($p < 0.05$) and the means compared by Tukey's test ($p < 0.05$). Data were expressed as means ± Standard Deviation (SD).

3 Results and discussion

3.1 Total phenolic compounds and antioxidant activities

The extracts obtained through the treatments T1 (water), T2 (ethanol pure), and T3 (ethanol 70%) are shown in Table 1. In this study, treatments T1 and T3 did not significantly differ in TPC. The TPC values ranged from 345.32 to 93.99 mg GAE/g. The highest TPC values were found in the treatment T3. Whereas the lowest TPC values were observed when the pure ethanol (T2) was used as a solvent, and significant

differences were found among the other treatments. This difference may be justified by the polarity of the solvents, in which this study showed that polar and medium polar solvents had a higher capacity to extract the phenolic compounds. According to Papoutsis et al. (2018), water can be an alternative solution for extraction; nonetheless, it can achieve extraction yields of phenolic compounds lower than those obtained by less-polar solvents.

Table 1. Total phenolic compounds and antioxidant activities in açaí juçara (*E. edulis* Mart.) extracts.

Treatments	Solvents	TPC (mg GAE*g^{-1})	DPPH (µmol Trolox g^{-1})	ABTS (µmol Trolox g^{-1})	FRAP (µmol de Fe^{2+} g^{-1})
T1	Water	311.37 ^{a} ± 28.94	99.65 ^{b} ± 5.66	16.53 ^{b} ± 0.20	755.08 ^{b} ± 5.10
T2	Ethanol 99.9%	93.99 ^{b} ± 13.27	214.49 ^{a} ± 11.36	15.96 ^{c} ± 0.07	241.36 ^{c} ± 12.02
T3	Ethanol 70%	345.32 ^{a} ± 21.78	211.90 ^{a} ± 3.54	17.52 ^{a} ± 0.01	1659.91 ^{a} ± 15.30

T1: extraction with water. T2: extraction with ethanol 99.9%. T3: extraction with ethanol 70%. *GAE: Gallic Acid Equivalent. Values of means of triplicates ± Standard Deviation (SD). Means followed by different letters in the same column differ statistically from each other by the Tukey's test ($p < 0.05$).

In the current study, the TPC values for açaí juçara (*E. edulis* Mart.) were higher than those previously reported (Reis et al., 2017; Bernardes et al., 2019). In other studies, other species of açaí juçara from different collection areas were used. Additionally, different extraction conditions were used in these experiments. Paz et al. (2015) studied açaí (*E. oleracea*) from the Amazon rainforest and reported 18.08 mg GAE/ g when ethanol (50% v/v) was used as a solvent at 25 °C for 1 h in a shaker. On the other hand, Caramês et al. (2020) used aqueous acetone solution (1:1 v/v), with agitation in a vortex for 90 seconds and microwave-assisted extraction for açaí *E. oleracea*, obtained 11.93 mg GAEq/g. Finally, Bernardes et al. (2019) found 1006.77 mg GAE/100 g in açaí *E. edulis* from Espirito Santo state, when 70% ethanol (1:10 m/v) acidified with hydrochloric acid (HCl) was used as a solvent. Thus, these previous studies demonstrated the importance of determining extraction efficiency.

Many studies have also focused on the polyphenol compounds and antioxidant activities of fruit extracts with different extraction solvents (Ribeiro et al., 2018; Martins et al., 2020). Several solvents may be used in the extraction process, and the solvent type is one of the first steps to increase the phenolic compounds recovery. Additionally, the solvent selection is the most critical parameters affecting their industrial application as a natural antioxidant (Anjos et al., 2019; Papoutsis et al., 2018).

One of the reasons for choosing ethanol in this study is associated with the already known characteristics of this matrix, principally regarding the main compounds solubility. Besides, the low toxicity for human health, easy evaporation, and incorporation into food and pharma products are other important reasons for ethanol choosing. Moreover, the ethanol has been chosen as a solvent in several food matrices, such as curry leaf (Sepahpour et al., 2018), Chinese sumac fruits (Zhang et al., 2018), chia (Alcântara et al., 2019) and apple pomace (Ferrentino et al., 2018).

