Current Plant Biology 29 (2022) 100233

Contents lists available at ScienceDirect

Current Plant Biology

journal homepage: www.elsevier.com/locate/cpb

Phenolic composition of peels from different Jaboticaba species determined by HPLC-DAD-ESI/MS^n and antiproliferative activity in tumor cell lines

Michelly Cristiane Paludo [a], Silvia Borges Pimentel de Oliveira [b], Luciana Fontes de Oliveira [c], Ronan Carlos Colombo [d], Sergio Gómez-Alonso [e], Isidro Hermosín-Gutiérrez [e], Rafaela Prata [a], Adriano Freitas Lima [a], José Teixeira Filho [f,g], Cristiano Augusto Ballus [h], Helena Teixeira Godoy [a,*]

[a] Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil
[b] Department of Structural and Functional Biology, State University of Campinas, Av. Bertrand Russel, CP 6109, 13083-865 Campinas, São Paulo, Brazil
[c] Institute of Chemistry, University of Campinas, P.O. Box 6194, 13084-971 Campinas, São Paulo, Brazil
[d] Departament of Agronomy, Londrina State University, Celso Garcia Cid Road, km 380, P.O. Box 10.011, 86057-970 Londrina, Paraná, Brazil
[e] Universidad de Castilla-La Mancha, Instituto Regional de Investigación Científica Aplicada, Avda. Camilo José Cela s/n, 13071 Ciudad Real, Spain
[f] Faculty of Agricultural Engineering, Av. Cândido Rondon, 501, 13083-875 Campinas, São Paulo, Brazil
[g] Institute of Geosciences, University of Campinas (UNICAMP), R. Carlos Gomes, 250, 13083-855 Campinas, São Paulo, Brazil
[h] Department of Food Science and Technology, Centre for Agrarian Sciences, Federal University of Santa Maria, Av. Roraima 1000, 97105-900 Santa Maria, Rio Grande do Sul, Brazil

ARTICLE INFO

Keywords:
Phenolic compounds
Jaboticaba residues
Cancer
Antiproliferative properties
Food industries

ABSTRACT

Jaboticaba has been studied extensively, mainly in the context of its phenolic composition. Although there are several varieties, research has been concentrated on Sabará and Paulista due to their greater dispersion. This study aimed to characterize five jaboticaba varieties in terms of their anthocyanin and nonanthocyanin phenolic compositions and their antiproliferative action on tumor cells of breast and prostate cancer. The most abundant anthocyanins were cyanidin-3-glycoside and delphinidin-3-glycoside. Three myricitin derivatives, 14 quercetin derivatives (including free quercetin), 13 ellagic acid derivatives (including free ellagic acid) and 4 methylellagic acid derivatives were detected. The variety that showed the best antiproliferative action was Pintada (PFP), which was harvested in the 2015 crop. The phenolic compounds showed differences in different crop years however, samples from both years decreased cellular proliferation.

1. Introduction

"Jaboticaba" as it is popularly known, is a Brazilian berry species cultivated throughout the country [1]. Its pulp presents desirable sensory characteristics and high concentrations of iron, copper, manganese, and vitamin C [2]. Moreover, fruits of jaboticaba are rich in phenolic compounds, mainly anthocyanins (responsible for its intense dark peel), flavonols, and hydrolyzable tannins (ellagitannins, gallotannins), which exhibit activity against free radicals resulting in potent antioxidant activity [3].

Usually, the peel is not consumed due to its stiffness and astringent taste thus the peel is responsible for the generation of large amounts of residues in the manufacture of products derived from jaboticaba fruit [4]. Use of the peel (up to 35% of the fruit weight) as a source of antioxidants is a sustainable alternative, and it has been used in food industries to produce jams, ice cream, and beverages [1,3,5].

Currently, there is growing interest in the antioxidant activity of the phytochemicals present in the diet since they play a very important role in an organism's defense [6]. According to Inada et al. [2], several studies show that jaboticaba presents in vitro and in vivo biological activities, which have been mainly associated with its phenolic compounds. Furthermore, phenolic compounds from jaboticaba extracts appear to contribute to potential health benefits, such as diabetes control and prevention of cardiovascular diseases and neurological disorders [7]. Thus, jaboticaba fruits have been consumed as a source of nutrients and bioactive compounds, and not only for their sensory properties and personal preference [6]. Additionally, there is evidence not only of the antioxidant capacity of jaboticaba fruits but also of the

* Corresponding author.
E-mail address: helenatg@unicamp.br (H.T. Godoy).

https://doi.org/10.1016/j.cpb.2021.100233
Received 13 September 2021; Received in revised form 6 December 2021; Accepted 13 December 2021
Available online 16 December 2021

Table 1
Climate parameter per month in cultivar regions for the crop years.

Region	Parameter	Crop year	Month											Annual
			Jan	Feb	Mar	Apr	May	Jun	Jul	Ago	Sep	Oct	Nov	Dec	
Lagoa Branca	Temperature (ºC)	2014	25.3	27.2	25.2	22.1	19.8	19.7	18.6	20.9	22.8	24.6	23.3	23.6	**22.8**
		2015	25.8	23.7	22.5	22.3	19.5	19.3	20.0	21.7	22.9	25.1	23.7	23.8	**22.5**
	Rainfall (mm)	2014	50.6	49.4	9.4	70.2	25.8	7.8	43.2	0.0	59.8	61.2	219.0	116.8	**59.4**
		2015	58.4	217.2	220.4	25.6	0.6	0.0	0.0	13.6	119.0	90.2	175.4	95.0	**84.6**
Araçoiaba da Serra	Temperature (ºC)	2014	24.8	25.2	23.0	20.9	18.5	18.0	17.0	18.4	20.7	22.3	21.9	23.1	**21.2**
		2015	25.1	22.9	22.0	20.8	18.4	17.7	17.3	19.3	209	22.3	22.5	23.7	**21.1**
	Rainfall (mm)	2014	79.4	157.8	109.6	88.2	27.4	15.2	26.8	40.8	832	19.8	151.2	227.8	**85.6**
		2015	199.8	242.0	165.0	33.8	66.8	5.8	124.0	19.2	190.4	100.6	284.2	72.8	**125.4**

effects of these compounds on different types of cancer [8,9] and the immune system [10,11].

Cancer in the broader sense refers to more than 277 different types of cancer disease. It is a serious problem affecting the health of all human societies and is considered the second leading cause of mortality worldwide [12]. Prostate cancer is the second most common and fifth most aggressive neoplasm among men worldwide. It is known that one man in 25 worldwide is likely to receive a prostate cancer diagnosis within his lifetime [13]. Another cancer of great worldwide occurrence is breast cancer. According to Heer et al. [14] among women, breast cancer is the most common malignant disease worldwide, accounting for 24% of new cancer cases.

Natural products, especially of plant origin have continuously served as major sources of drugs against diseases such as cancer, diabetes, and microbial infections among others. Currently, over 60% of anticancer drugs are derived from plants [15]. Thus, the control and treatment of cancer have prompted researchers to seek new control strategies, aimed at the improvement of nonconventional therapies [16]. Hou et al. [17] showed that anthocyanin cyanidin-3-glycoside can inhibit the proliferation of breast cancer cells and similar results were described by Fang [18]. Moreover, the potential of jaboticaba peel to inhibit tumor cell growth was observed in four different tumor cell lines (lung, breast, hepatocellular, and cervical carcinoma) [4]. The breast cancer cell lineage used in many studies is MDA-MB-231. According to Fung et al. [19], this cell lineage is more susceptible to dietary interventions than other lines. A possible synergistic effect between anthocyanin and ellagitannin metabolites in reducing MDA-MB-231 cell proliferation was reported by Teixeira et al. [20].

Considering all possible health benefits of anthocyanin and nonanthocyanin phenolic compounds, the identification and quantification of these compounds in foods is extremely important. To carry out these studies with sensitivity and efficiency, it is necessary to use modern techniques [21,22]. Therefore, advanced separation techniques such as high-performance liquid chromatography (HPLC), coupled to photodiode array detection (PDA) and/or to mass spectrometry (MS) have been used to analyze anthocyanins and nonanthocyanin phenolic compounds that always coexist in plant extracts [23]. Thus, the present study was performed using HPLC coupled to a UV–vis diode array detector (DAD) in tandem with an electrospray ionization multidimensional mass spectrometry (ESI-MS^n) detector.

The present study aimed to characterize the phenolic profiles of five jaboticaba peel varieties (in two crop seasons): *Myrciaria jaboticaba* (Vell.) O. Berg., popularly known as jaboticaba Sabará, *Myrciaria cauliflora* (DC.) O. Berg., popularly known as jaboticaba Paulista, *Myrciaria coronata Mattos*, popularly known as jaboticaba Coroada, Híbrida (*Myrciaria cauliflora* (DC.) O. Berg) and Pintada (*Plinia* spp.). Furthermore, the bioactive potential of jaboticaba peel extracts was also assessed to decrease the proliferation of breast and prostate cancer cells.

2. Materials and methods

2.1. Chemicals

All solvents were HPLC-MS quality, all chemicals were analytical grade (> 99%), and water was ultrapure (Milli-Q Direct 3 system (Millipore, Billerica, MA, USA)). To identify phenolic compounds, commercial standards from Phytolab (Vestenbergsgreuth, Germany): malvidin-3-glycoside chloride and (-)-gallocatechin; Extrasynthese (Genay, France): cyanidin-3-glycoside chloride, quercetin, myricetin and quercetin-3-glycoside; and Sigma-Aldrich (Tres Cantos, Madrid, Spain): (+)-catechin and (-)-gallocatechin 3-gallate were used. Some other, noncommercial flavonol standards (myricetin 3-glucoside and quercetin 3-glucuronide) were previously isolated from 'Petit Verdot' grape peel [24].