Antioxidant properties assay of these extracts are required steps in this study and cannot be evaluated satisfactorily by an only method without highlighting the many variables that influence the results (Casagrande et al., 2019; Zielinski et al., 2016). Thus, the antioxidant activities of the açaí extracts were evaluated by the DPPH, ABTS, and FRAP methods. The antioxidant activity evaluated by free radical scavenging assays ranged from 99.65 to 214.49 µmol Trolox/ g ($DPPH^{\bullet}$) and from 15.96 to 17.52 µmol Trolox/ g ($ABTS^{\bullet+}$). However, concerning the FRAP method, which is characterized only by electron transferability, the antioxidant activity values ranged from 241.36 to 1659.91 µmol Fe^{2+}/g. The highest antioxidant activity level by DPPH, ABTS and FRAP methods were found in the extract of treatment T3. However, with respect to the DPPH method, no significant difference was found in treatment T2. On the other hand, for this parameter, the lowest values of antioxidant activities were found in the extract of treatment T1. Differences among the three treatments were significant for antioxidant activity by ABTS and

FRAP methods. For these methods, the extracts with the highest and lowest values were related to treatments T3 and T2, respectively (Table 1).

Antioxidant activity in this study was higher than found by other authors who studied açaí with treatments at a temperature above 100 °C and found 5.28 µmol Trolox/g by the DPPH method (Castro et al., 2016). In fact, degradation of bioactive compounds can be attributed to extreme extraction temperatures. Borges et al. (2013) used hexane as a solvent on açaí juçara (*E. edulis* Mart.) pulp and obtained lower results for DPPH when compared with this study. This lower antioxidant capacity can be related to the solvent polarity once those antioxidants have a greater ability to capture DPPH radicals in more polar solvents.

TPC and AA's values by ABTS and FRAP methods for the treatment T3 were higher than for other treatments (Table 1). However, the increase in the concentration of phenolic compounds and antioxidant activity by these methods is polarity-dependent. This specific study showed a higher affinity for the intermediate polarity solvent. In fact, the polarity of the solvents used to extract these compounds and antioxidant activity is directly related to the extractive efficiency of the compounds in the plant material (Casagrande et al., 2019; Moura et al., 2018). Therefore, solvents that present a small percentage of water showed more extractive efficiency of compounds with antioxidant potential than the monocomponent solvent systems (Zielinski et al., 2016).

Several antioxidant activity techniques have been used successfully in many fruit species (Amorati & Valgimigli, 2018; Ferrentino et al., 2018). However, in this study, the ABTS method was more efficient than the DPPH method for quantifying antioxidant activity in açaí juçara due to both hydrophilic and lipophilic characteristics of this reagent (Ácsová et al., 2019). In fact, the treatment T3, in which aqueous mixture containing ethanol was used, showed the highest content of phenolic compounds with antioxidant activities. Moura et al. (2018) also observed this positive correlation during the optimization of bioactive compounds extraction in fruits such as açaí from Amazon Forest, blueberry, and goji berry. Thus, according to the results, it is clear that the compounds present in the açaí juçara originated from Atlantic Forest can be easily extracted using medium polarity solvent, i.e., 70% of ethanol.

3.2 Phenolic profile by HPLC and Principal Component Analysis (PCA)

The profile of phenolic compounds by HPLC in the açaí extracts obtained from the different treatments (T1, T2 and T3) was performed with different channels and wavelengths ranging from 276 to 371 nm. The regression equation data, correlation coefficient, wavelengths, and retention time are shown in Table 2.

Table 2. Phenolic profile determined by HPLC in the extracts of açaí juçara (*E. edulis* Mart.).