Quantification (mg kg^{-1} of dry peel) was express as the most representative compounds for each family of phenolic compounds equivalent: cyaniding-3-glycoside was used for anthocyanins; ellagic acid for ellagic acid and methylellagic acid derivatives; quercetin-3-rhamnoside for flavonols, flavonol 3-glycoside and its free-aglycones; (+)-catechin for flavan-3-ols (total proanthocyanidins); corresponding standards for flavan-3-ol monomers and dimers; and the total using the sum of (+)-catechin equivalents.

2.2. Samples

Sabará (*Myrciaria jaboticaba* (Vell.) O. Berg) (SF) and Paulista (*Myrciaria cauliflora* (DC.) O. Berg) (PF) jaboticaba samples were provided by the Faggan Farmers Group, located in Lagoa Branca, São Paulo (21°46'26" S, 47°05'11" W and 684 m of elevation) in October 2014 and 2015. Other samples of Sabará (*Myrciaria jaboticaba* (Vell.) O. Berg) (SFP), Coroada (*Myrciaria coronata* Mattos) (CFP), Híbrida (*Myrciaria cauliflora* (DC.) O. Berg) (HFP) and Pintada (*Plinia* spp.) (PFP) were provided by F.P. Frutas e Plantas, located in Araçoiaba da Serra, São Paulo, (23°30'19" S, 47°36'51" W and 625 m of elevation), in October 2014 and 2015. The region of Lagoa Branca showed average annual temperatures of approximately 22.5ºC (2014) and 22.8ºC (2015) and average annual rainfall of approximately 59.4 mm (2014) and 84.6 mm (2015). In the region of Sabará, the average annual temperatures were approximately 21.2ºC (2014) and 21.1ºC (2015), and the average annual rainfall was approximately 85.6 mm (2014) and 125.4 mm (2015). Both locations have a tropical climate. More detailed climatological data for the crop years are reported in Table 1.

Fruits were harvested at the maturity index, determined according to the external color, when they were dark purple and full-ripe. Jaboticaba samples were selected after ascertaining the presence of defects or disease, washed, and manually peeled off to separate flesh and peels, which were frozen at -22 °C, freeze-dried, vacuum-packed and stored at -22 °C. The freeze-dried samples were ground to a fine and homogeneous powder to be used to prepare the extracts.

2.3. Preparation of extracts and chromatographic analysis

The extracts of the five jaboticaba varieties were prepared according to Paludo et al. [25]. The extracts were dried in a speed vac (Eppendorf Vacufuge Plus Vacuum Concentrator, Eppendorf) and resuspended in a specific solvent mixture for each chromatographic trial.

For anthocyanin compound analysis, the peel extracts were resuspended in HCl 0.1 N (1:10, v/v), filtered through a polyester membrane (0.20 μm, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany), and directly injected into the HPLC equipment.

To reduce the interference of anthocyanin compounds in flavonol analysis, the extracts from five jaboticaba varieties were previously purified by solid-phase extraction (SPE) according to Castillo-Munõz et al. [24] with some adaptation. The extracts were passed through cartridges (6 mL, 500 mg, Bond Elute Plexa PCX, Agilent®); the anthocyanin-free fractions were eluted in ethanol. These eluates were dried in a rotary evaporator (35 °C), redissolved in 1.5 mL of 20% methanol, and directly injected into the HPLC equipment.

For flavan-3-ol isolation, monomers, B-type dimers, and polymeric proanthocyanidins were isolated from peels of the five jaboticaba varieties using SPE C18 (Sep-Pak Plus C18, Waters Corp., Milford, MA; 820 mg). A mixture of 2 mL of peel extract and 12 mL of ultrapure water was passed through a C18 cartridge previously conditioned with 5 mL of methanol and 5 mL of water. After the cartridge was dried under reduced pressure, methanol (15 mL) and ethyl acetate (5 mL) were added to recover adsorbed phenolics. Later, the solvent was evaporated in a rotary evaporator (35 °C), and the residue was dissolved in methanol (2 mL) and stored at -18 °C until needed.

2.4. Tannins condensed by precipitation with methylcellulose

The tannins were quantified according to the method for condensing tannins by precipitation with methylcellulose [26].

2.5. HPLC–DAD–ESI-MSn identification and quantification of phenolic compounds from five jaboticaba varieties

2.5.1. Analysis of anthocyanins

HPLC separation, identification and quantification of the anthocyanins were performed in an Agilent 1100 Series liquid chromatograph equipped with an ion trap mass spectrometer. First, 10 μL of extract was injected on a C18 reverse-phase column (Zorbax Eclipse, 2.1 × 150 mm; 3.5 μm particle size, Agilent) at a controlled temperature of 40 °C [27]. The solvents used were a mixture of water:acetonitrile: formic acid (88.5:3:8.5, v/v/v, solvent A; 41.5:50:8.5, v/v/v, solvent B) with a flow rate of 0.19 mL min^{-1}. The linear solvent gradient for anthocyanin analysis was 0 min, 6%; 10 min, 30%; 30 min, 50%; 34 min, 100%; 36 min, 100%; and 42 min, 6%. The ESI-MS/MS parameters were as follows: positive ionization mode; dry gas, N_2, 11 L min^{-1}; drying temperature, 350 °C; nebulizer, 65 psi; capillary, -2500 V; capillary out, 70 V; skimmer 1, 20 V; skimmer 2, 6 V; and scan range, 50–1200 *m/z*. For quantification we used the extracted chromatograms obtained at 520 nm, and the total anthocyanin concentration is expressed as cyanidin-3-glycoside equivalent (mg kg^{-1} of dry peel).

2.5.2. Analysis of nonanthocyanin

To analyze nonanthocyanin compounds, the same equipment described for anthocyanin analysis was used. First, 20 μL of SPE extract was injected on a C18 reverse-phase column (Zorbax Eclipse, 2.1 × 150 mm; 3.5 μm particle size, Agilent) at a controlled temperature of 40 °C [27]. The solvents used were solvent A (acetonitrile:water:formic acid, 3:88.5:8.5, v/v/v), solvent B (acetonitrile:water:formic acid, 50:41.5:8.5, v/v/v), and solvent C (methanol:water:formic acid, 90:1.5:8.5, v/v/v). The flow rate was 0.19 mL min^{-1}, and the linear solvent gradient was 0 min, 98% A and 2% B; 8 min, 96% A and 4% B; 37 min, 70% A, 17% B, and 13% C; 51 min, 50% A, 30% B, and 20% C; 51.5 min, 30% A, 40% B, and 30% C; 56 min, 50% B and 50% C; 57 min, 50% B and 50% C; 64 min, 98% A and 2% B. For quantification we used the extracted chromatograms obtained at 360 nm, and the total concentrations of flavonols and ellagic derivatives were expressed as quercetin-3-rhamnoside (mg kg^{-1} of dry peel) and ellagic acid (mg kg^{-1} of dry peel) equivalents, respectively.

2.5.3. Identification and quantification of flavan-3-ol monomer content and the total content and structural characteristics of oligomers (proanthocyanidins) using multiple reaction monitoring (MRM) in HPLC-DAD-ESI-MS/MS

The individual contents of flavan-3-ol monomers were analyzed directly in the extracts. Structural information and estimation of the total proanthocyanidin (PA) content were obtained following the acid-catalyzed depolymerization method and were assisted by pyrogallol [27–29].

2.6. Antiproliferative activity in tumor cell lines

The extracts of the five varieties of jaboticaba peel were prepared according to Paludo et al. [25]. The extracts were dried in a speed vac (Eppendorf Vacufuge Plus Vacuum Concentrator, Eppendorf) and resuspended in ultrapure water/dimethylsulfoxide (DMSO). For this study cellular lineages established from prostate (DU-145) and breast cancers (MDA-MB-231) were used. Stock cultures were maintained in RPMI culture medium with 1 g/L glucose containing 10% fetal bovine serum (FBS) and supplemented with 1% penicillin-streptomycin. Cell lineages were kept in a humidified oven at 35 °C and 5% CO_2. Cell lineages were obtained from American Type Culture Collection (ATCC) (Rockville, MD) and kindly provided by Prof. Dr. Hernandes Faustino de Carvalho, Universidade Estadual de Campinas, UNICAMP, SP, Brazil.

Cell lineages of prostate (DU-145) and breast cancers (MDA-MB-231) were plated at a concentration of 9×10^4 cells per well (well trays = 96) and filled with 100 μL of culture medium (with FBS). After 24 hours, the culture medium was changed, and the cells were subjected to treatments with jaboticaba peel extracts at concentrations of 2.5, 25, 50 and 250 μg mL^{-1}. Cells were treated with doxorubicin for 24, 48 [6,11] or 72 hours. The extracts were diluted in culture medium without FBS, and the final concentration of DMSO was at most 0.2% to avoid harming cellular viability. Control groups consisted of cells cultivated in culture medium with DMSO and without FBS.

After each incubation period, the cell viability was assessed with the colorimetric thiazolyl blue tetrazolium bromide method (mitochondrial activity assay (MTT)), which is based on the cleavage of tetrazolium salt. This cleavage produces formazan, which is insoluble in water and presents a blue color. The formazan is then solubilized in isopropanol and its absorbance is measured in a spectrophotometer. The formazan produced in this reaction is directly proportional to the number of viable cells present in the culture medium at the moment, MTT is added [30]. Here 10 μL of solution prepared from 5 mg mL^{-1} MTT was added to the culture medium. Then, the plate was kept in a humidified oven (95%) at 37 °C and 5% CO_2 for three hours, after wich 100 μL of acidified isopropanol (isopropyl alcohol and hydrochloric acid) was added. Readings were performed after 10 min at 540 and 570 nm in a microplate reader [31].

2.7. Statistical analysis

Analysis of variance (ANOVA) was applied to the experimental data, and the averages were compared by Tukey's test and the Student-Newman-Keuls test with a 5% significance level ($\alpha = 0.05$) using the statistical software Statistica 7.0 (Statsoft Inc., Tulsa, OK, U.S.A). For cellular trials, the inhibitory concentration that decreased the amount of reactive species in the tested medium by 50% (IC_{50}) was determined using Graph Pad Prism 5 software. Phenolic compound groups also underwent principal component analysis (PCA) using the statistical

Table 2
Anthocyanin and nonanthocyanin phenolic compounds identified by HPLC-DAD-ESI-MS/MS in the peels of five jaboticaba varieties.

Peak	Assignation[a]	Rt (min)	UV-vis (nm)	MS (*m/z*)	MS^2 (*m/z*)
Flavonols					
4	**M-hex-1 (M-3-gal)**	20.5	255; 265 sh; 305 sh; 352	479.2	316.9; 315.8
2	**M-hex-2 (M-3-glu)**	19.0	352,0	479.4	316.8; 315.8
5	**M-rhm**	24.6	255 sh; 261; 305 sh; 348	463.4	316.7; 316.0
9	**Q-hex-1 (Q-3-gal)**	27.5	256 sh; 262; 305 sh; 352	463.4	300.8; 300.1
11	**Q-hex-2 (Q-3-glu)**	29.6	353,0	463.4	300.8; 300.0
17	**Q-glcu (Q-3-glucur)**	39.6	352,0	463.4	300.8; 300.0
12	**Q-pent-1**	30.6	351,0	433.2	300.8
13	**Q-pent-2**	32.3	354,0	433.3	300.8
14	**Q-pent-3**	33.2	256; 262 sh; 310 sh; 350	433.4	300.8
15	**Q-rhm (Q-3-rhm)**	35.0	255; 260 sh; 305 sh; 348	447.4	300.8
16	**Q-galloyl-pent-1**	37.9	357,0	585.2	(433.4); 300.9
18	**Q-galloyl-pent-2**	40.7	357,0	585.2	(433.4); 300.9
23	**Q-cm-hex-1**	47.6	255 sh; 265; 295 sh; 314; 352 sh	609.3	463.0; 301.0
24	**Q-cm-hex-2**	47.7	265 sh; 295 sh; 314; 352 sh	609.3	462.9; 300.9
22	**Q-fer-hex-1**	47.0	255; 265 sh; 300 sh; 325; 352 sh	639.2	476.9; 462.9; 314.9; 300.8
25	**Q-fer-hex-2**	49.3	255; 265 sh; 295 sh; 328; 352 sh	639.2	477.0; 462.9; 315.1; 300.9
19	**Free-Q**	44.7	255; 265 sh; 300 sh; 371	301.0	299.8; 270.8; 254.7; 228.8; 178.7; 150.7
Ellagic derivatives					
1	**EA-Hex**	15.6	254; 292 sh; 345 sh; 360	463.2	300.8
3	**EA-pent-1**	19.4	253; 292 sh; 345 sh; 358	433.3	300.8; 299.8
7	**EA-pent-2**	26.3	254; 295 sh; 348 sh; 359	433.4	300.7
8	**EA-rhm-1**	27.2	254; 261 sh; 303 sh; 348 sh; 359	447.5	300.8
10	**EA-rhm-2**	28.5	254; 260 sh; 290 sh; 348 sh; 362	447.4	300.6; 299.9
20	**EA-ac-rhm-1**	45.8	252; 262 sh; 308 sh; 345 sh; 361 sh; 376	489.3	299.8; 300.8
26	**EA-ac-rhm-2**	50.0	254; 260 sh; 295 sh; 336 sh; 345 sh; 358	489.3	299.8; 300.8
27	**EA-valeryl-rhm-1**	52.9	254; 260 sh; 300 sh; 348 sh; 361	531.4	488.9; 470.8; 300.8; 299.8
28	**EA-valeryl-rhm-4**	55.2	254; 260 sh; 300 sh; 348 sh; 361	531.2	488.9; 470.8; 300.8; 299.8
30	**EA-valeryl-rhm-2**	57.8	254; 260 sh; 305 sh; 355 sh; 373	531.3	470.9; 300.8; 299.7
31	**EA-valeryl-rhm-3**	57.9	254; 260 sh; 300 sh; 348 sh; 361	531.3	488.9; 471.0; 300.8; 299.8
33	**EA-caprilyl-rhm**	60.3	256; 265 sh; 297 sh; 345 sh; 360	573.3	531.5; 513.0; 300.7; 299.8
6	**Free-EA**	25.5	253; 292 sh; 355 sh; 367	301.3	300.8; 256.8; 228.7; 184.7
32	**Me-EA-valeryl-rhm**	59.4	253; 265 sh; 290 sh; 348 sh; 365	545.5	503.3; 485.0; 470.0; 442.3; 314.9; 299.9
34	**Me-EA-caprilyl-rhm**	61.6	255; 265 sh; 297 sh; 344 sh; 357	587.6	544.9; 527.0; 467.1; 315.2; 314.0; 300.1
21	**Me-EA-rhm**	46.8	253; 262 sh; 290 sh; 352 sh; 365	461.4	314.9; 300.3
29	**Me-EA-ac-rhm**	57.0	253; 260 sh; 290 sh; 351 sh; 365	503.2	460.7; 442.9; 428.2; 314.8; 299.9
Anthocyanins					
a	**dp-3-glc**	6.207	287; 523	465	303
b	**cy-3-glc**	9.284	280; 516	449	287
c	**pt-3-glc**	11.124	284; 506	479	317
d	**pg-3-glc**	11.224	283; 520	433	271
e	**pn-3-glc**	12.925	283; 518	463	301
f	**dp-3-acglc**	14.393	280; 524	507	303
g	**cy-3-acglc**	16.127	281; 519	491	287
h	**dp-3-cmglc**	18.185	299; 528	611	303
i	**cy-3-cmglc**	20.313	284; 314 sh, 440 sh; 520	595	287
j	**cy-3-ferglc**	20.86	270; 331, 440 sh; 520	625	287

[a] M (myricetin derivatives): M-hex-1 (myricetin-hexose), M-hex-2 and M-rhm (myricetin-rhamnoside); Q (quercetin derivatives): Q-hex-1 (quercetin-hexose), Q-hex-2, Q-glcu (quercetin-glucuronide), Q-pent-1, Q-pent-2 and Q-pent-3 (quercetin-pentose isomers), Q-rhm (quercetin- rhamnoside), Q-galloyl-pent-1 and Q-galloyl-pent-2 (quercetin-galloyl-pentose isomers), Q-cm-hex-1 and Q-cm-hex-2 (quercetin-coumaroyl-hexose isomers), Q-fer-hex-1 and Q-fer-hex-2 (quercetin-feruloyl-hexose isomers), and Free-Q (free-quercetin); EA (ellagic acid): EA-hex (ellagic acid-hexose), EA-pent-1 and EA-pent-2 (ellagic acid-pentose isomers), EA-rhm-1 and EA-rhm-2 (ellagic acid-rhamnoside isomers), EA-ac-rhm-1 and EA-ac-rhm-2, (ellagic acid-acetyl-rhamnoside isomers), EA-valeryl-rhm-1, EA-valeryl-rhm-4, EA-valeryl-rhm-2 and EA-valeryl-rhm-3 (ellagic acid-valeryl-rhamnoside isomers), EA-caprilyl-rhm (ellagic acid-caprilyl-rhamnoside), and Free-EA (free-ellagic acid); Me-EA (methylellagic acid): Me-EA-valeryl-rhm (methylellagic acid-valeryl-rhamnoside), Me-EA-caprilyl-rhm (methylellagic acid-caprilyl- rhamnoside), Me-EA-rhm (methylellagic acid-rhamnoside), and Me-EA-ac-rhm (methylellagic acid-acetyl-rhamnoside); hex: hexose; pent: pentose; rhm: rhamnosis; gal: galactose; glu: glucose; ac: acetic acid; cm: *p*-coumaric acid; fer: ferulic acid; dp: delphinidin; cy: cyanidin; pg: pelargonidin; pn: peonidin; pt: petunidin; glc: glycoside; cmglc: coumaryl-glicoside; acglc: acetylglicoside; ferglc: feruloylglycoside.

software SAS 9.0 (SAS Institute, Cary, NC, U.S.A) and SPSS Statistics (IBM Corporation, NY, U.S.A.).

3. Results and discussion

3.1. Nonanthocyanin phenolic compounds from peels of five jaboticaba varieties

Applying HPLC procedures to nonanthocyanins phenolic compounds

Table 3
Anthocyanin and nonanthocyanin phenolic compounds contents obtained by HPLC-DAD-ESI-MS/MS for the peels of five jaboticaba varieties (mean ± standard deviation, n = 3).