Compounds	RT (min)	ƛ (nm)	R^2 (Correlation coefficient)	T1 (µg/g)	T2 (µg/g)	T3 (µg/g)
Catechin (CAT)	10.00	276	y=0.1794x – 0.156 (R^2=0.9311)	nd	nd	13.82 ± 2.70
Caffeic acid (CAA)	12.50	320	y=1.0138x – 1.229 (R^2= 0.9679)	12.18 ± 0.40	nd	nd
Coumaric acid (COA)	18.90	309	y=1.7284x – 3.223 (R^2= 0.9094)	37.07^{b} ± 2.63	19.31^{c} ± 1.34	42.63^{a} ± 2.12
Salicylic acid (SAA)	22.70	303	y=0.616x – 0.525 (R^2= 0.9453)	91.67^{b} ± 7.08	72.83^{b} ± 9.94	166.89^{a} ± 10.71
Epicatechin (EPI)	15.00	276	y=0.2588x – 0.278 (R^2= 0.9381)	nd	nd	13.44 ± 1.19

T1: extraction with water. T2: extraction with ethanol 99.8%. T3: extraction with ethanol 70% with time of 30 minutes and temperature of 60 °C. Values of means of triplicates ± Standard Deviation (SD). Means followed by different letters on the same line differ statistically from each other by the Tukey's test ($p < 0.05$). nd: not detected

Five phenolic compounds were found in the açaí extracts as following: CAA; CAT; COA; EPI; and SAA. However, CAT and EPI were not found in the extract in treatment T1. In this aqueous extract,

CAA (12.18 µg/g) and SAA (91.67 µg/g) were present in lower and higher concentrations, respectively. CAT (13.82 µg/g) and EPI (13.44 µg/g) were found only in treatment T3 (ethanol 70%), however not was found significant differences among the results.

In this study, COA and SAA were found in all treatment and ranged from 19.31 to 42.63 µg/g and from 72.83 to 166.89 µg/g, respectively. However, there was a significant difference ($p < 0.05$) between the treatments. On the other hand, CAA was found only in treatment T1 at a lower concentration of 12.18 µg/g (Table 2).

Several authors have found different phenolic compounds in açaí (*E. oleraceae*), such as VA, CAA, and GA (Garzón et al., 2017). CAT, EPI, rutin, and myricetin were also reported by Moura et al. (2018) in açaí species grown in the Brazilian Amazon forest. However, these values were in lower concentrations than those highlighted herein in this study. The divergences found between the studies regarding concentrations of the compounds identified or their absence are directly related to the differences in the extraction conditions, the equipment sensitivity, and the methodologies used.

The correlation between the treatment conditions and the extractability of individual phenolic compounds from açaí extracts were investigated using HPLC and PCA (Figure 1). To examine the correlation of the treatment conditions, and the extractability of individual phenolic compounds from açaí extracts using HPLC, the PCA was used. The first principal component (PC1) described 61.43% of the variation of extraction experiments, whereas the second principal component (PC2) showed 32.80%, thus contributing to 94.23% of the total variation of treatments. Three groups represented by the different extraction treatments and phenolic compounds identified by HPLC were formed. Indeed, treatment T2 represented the first group (first quadrant) (Figure 1), the second group (third quadrant) was represented by treatment T3 and phenolic compounds: CAT; EPI; SAA; and COA and finally the third group (fourth quadrant) was represented by T1 and CAA.