Non-anthocyanin phenolic compounds													
Peak	**Assignation**[†]	**SF molar%**		**PF molar%**		**SFP molar%**		**PFP molar%**		**HFP molar%**		**CFP molar%**	
		2014 Crop	**2015 Crop**	**2014 Crop**	**2015 Crop**	**2014 Crop**	**2015 Crop**	**2014 Crop**	**2015 Crop**	**2014 Crop**	**2015 Crop**	**2014 Crop**	**2015 Crop**
4	**M-hex-1**	ND	0.96 ± 0.05 b	ND	ND	1.10 ± 0.04 b	0.35 ±0.03 d	ND	1.55 ± 0.33 a	0.58 ± 0.02 c	ND	1.32 ± 0.24 a	0.88 ± 0.15 b
2	**M-hex-2**	ND	ND	ND	ND	ND	ND	5.54 ± 1.69 b	14.28 ± 1.06 a	ND	ND	ND	ND
5	**M-rhm**	26.18 ± 2.22 c d	24.36 ± 2.20 d	38.93 ± 0.67 a	27.14 ±1.12 c d	32.12 ± 1.36 b	29.26 ± 0.69 b c	8.14 ± 1.41g	14.70 ± 2.35f	21.04 ± 0.47 e	16.64 ± 0.51f	37.43 ± 3.30 a	29.44 ± 2.86 bc
9	**Q-hex-1**	2.15 ± 0.27 d	2.37 ± 0.33 d	ND	ND	2.29 ± 0.08 d	6.74 ± 0.42 c	9.21 ± 0.49 b	1.40 ± 0.18 e	13.22 ± 0.52 a	ND	ND	ND
11	**Q-hex-2**	11.30 ± 0.68 d	13.49 ± 0.86 c	6.97 ± 1.06 e	10.12 ± 1.51 d	11.30 ± 0.13 d	10.09 ± 0.61 d	13.63 ± 0.46 c	15.36 ± 1.79 b	4.87 ± 0.04f	6.29 ± 0.11 e	18.10 ± 0.67 a	18.22 ± 0.52 a
17	**Q-glcu**	ND	ND	ND	ND	0.40 ± 0.01 e	ND	ND	ND	1.90 ± 0.03 b	5.69 ± 0.05 c	ND	ND
12	**Q-pent-1**	1.65 ± 0.07fg	1.54 ± 0.13fg	6.52 ± 0.45 b	7.83 ± 0.27 a	1.63 ± 0.09fg	2.07 ± 0.61 e f	4.12 ± 0.42 d	2.72 ± 0.81 e	4.70 ± 0.18 c d	4.97 ± 0.20 c	0.76 ± 0.11g	1.56 ± 0.50fg
13	**Q-pent-2**	2.07 ± 0.26 d e	1.88 ± 0.23 d e	6.94 ± 1.62 b c	9.22 ± 2.35 a	2.10 ± 0.16 d e	3.65 ± 0.21 d	5.78 ± 0.51 c	8.30 ± 0.85 a b	5.78 ± 0.39 c	9.07 ± 0.40 a	0.75 ± 0.10 e	2.11 ± 0.11 d e
14	**Q-pent-3**	6.71 ± 0.86 d e	9.60 ± 2.72 d	15.02 ± 1.32 c	21.97 ± 5.05 a b	8.38 ± 0.93 d e	6.71 ± 0.45 d e	25.19 ± 2.18 a	21.20 ± 1.76 b	4.21 ± 0.33 e	6.04 ± 0.38 d e	ND	ND
15	**Q-rhm**	27.19 ± 2.08 b	22.16 ± 1.98 c d	19.64 ± 4.76 d	20.75 ±1.22 d	21.67 ± 0.92 c d	20.08 ± 0.64 d	8.48 ± 1.54 e	3.88 ± 0.86f	23.84 ± 0.51 b c d	26.31 ± 0.86 b c	26.46 ± 2.74 b c	38.43 ± 2.33 a
16	**Q-galloyl-pent-1**	1.00 ± 0.09 b c	ND	3.59 ± 1.28 a	ND	0.86 ± 0.01 b c	0.25 ± 0.03 c	0.69 ± 0.21 b c	1.37 ± 0.19 b	0.43 ± 0.15 c	0.37 ± 0.10 c	ND	ND
18	**Q-galloyl-pent-2**	1.56 ± 0.05 b	1.58 ± 0.33 b	ND	ND	1.77 ± 0.08 b	0.78 ± 0.12 c	ND	ND	0.22 ± 0.02 d	0.43 ± 0.04 d	9.76 ± 0.07 b	6.98 ± 4.81 a
23	**Q-cm-hex-1**	0.77 ± 0.04 c d	1.52 ± 0.03 b	ND	2.93 ± 0.69 a	1.54 ± 0.03 b	1.58 ± 0.05 b	1.30 ± 0.67 b c	1.32 ± 0.06 b c	1.02 ± 0.05 b c d	0.59 ± 0.01 d	1.76 ± 0.16 b	1.78 ± 0.24 b
24	**Q-cm-hex-2**	ND	0.52 ± 0.04 c	ND	ND	0.66 ± 0.07 b	0.95 ± 0.03 a	ND	ND	0.95 ± 0.01 a	0.41 ± 0.01 d	ND	ND
22	**Q-fer-hex-1**	ND	ND	2.35 ± 0.20 a	ND	ND	ND	2.19 ± 0.57 a	ND	ND	ND	ND	0.57 ± 0.03 b
25	**Q-fer-hex-2**	ND	ND	ND	ND	ND	ND	0.87 ± 0.11 a	ND	ND	ND	ND	ND
19	**Free Q**	19.37 ± 3.64 a	19.25 ± 2.93 a	ND	ND	14.10 ± 2.45 a b	17.08 ± 1.10 a	10.11 ± 4.32 b c	ND	6.15 ± 1.61 c	7.31 ± 2.82 c	8.96 ± 3.06 c	ND
Total flavonols (mg kg^{-1} DW skin, eq Q-rhm)		**349.59 ± 13.33 e**	**679.57 ± 37.11 c**	**121.67 ± 6.87 g**	**140.84 ± 8.91 g**	**648.81 ± 13.87 c**	**1147.87 ± 27.04 b**	**449.06 ± 146.24 d**	**241.56 ± 42.82 f**	**1195.21 ± 60.42 b**	**1521.29 ± 58.10 a**	**522.67 ± 69.61 d**	**469.98 ± 28.63 d**
1	**EA-hex**	6.99 ± 0.37 b	6.43 ± 0.17 b	1.41 ± 0.10 e	4.04 ± 1.22 c	6.24 ± 0.11 b	ND	2.45 ± 0.01 d	ND	12.44 ± 0.25 a	ND	5.70 ± 1.47 b	ND
3	**EA-pent-1**	1.95 ± 0.11 b	2.04 ± 0.21 b	ND	ND	2.50 ± 0.09 a	ND	ND	ND	ND	ND	1.57 ± 1.09 d	1.58 ± 0.02 c
7	**EA-pent-2**	18.21 ± 0.79 e	19.94 ± 0.66 e	13.60 ± 1.18f	20.31 ± 3.78 e	17.85 ± 0.35 e	36.59 ± 1.74 c	ND	44.84 ± 1.96 b	36.42 ± 0.59 c	50.33 ± 1.11 a	24.58 ± 3.96 d	49.91 ± 3.32 a
8	**EA-rhm-1**	0.28 ± 0.01 e	0.54 ±0.09 e	2.05 ± 0.18 d	3.97 ± 0.98 b	0.21 ± 0.01 e	ND	0.67 ± 0.37 e	ND	6.04 ± 0.10 a	ND	3.21 ± 0.68 c	ND
10	**EA-rhm-2**	3.21 ± 0.05 b c d	3.23 ± 0.16 b c d	6.13 ± 0.12 b	4.19 ± 1.03 b c	3.78 ± 0.23 b	2.19 ± 0.39 b c d	16.89 ± 3.68 a	1.34 ± 0.44 c d	2.51 ± 0.05 b c d	ND	0.65 ± 0.28 d	ND
20	**EA-ac-rhm-1**	0.38 ± 0.04 b c	0.41 ± 0.01 b c	1.67 ± 0.08 a	1.32 ± 0.44 a	0.46 ± 0.02 b c	0.78 ± 0.05 b	1.31 ± 0.66 a	0.60 ± 0.05 b c	ND	ND	0.41 ± 0.17 c	0.30 ± 0.02 b c
26	**EA-ac-rhm-2**	0.92 ± 0.05 e	1.09 ± 0.05 d	3.00 ± 0.02 a	2.09 ± 0.06 b	0.89 ± 0.01 e	0.82 ± 0.02 e	ND	1.29 ± 0.0 c	0.26 ± 0.12f	ND	ND	0.19 ± 0.0f
27	**EA-valeryl-rhm-1**	0.27 ± 0.00 b	0.21 ± 0.01 b	0.55 ± 0.01 a	0.16 ± 0.01 b	0.20 ± 0.01 b	0.23 ± 0.01 b	0.63 ± 0.38 a	0.20 ± 0.01b	0.26 ± 0.14 b	ND	ND	0.21 ± 0.01 b

(*continued on next page*)

Table 3 (*continued*)

Non-anthocyanin phenolic compounds													
Peak	Assignation[†]	SF molar% 2014 Crop	2015 Crop	PF molar% 2014 Crop	2015 Crop	SFP molar% 2014 Crop	2015 Crop	PFP molar% 2014 Crop	2015 Crop	HFP molar% 2014 Crop	2015 Crop	CFP molar% 2014 Crop	2015 Crop
28	EA-valeryl-rhm-4	0.37 ± 0.02 c d	0.38 ± 0.01 b c	0.56 ± 0.01 a	0.16 ± 0.0f	0.20 ± 0.00 e	0.36 ± 0.01 d	ND	0.40 ± 0.01 b	ND	ND	ND	ND
30	EA-valeryl-rhm-2	0.27 ± 0.00 d e	0.30 ± 0.02 c d	0.86 ± 0.07 a	0.43 ± 0.02 b	0.34 ± 0.06 c	0.25 ± 0.01 d e	ND	0.22 ± 0.01 e	ND	ND	ND	ND
31	EA-valeryl-rhm-3	ND	ND	0.55 ± 0.01 a	0.32 ± 0.02 b	0.27 ± 0.03 c	ND	ND	ND	ND	ND	ND	ND
33	EA-caprilyl-rhm	0.81 ± 0.03 d	0.96 ± 0.04 c	1.80 ± 0.07 a	1.03 ±0.0 b	0.83 ± 0.02 d	1.05 ± 0.06 b	ND	0.68 ± 0.03 e	ND	ND	ND	ND
6	Free EA	66.29 ± 1.38 a b c	64.41 ± 1.33 a b c	69.71 ± 3.22 a b	60.51 ± 4.38 b c	66.15 ± 0.74 a b c	57.74 ± 1.66 c d	73.60 ± 11.07 a	49.40 ± 4.03 d e	42.56 ± 0.75 e	49.66 ± 1.11 d e	63.31 ± 5.71 a b c	48.00 ± 3.00 e
Total	**EA derivatives (mg kg^{-1} DW skin, eq. EA)**	**960.30 ± 37.50 d**	**1265.60 ± 22.60 b c**	**472.16 ± 7.15 f**	**1659.65 ± 61.32 a**	**1314.49 ± 25.83 b c**	**1123.68 ± 60.95 c d**	**376.57 ± 120.05 f**	**1210.01 ± 19.94 c**	**462.41 ± 23.98 f**	**683.33 ± 10.22 e**	**1497.69 ± 337.12 a b**	**1372.42 ± 15.80 b c**
32	Me-EA-valeryl-rhm	ND	47.27 ± 0.43 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
34	Me-EA-caprilyl-rhm	43.25 ± 1.52f	52.72 ± 0.43 d	49.19 ± 1.19 e	60.87 ± 5.25 c	100.00 ± 0.00 a	100.00 ± 0.00 a	ND	72.97 ± 1.94 b	ND	ND	ND	ND
21	Me-EA-rhm	56.74 ± 1.52 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
29	Me-EA-ac-rhm	ND	ND	50.80 ± 1.19 b	39.12 ± 5.25 c	ND	ND	ND	27.02 ± 1.94 d	100.00 ± 0.00 a	ND	ND	ND
Total	**Me-EA derivatives (mg kg^{-1} DW skin, eq. EA)**	**6.19 ± 0.15 c**	**5.39 ± 0.17 d**	**5.17 ± 0.14 d**	**8.26 ± 0.52 b**	**2.79 ± 0.04 e**	**2.58 ± 0.03 e**	**ND**	**9.58 ± 0.78 a**	**0.04 ± 0.00 f**	**ND**	**ND**	**ND**
	Flavan-3-ols monomers												
	(+)-catechin	53.15 ± 5.65 b c d	61.76 ± 3.50 a b	45.79 ± 5.11 d e	69.05 ± 7.48 a	59.54 ± 3.84 a b c	62.73 ± 3.55 a b	49.73 ± 2.47 b c d e	39.30 ± 2.54 e	48.48 ± 6.53 c d e	52.76 ± 3.53 b c d	62.13 ± 1.58 a b	60.79 ± 7.86 a b c
	(−)-gallocatechin	36.61 ± 3.42 a b	27.85 ± 6.48 a b	40.35 ± 8.24 a	ND	28.95 ± 2.03 a b	26.99 ± 4.11 a b	29.18 ± 7.62 a b	22.26 ± 3.76 b	41.37 ± 7.43 a	33.66 ± 6.29 a b	25.74 ± 2.21 a b	25.30 ± 9.56 a b
	Epicatechin 3-gallate	4.59 ± 0.68f	6.74 ± 1.65 d e f	10.45 ± 0.83 c d	24.14 ± 3.94 b	5.64 ± 0.21 e f	8.70 ± 1.04 c d e	6.31 ± 1.43 e f	24.68 ± 1.70 b	9.04 ± 0.58 c d e	10.78 ± 1.69 c	12.57 ± 1.07 c	28.88 ± 1.87 a
	Gallocatechin 3-gallate	1.51 ± 0.23 d	1.90 ± 0.13 c d	ND	10.15 ± 1.30 b	2.33 ± 0.53 c d	3.22 ± 0.25 c	ND	12.67 ± 2.35 a	1.69 ± 0.18 c d	3.80 ± 1.66 c	ND	10.12 ± 1.22 b
	Epigallocatechin 3-gallate	6.08 ± 1.04 a	ND	6.31 ± 2.21 a	ND	2.87 ± 0.77 b	ND	ND	ND	ND	ND	ND	ND
Total	**monomers (mg kg^{-1} DW skin, eq. Catechin)**	**0.21 ± 0.03 a**	**0.14 ± 0.03 b c**	**0.08 ± 0.02 d e**	**0.03 ± 0.01 f**	**0.15 ± 0.01 b**	**0.12 ± 0.02 b c d**	**0.06 ± 0.01 e f**	**0.04 ± 0.01 f**	**0.19 ± 0.03 a**	**0.11 ± 0.02 c d**	**0.10 ± 0.01 d**	**0.03 ± 0.01 f**
	Proanthocyanidins												
	mDP	11.37 ± 1.14 a	1.92 ± 0.04 c	2.35 ± 0.54 c	1.82 ± 0.10 c	4.66 ± 0.58 b	2.84 ± 0.30 c	2.54 ± 0.41 c	2.18 ± 0.07 c	2.84 ± 0.17 c	3.04 ± 0.83 c	2.75 ± 0.86 c	1.76 ± 0.27 c
	Galloylation %	2.45 ± 0.39 c	5.95 ± 0.79 a b	8.80 ± 2.28 a	8.03 ± 1.70 a	4.51 ± 0.47 b c	5.80 ± 1.44 a b	3.95 ± 0.91 b c	8.21 ± 0.97 a	8.67 ± 0.13 a	8.81 ± 1.74 a	4.67 ± 1.42 b c	5.71 ± 0.34 a b
	Prodelphinidin %	26.23 ± 0.60 a b	21.02 ± 0.19 a b	22.53 ± 3.72 a b	19.59 ± 3.57 b	27.49 ± 0.62 a	24.26 ± 2.37 a b	20.48 ± 2.72 a b	26.60 ± 2.42 a b	25.88 ± 0.40 a b	20.51 ± 1.20 a b	22.27 ± 3.77 a b	22.24 ± 4.43 a b
Total	**Proanthocyanidins (mg kg^{-1} DW skin, eq. Catechin)**	**6.15 ± 0.61 a**	**1.10 ± 0.11 c d**	**0.59 ± 0.01 d**	**0.51 ± 0.10 d**	**2.56 ± 0.56 b**	**1.73 ± 0.62 b c**	**0.57 ± 0.07 d**	**0.52 ± 0.03 d**	**2.07 ± 0.42 b**	**2.27 ± 0.37 b**	**1.61 ± 0.84 b c d**	**0.56 ± 0.03 b c**

Anthocyanin phenolic compounds													
Peak	Assignation[†]	SF molar% 2014 Crop	2015 Crop	PF molar% 2014 Crop	2015 Crop	SFP molar% 2014 Crop	2015 Crop	PFP molar% 2014 Crop	2015 Crop	HFP molar% 2014 Crop	2015 Crop	CFP molar% 2014 Crop	2015 Crop
a	dp-3-glc	16.46 ± 0.43 c	12.62 ± 0.07h	27.15 ± 0.22 a	15.40 ± 0.33 e d	14.53 ± 0.19f	13.97 ± 0.05g	15.16 ± 0.35 e	23.13 ± 0.09 b	15.67 ± 0.20 d	13.99 ± 0.27g	13.87 ± 0.23 g	12.21 ± 0.06 i
b	cy-3-glc	82.52 ± 0.37f	86.31 ± 0.11 b	72.13 ± 0.27h	83.76 ± 0.36 d	84.63 ± 0.21 c	84.83 ± 0.06 c	84.15 ± 0.35 d	76.21 ± 0.09g	83.26 ± 0.15 e	84.91 ± 0.26 c	85.09 ± 0.20 c	86.80 ± 0.04 a
c	pt-3-glc			ND			0.36 ± 0.01 b		0.36 ± 0.09 b	0.27 ± 0.006 c	0.36 ± 0.03 b		0.46 ± 0.04 a

(*continued on next page*)

Table 3 (*continued*)

Non-anthocyanin phenolic compounds														
Peak	Assignation[†]	SF molar%		PF molar%		SFP molar%		PFP molar%		HFP molar%	2015 Crop	CFP molar%	
		2014 Crop	2015 Crop	2014 Crop	2015 Crop	2014 Crop	2015 Crop	2014 Crop	2015 Crop	2014 Crop		2014 Crop	2015 Crop
		0.23 ± 0.02 c d	0.24 ± 0.001 c d		0.17 ± 0.01 d	0.05 ± 0.001 e		0.21 ± 0.009 c d				0.29 ± 0.008 b c	
d	pg-3-glc	0.21 ± 0.01 a	0.13 ± 0.007 b	ND	ND	0.14 ± 0.009 b	0.07 ± 0.007 c	ND	ND	ND	ND	ND	ND
e	pn-3-glc	0.35 ± 0.01 b	0.40 ± 0.005 a	ND	0.26 ± 0.00f	0.33 ± 0.002 c	0.33 ± 0.004 c d	0.16 ± 0.002h	0.20 ± 0.003g	0.31 ± 0.00 d	0.22 ± 0.01g	0.27 ± 0.003 e	0.21 ± 0.01 g
f	dp-3-acglc	ND	ND	ND	ND	ND	0.06 ± 0.001 b	ND	ND	ND	0.10 ± 0.007 a	ND	ND
g	cy-3-acglc	0.09 ± 0.01 b	0.12 ± 0.03 b	0.11 ± 0.02 b	ND	0.16 ± 0.06 b	0.15 ± 0.02 b	0.16 ± 0.03 b	ND	0.39 ± 0.07 a	0.34 ± 0.04 a	0.11 ± 0.01 b	0.11 ± 0.003 b
h	dp-3-cmglc	ND	ND	0.21 ± 0.02 a	ND	ND	0.03 ± 0.003 c	ND	ND	ND	ND	0.05 ± 0.00 b	ND
i	cy-3-cmglc	0.10 ± 0.01 c	0.11 ± 0.002 c	0.28 ± 0.02 a	0.30 ± 0.02 a	0.12 ± 0.001 c	0.12 ± 0.004 c	0.13 ± 0.01 c	ND	0.07 ± 0.006 d	ND	0.18 ± 0.003 b	0.11 ± 0.003 c
j	cy-3-ferglc	ND	ND	0.10 ± 0.004 a	ND	ND	0.03 ± 0.006 c	ND	ND	ND	ND	0.04 ± 0.006 b	ND
Total mg kg^{-1} DW skin,		4295.63 ± 593.16 e	10546.4 ± 289.98 b	1496.53 ± 68.86g	1663.47 ± 25.91g	7659.33 ± 1210.97 c	12174.33 ±480.39 a	6341.68 ± 685.69 d	3237.88 ± 355.60f	4553.73 ± 304.71 e	5880.23 ± 113.85 d	7198.46 ± 211.69 c	4613.01 ± 307.74 e
Eq. Cy-3-glc													