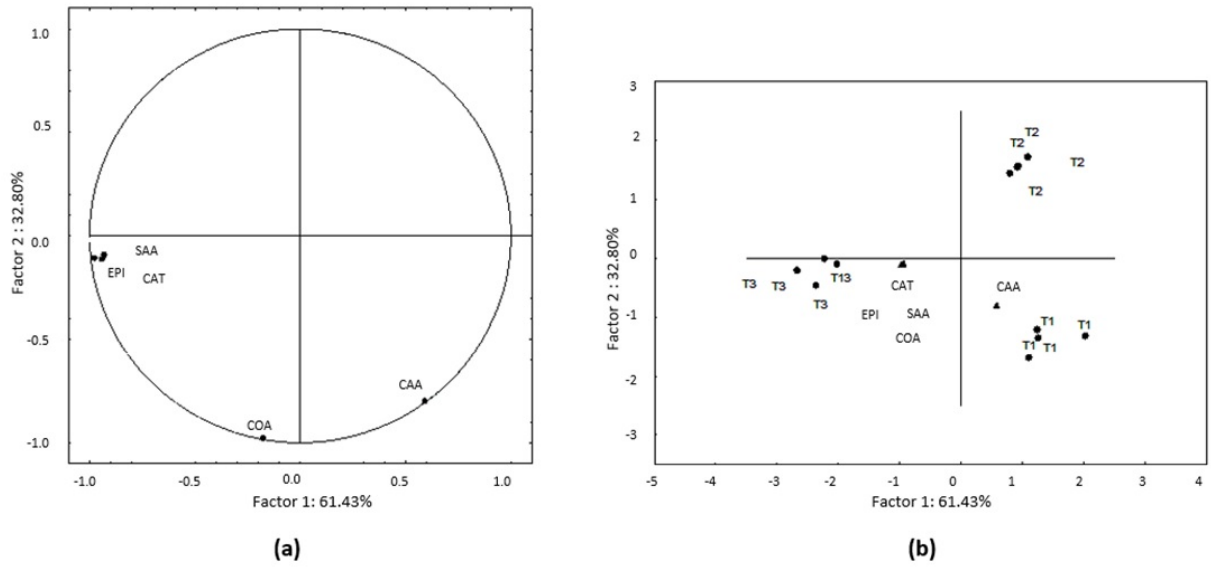

Figure 1. Principal component analysis chart. (a) scores of identified phenolic compounds; (b) Projection of identified phenolic compounds and different types of extraction. EPI (epicatechin); SAA (salicylic acid); CAT (catechin); COA (coumaric acid); CAA (caffeic acid). T1: extraction with water; T2: extraction with ethanol 99.8%; T3: extraction with ethanol 70% for 30 minutes and at 60 °C.

From PCA analysis, it was concluded that treatment T3 was more efficient in extracting polyphenols since four different compounds could be identified in this extract. This difference was denoted by the solvent polarity, which medium polar compounds such as CAT and EPI that were easily extracted. Furthermore, it is

important to note that polarity of phenolic compounds can vary significantly. They may have different hydroxyl groups (position and quantity) conjugated to other compounds such as alkyl, acid, or sugar groups. However, it is difficult to develop an ideal method for extraction of all polyphenols (Ameer et al., 2017). In this context, we could consider the extraction with the largest range of compounds.

Based on previous studies that the phenolic compounds are significant contributors to the antioxidant properties of fruits, the extracts containing these bioactive compounds have become an alternative source for the pharmaceutical and food industry to replace synthetic antioxidants (Carpes et al., 2020; Anjos et al., 2019; Neha et al., 2019; Almeida et al., 2017). The toxicological effects could potentially result in health implications of these antioxidants commonly employed in the industry, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) (Gulcin, 2020).

The search strategies for identifying natural substances with antioxidant potential has become more frequent in studies due to the significant importance of relating, economy, diet, and health (Casagrande et al., 2019).

4 Conclusion

The effects of solvent type on total and individual phenolic compounds, antioxidant activities were determined using a Completely Randomized Design and Principal Component Analysis. These tools were useful to optimize the extraction of phenolic compounds in a simplified way. In this work, the best condition for the extraction of polyphenols occurred using ethanol 70% at 60 °C for 30 min. Remarkably, the açaí juçara contained much stronger ethanol 70% soluble antioxidants than pure ethanol or pure water. The use of this solvent presented better results towards the total phenolic compounds and antioxidant activity by different methods. In addition, it was also able to extract phenolic compounds identified by HPLC, such as catechin, epicatechin, salicylic acid, and coumaric acid, which are known for the high antioxidant activity. This study reported a simple and rapid method to extract phenolic compounds from açaí juçara. These results suggested that the açaí juçara from Atlantic Forest is a potential source of natural antioxidants both for consumption and as an alternative for use in several chemistry and food areas.

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