[†] M: myricetin derivatives, Q: quercetin derivatives, EA: ellagic acid, Me-EA: methylellagic acid, hex: hexose, pent: pentose, rhm: rhamnosis, gal: galactose, glu: glucose, ac: acetic acid, cm: *p*-coumaric acid, fer: ferulic acid, dp: delphinidin, cy: cyanidin, pg: pelargonidin, pn: peonidin, pt: petunidine, glc: glycoside, cmglc: coumaryl-glicoside, acglc: acetylglicoside, ferglc: feruloylglycoside. Means followed by the different letters in the same row differ by *t* test's $p > 0.05$. ND: non-detected.

resulted in the identification of several flavonoids, ellagic acid, and methylellagic acid. Among the identified flavonoids, quercetin and myricetin derivatives were the most numerous. As shown in Tables 2 and 3, the identified and quantified compounds were three flavonol derivatives from myricetin (M), 14 flavonol derivatives from quercetin (Q), 13 derivatives from ellagic acid (EA), and four derivatives from methylellagic acid (Me-EA), which are described in Table 2.

The flavan-3-ol monomers that were identified and quantified were the phenolic compounds catechin, gallocatechin, epicatechin 3-gallate, gallocatechin 3-gallate and epigallocatechin 3-gallate. Three proanthocyanidins were estimated: mDP, galloylation (%) and prodelphinidin (%).

The major compound derivative from myricetin was M-rhm (peak 5), which presented an *m/z* ratio of 463 and an MS/MS fragmentation of 316. These results are in agreement with Neves et al. [32]. The jaboticaba varieties that presented the highest concentration of this compound in the peel were PF and CFP, both harvested in the 2014 crop season, whereas the lowest concentrations were recorded in PFP in both crop seasons and HFP in the 2015 crop season. Silva et al. [33] found 0.06 μg mL^{-1} myricetin in Sabará jaboticaba peels; on the other hand, Inada et al. [34] reported 4.3 mg myricetin per 100 g of dried peel in Sabara jaboticaba.

Peak 14, which presented a *m/z* ratio of 433 and an MS/MS fragmentation of 301, was a quercetin derivative (Q-pent-3). Peak 15 (*m/z* 447/301) was also a quercetin derivative (Q-rhm). These results are in agreement with Neves et al. [35] Q-pent-3 and Q-rhm were the most representative quercetin derivatives in the five studied jaboticaba varieties. PFP in both crop seasons and PF harvested in 2015 presented the highest Q-pent-3 concentrations. On the other hand, this compound was not detected in the CFP jaboticaba variety. However, CFP presented the highest Q-rhm concentration, whereas the SFP and PF varieties in both crop seasons presented the lowest concentrations of this compound.

Paulista and Sabará jaboticaba fruits presented, in molar percentages, 11.6 and 12.6% Q-pent-3 and 26.5 and 26.9% Q-rhm, respectively [32], similar to the molar percentage values reported in the present study for the same jaboticaba varieties. In Sabará jaboticaba peel extracts, da Silva et al. [33] reported that the quercetin content was 0.09 μg mL^{-1}, whereas Inada et al. [34] reported 3.5 mg of quercetin per 100 g of peel in the same jaboticaba variety, and Wu et al. [36] detected 11.57 mg of quercetin per 10 g of jaboticaba fruit extracts in Sabará jaboticaba.

The total flavonols (myricetin and quercetin derivatives) were expressed in mg per kg of dried peels as equivalents of Q-rhm. The highest total flavonol content was recorded in HFP jaboticaba harvested in 2015. In contrast, the PF jaboticaba variety presented the lowest concentration of total flavonols in both crop seasons. However, in all jaboticaba varieties studied, there were high concentrations of myricetin and quercetin derivatives, as well as total flavonols. Due to the wide variety of standards used to express the concentrations of these compound classes, as well as the numerous techniques used to perform the chromatographic analyses, comparison with results reported in other studies would is not satisfactory.

Among ellagic acid (EA) derivatives, the principal compound recorded in all studied jaboticaba varieties was free ellagic acid (*m/z* 301/257). These *m/z* and fragmentation values were also described by Neves et al. [32]. PFP and PF jaboticaba varieties, both harvested in the 2014 crop season, presented the highest free EA concentrations. On the other hand, HFP jaboticaba in both crop seasons and the PFP and CFP jaboticaba harvested in 2015 showed the lowest free EA concentrations. The molar percentages of free EA reported in Paulista and Sabará jaboticaba fruit extracts were 64.7 and 72.4%, respectively [32]. Similar results were described in our study for peel extracts from Paulista and Sabará jaboticaba. Alezandro et al. [37] reported that Sabará jaboticaba had 40 mg of EA per 100 g of dried sample. Greater concentrations were reported by Plaza et al. [38] and Inada et al. [34] in the same variety: 142.8 and 178 mg of EA per 100 g of dried samples.

Table 4
Total condensed tannins contents in the peels of five jaboticaba varieties (mean ± standard deviation, n = 3) [†].

	2014 Crop	2015 Crop
Sabará Fagan (SF)	123.72±9.44A d	134.35±18.28A c d
Paulista Fagan (PF)	137.42±20.07A c d	183.70±5.08 B b
Sabará F.P (SFP)	180.20±8.33A b	194.30±28.47A a b
Pintada F.P (PFP)	57.18±4.48A e	217.37±4.84 B a
Hibrida F.P (HFP)	109.32±5.94A d	155.32±13.96 B c
Coroada F.P (CFP)	115.61±7.89A d	113.80±13.92A d

[†] Mean values expressed in mg g^{-1} of DW peels, eq. Epicatechin. Columns followed by the same capital letters did not differ statistically (comparison between crop seasons, $p < 0.05$), and rows followed by the same lower-case letters did not differ statistically (comparison among varieites, $p < 0.05$).

The total content of ellagic acid derivatives (mg kg^{-1} of dried peel, eq. EA) was measured in all studied varieties but was highest in PF jaboticaba harvested in 2015 and in CFP jaboticaba harvested in 2014. The PF, PFP and HFP jaboticaba varieties harvested in 2014 presented the lowest concentrations of total ellagic acid. In Paulista and Sabará jaboticaba fruits, Neves et al. [35] reported a total concentration of ellagic acid equivalents of 152.7 and 294.3 mg kg^{-1}. The concentrations reported by Neves et al. [32] are lower than those recorded in this study, possibly due to the plants' environmental conditions and cultural practices.

Methylellagic acid derivatives (Me-EA) were also detected, with substituents similar to those found for ellagic acid derivatives. Quantitatively, all jaboticaba varieties presented higher concentrations of ellagic acid derivatives than methylellagic acid derivatives. In PFP (2014 crop season), HFP (2015 crop season) and CFP (2014 and 2015 crop seasons), Me-EA derivatives were not detected. The highest concentration of total Me-EA was recorded in PFP jaboticaba harvested in 2015. Me-EA (EA equivalent) total concentrations of 2.85 and 1.96 mg kg^{-1} jaboticaba fruits were reported for Paulista and Sabará jaboticaba [32]. The results presented by the authors were considerably lower than the results found for the same varieties in the present study.

A few condensed tannins (flavan-3-ols) were detected. Catechin was the main monomeric flavan-3-ol detected in all jaboticaba varieties, and the varieties that presented the highest concentrations of this compound were PF (harvested in 2015), SFP and CFP (both harvested in 2014 and 2015). On the other hand, PF (harvested in 2014) and PFP (harvested in 2014 and 2015) jaboticaba varieties presented the lowest concentrations of catechin. In Sabará jaboticaba, da Silva et al. [33] reported a catechin concentration of 0.13 μg mL^{-1} in peel aqueous extract.

The total monomeric flavan-3-ols were expressed as catechin equivalents in mg kg^{-1} of dried peel. The varieties that presented the highest concentration were SF and HFP, both from the 2014 harvests. The 2015 PF and CFP and both PFP crops presented the lowest concentrations of this compound.

The reaction with pyrogallol induces the depolymerization of condensed tannin molecules. After this reaction, the catechin concentration increased, suggesting that these compounds can be polymerized in the form of larger molecules. Polymerization is related to loss of astringency [39], which is favorable for improving fruit flavor. It is especially interesting that this was observed in Sabará jaboticaba, which is widely used in technological procedures to manufacture food products.

The main proanthocyanidin isolated was prodelphinidin. No studied jaboticaba varieties differed statistically in their concentrations of these compound. The total proanthocyanidinins (catechin equivalents) were expressed in mg kg^{-1} dried peel. SF jaboticaba harvested in 2014 had the highest concentration of total proanthocyanidinins. PF and PFP jaboticaba (in both crop seasons) presented the lowest concentrations of proanthocyanidinins. In Sabará jaboticaba a proanthocyanidin A2 concentration of 0.12 μg mL^{-1} peel aqueous extract has been reported [33].

The total condensed tannin content (Table 4) was also measured, and the varieties that presented the highest concentration were SFP and PFP, both from the 2015 harvest, while PFP (2014 crop season) showed the lowest content of this compound.

Analyses of total flavan-3-ols (total proanthocyanidins) and total condensed tannins were performed because although these denominations are theoretically equivalent, the structural complexity of the polymerized flavan-3-ols is enormous, and these denominations try to describe a complex group of substances with chemical properties that differentiate them. Thus, proanthocyanidins are polymers of flavan-3-ols, which are depolymerized under strong oxidative conditions (6 N HCl medium and temperature at 100 °C for 2 hours). This behavior shows the proanthocyanidins in which the flavan-3-ol units are bound by "B-type" bonds (interflavan bonds between positions C-4 and C-8, especially the different flavan-3-ol molecules). This type of bond breaks down in the depolymerization assay with pyrogallol. There are also other types of interflavan bonds, such as those of type "A", which do not break under the conditions of the pyrogallol assay. The condensed tannins, however, are polymers of flavan-3-ols, which have the property of precipitating proteins and other polymers, such as methylcellulose. For this reason, we performed a precipitation assay with methylcellulose to estimate the total content of condensed tannins. Oligomers (polymers with few structural units) only cause precipitation of methylcellulose.

For all nonanthocyanin phenolic compounds, differences were observed between the harvest years in most of the samples studied. This occurred due to the edaphoclimatic factors that strongly influenced the synthesis of these compounds in the plants; in 2014, a severe drought occurred throughout Brazil. Thus, the fruits collected in the 2014 harvest suffered a much greater stress than the fruits collected in the harvest of 2015, which was a relatively normal year in terms of rainfall. Studies report that abiotic factors such as temperature, exposure to UV light, and drought stress, among others, can corroborate the accumulation of several phenolic compounds [40,41].

3.2. *Anthocyanin phenolic compounds from peels of five jaboticaba varieties*

The identified anthocyanins were delphinidin-3-glycoside (dp-glc), cyanidin-3-glycoside (cy-glc), petunidin-3-glycoside (pt-3-glc), pelargonidin-3-glycoside (pg-3-glc), peonidin-3-glycoside (pn-3-glc), delphinidin-3-acetylglycoside (dp-3-acglc), cyanidin-3-acetylglycoside (cy-3-acglc), delphinidin-3-coumaroylglycoside (dp-3-cmglc), cyanidin-3-coumaroylglycoside (cy-3-cmglc) and cyanidin-3-feruloylglycoside (cy-3-ferglc). The presence of anthocyanins derived from the anthocyanidins delphinidin (Dp), cyanidin (Cy), petunidin (Pt), pelargonidin (Pg) and peonidin (Pn) was detected by molecular fragmentation, resulting in ions with m/z values of 303, 287, 271, 317 and 301, respectively.

The identification of monoglycosylated structures occurred due to the fragmentation patterns observed in the MS/MS spectra, in which only one type of fragment was observed, characterizing the loss of a neutral fragment corresponding to one glucose (162 Da). In the case of cy-3-coumaroylglycoside, the loss of a neutral fragment corresponding to cumaroylglycosis (308 Da) was observed. The identification of cy-3-coumaroylglycoside was confirmed by the corresponding UV-Vis spectrum, in which a typical peak of coumaryl residue appeared at 314 nm. All results from the identification of anthocyanin phenolic compounds can be found in Table 2. The chromatogram of these compounds is available in Fig. 1.

The most commonly detected anthocyanin was cy-3-glc, followed by dp-3-glc. These results corroborate those obtained by Inada et al. [34], Leite-Legatti et al. [6] and Plaza et al. [38]. The same result was reported for pn-3-glc in jaboticaba fruits by Neves et al. [35]. CFP jaboticaba harvested in 2015 presented the highest concentration of cy-3-glc, and PF jaboticaba harvested in 2014 showed the lowest concentration of this compound. For dp-3-glc, the highest concentration was recorded in PF jaboticaba harvested in 2014, whereas the lowest concentration of this

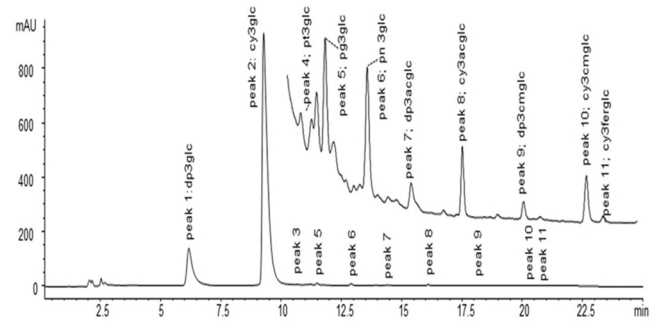

Fig. 1. Anthocyanins profile (DAD, 520 nm) recorded in SFP jaboticaba peels from the 2015 crop (all anthocyanins).

compound was observed in CFP jaboticaba harvested in 2015 (Table 3).

Neves et al. [35] reported cy-3-glc and dp-3-glc molar percentages of 75.1 and 23.7% in Paulista jaboticaba peels and 80.5 and 18.4% in Sabará jaboticaba peels, respectively. These results corroborate those recorded in the present study. Alezandro et al. [37] reported 123 mg of cy-3-glc per 100 g of dried sample and 23.5 mg of dp-3-glc per 100 g of dried sample in Sabará jaboticaba. Silva et al. [33] found 8.96 μg mL^{-1} (cy-3-glc) and 0.465 μg mL^{-1} (dp-3-glc) in Sabara jaboticaba peels. Inada et al. [34] reported values of 1261 mg of cy-3-glc per 100 g of dried peel and 269 mg of dp-3-glc per 100 g; and Wu et al. [36] reported 29.80 mg of cy-3-glc per 10 g of jaboticaba fruits and 7.36 mg per 10 g of dp-3-glc.

Anthocyanin phenolic compounds were expressed in cy-3-glc equivalents (mg kg^{-1} of dry peel). SFP jaboticaba harvested in 2015 presented the highest concentration of total anthocyanins whereas the lowest concentration of this compound was recorded in PF jaboticaba from both crop seasons. In Paulista jaboticaba, Neves et al. [32] reported 331.7 mg of cy-3-glc eq. per kg of fruits and 1057.7 mg of cy-3-glc eq. per kg of fruits. These results are lower than those found in the present study.

For the anthocyanin phenolic compounds, the same phenomena mentioned above for nonanthocyanin phenolic compounds were observed. That is, differences were observed between the years of harvest in most of the samples studied. This was due to edaphoclimatic factors that strongly influence the synthesis of these compounds in plants. Many phenolic compounds are synthesized by the phenylpropanoid pathway. Under drought conditions, some genes encoding this pathway are regulated, stimulating the biosynthesis of compounds such as anthocyanins. A review on this topic shows that many plant species increase the contents of phenolics, flavonoids, and anthocyanins under drought stress. On the other hand, UV light also enhances phenolic accumulation [41].

3.3. Principal component analysis (PCA) of anthocyanin and nonanthocyanin phenolic compounds from peels of five jaboticaba varieties

Principal component analysis was used in this work to compare the samples in relation to the analyzed compounds. As there were 36 samples and the number of variables was relatively large, the use of tables alone without the application of PCA would be laborious and in some cases very difficult.

The results obtained for the analysis of anthocyanins were organized in a data matrix where all of the samples of the different varieties analyzed in the two harvests were listed in the rows and the 10 anthocyanins found were listed in the columns, creating a 36 × 10 matrix (samples x variables). The data matrix was used in the PCA, and the different varieties were used as a class in the score graphic (Fig. 2(a)) to determine if there was a difference and what variables were responsible for this difference based on the loading plot analysis (Fig. 2(b)). The preprocessing used was self-scaling, and the number of main components used was 4, which explained 86.5% of the variation in the data.

The first principal component (PC1) represented 45.3% of the total

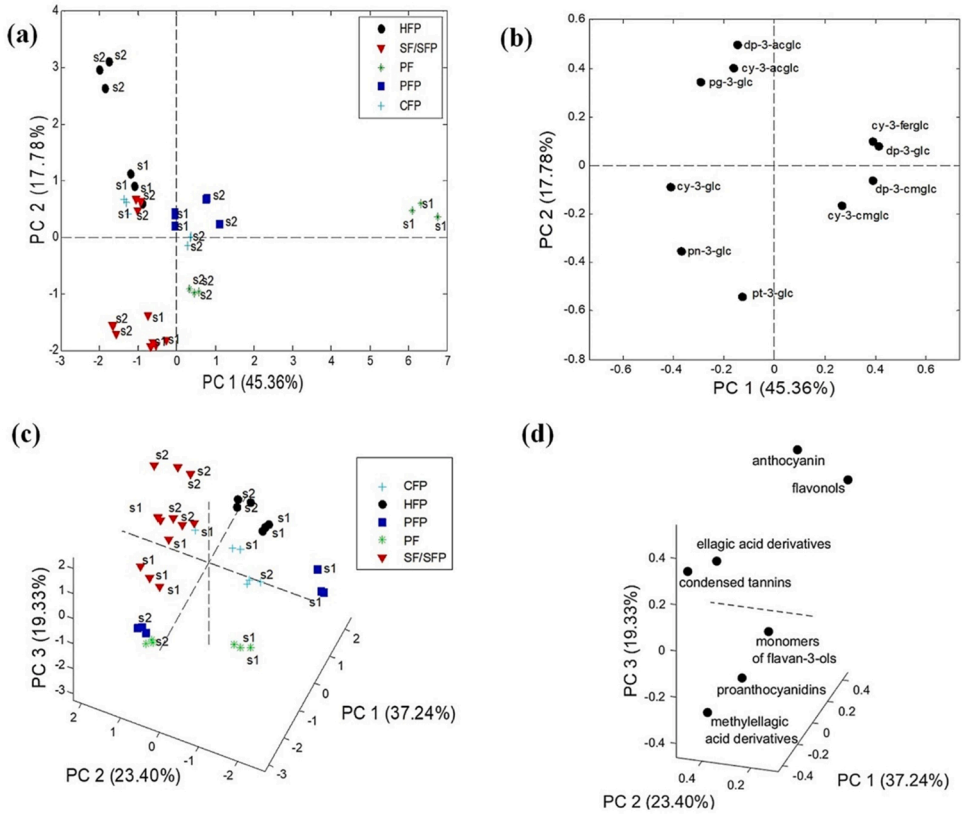

Fig. 2. (a) Scores (PC1xPC2) of anthocyanin phenolic compounds from five jaboticaba varieties, 2014 crop (S1) and 2015 crop (S2). (b) Loading graphic (PC1xPC2) of anthocyanin phenolic compounds from five jaboticaba varieties, 2014 crop (S1) and 2015 crop (S2). (c) Scores (PC1xPC2) of total anthocyanin and nonanthocyanin phenolic compounds from five jaboticaba varieties, 2014 crop (S1) and 2015 crop (S2). (d) Loading graphic (PC1xPC2) of total anthocyanin and nonanthocyanin phenolic compounds from five jaboticaba varieties, 2014 crop (S1) and 2015 crop (S2).

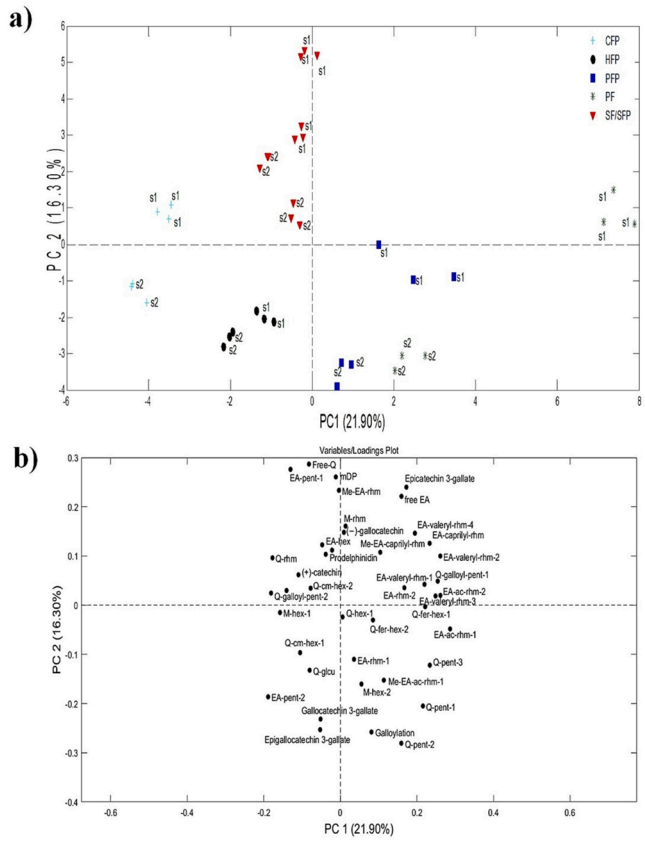

Fig. 3. (a) Scores (PC1xPC2) of nonanthocyanin phenolic compounds from five jaboticaba varieties, 2014 crop (S1) and 2015 crop (S2). (b) Loading graphic (PC1xPC2) of nonanthocyanin phenolic compounds from five jaboticaba varieties, 2014 crop (S1) and 2015 crop (S2).

variation. PC1 divided all jaboticaba varieties as a function of the different crop seasons; this effect was more evident in the PF jaboticaba samples. However, PC1 could be used to explain the PF sample replicates from the 2014 crop. The 2014 crop was predominantly on the right side of the graphic, except for PFP jaboticaba, which showed the highest proportions of cy-3-cmglc, dp-3-cmglc, cy-3-ferglc and dp-3-glc. The second principal component explained HFP jaboticaba from the 2015 crop. In relation to anthocyanin groups, these samples (PF jaboticaba from the 2014 crop and HFP jaboticaba from the 2015 crop) differed from the other analyzed samples.

The PF sample from the 2014 crop contained high values of the anthocyanins cy-3-cmglc, dp-3-cmglc, cy-3-ferglc and dp-3-glc, as verified in the loading graphic (Fig. 2(b)). However, in the 2015 crop, this did not hold true for the same variety. The HFP sample presented high dp-3-acglc, cy-3-acglc and pg-3-glc concentrations when harvested in 2015, which was not true for the first harvest (2014 crop).

Principal component analysis was also used for better visualization of nonanthocyanin phenolic compounds. In this case the data matrix used was 36×42 dimensions: 36 samples and 42 variables (nonanthocyanic phenolic compounds). The data were self-scaled prior to analysis, and the number of principal components used was 5, which explained 71% of the data variation. The scores and loading graphics are available in Fig. 3(a) and (b), respectively.

By analyzing the score graphic (Fig. 3(a)), a trend of separation between the different varieties of jaboticaba analyzed in relation to nonanthocyanin phenolic compounds was observed. PF jaboticaba (2014 crop) showed the greatest difference in relation to the other analyzed varieties. In addition, the CFP jaboticaba (for both crops) showed a subtle difference from the other samples. Another relevant piece of information that can be obtained from the graphic of scores (Fig. 3(a)) is the difference in PC1 between the different harvests. All samples from 2014 are shifted to the right of the graphic. In regard to the compounds that were responsible for this differentiation (loading graphic analysis – Fig. 3(b)), it can be seen that the weights for most of the compounds were close, and it was not possible to identify a single compound that was responsible for the differentiation among samples.

A third PCA was performed using the total compounds: anthocyanin phenolic compounds and nonanthocyanin phenolic compounds (flavonols, ellagic acid derivatives, methylellagic acid derivatives, monomers of flavan-3-ols, proanthocyanidins and condensed tannins) from different jaboticaba varieties (2014 and 2015 crops). The data matrix used in this analysis comprised 36 lines (samples) and 7 variables (36×7). The preprocessing used was self-scaling, and the number of components used was 4, which explained 90% of the total variation in data. Fig. 2(c) and (d) show the scores and loading graphics for PCA (PC1xPC2xPC3). The percentage of variation explained was 37%, 23% and 19% for PC1, PC2 and PC3, respectively.

By analyzing the score graphic (Fig. 2(c)), it can be seen that the SF and SFP samples (both crops) were well grouped, and the loading graphic shows that the variables responsible for this grouping were anthocyanins, flavonols, ellagic acid derivatives and condensed tannins. In addition, the PFP and PF jaboticaba varieties from the 2015 crop showed differences relative to the others due to the proanthocyanidins and methylellagic acid derivatives. All of the other samples presented contrasting behavior to that of the SF and SPF jaboticaba varieties that is, they had a smaller influence in relation to the total compounds considered in the analysis.

Regarding both crop seasons, the crops showed great differentiation in certain varieties, e.g., the PFP jaboticaba variety, and for other jaboticaba varieties this differentiation was more subtle, e.g., the HFP jaboticaba variety. However, it must be noted that it was possible to observe this distinction between the different crops in all varieties.

3.4. Anti-proliferative activity on prostate and breast tumor cells

The potential of jabuticaba peel extracts to inhibit tumor cell growth was evaluated in two different tumor cell lines (prostate (DU-145) and breast (MDA-MB-231)) and the results obtained are available in Table 5.

The extracts obtained from 2015 showed a greater decrease in cellular proliferation in DU-145 and MDA-MB-231 tumor cells at the three treatment times tested (24, 48 and 72 hours). For DU-145, the best treatment times were 48 and 72 hours. PFP jaboticaba extracts from 2015 presented the highest efficiency at all studied times for controlling DU-145 and MDA-MB-231 tumor cell proliferation, whereas unsatisfactory results were recorded for this same jaboticaba variety harvested in 2014. In MDA-MB-231 tumor cells after 24 and 48 hours of treatment, the extracts of peel from all jaboticaba varieties analyzed in both crop seasons were more efficient in controlling cellular proliferation than the drug doxorubicin. Previously, the antiproliferative effect of the Sabará jaboticaba peel extract (polar extraction with 80% ethanol) in a breast cancer cell lineages (MCF-7) and prostate cancer line (PC-3) was achieved with 181.2 GI50 μg mL^{-1} extract and > 250 GI50 μg mL^{-1} extract, respectively [6]. In breast carcinoma (MDA-MB-231) cells, there was a decrease in carcinogenic cells at concentrations of 1000 mg mL^{-1} and 500 mg mL^{-1} [42]. Additionally, Albuquerque et al. [4] observed that the antiproliferative activity of jaboticaba peel against MCF-7 (breast carcinoma) tumor cells was also achieved at 300 GI_{50} μg mL^{-1}. Thus, the results obtained for the two cell linages analyzed are very promising, since all varieties analyzed in both crops showed an effect of decreasing cellular proliferation however, pharmacological activity is not yet fully elucidated and more investigative studies need to be performed. This encourages new studies to derive a drug from this fruit which is so well known and abundant in Brazil.

Table 5
Results of treatments carried (MTT) out with skin extracts from five jaboticaba varieties and reference drug expressed in IC_{50} (μg mL^{-1} of extract) †.

Variety	DU-145						MDA-MB-231					
	24 hours		48 hours		72 hours		24 hours		48 hours		72 hours	
	2014 Crop	2015 Crop	2014 Crop	2015 Crop	2014 Crop	2015 Crop	2014 Crop	2015 Crop	2014 Crop	2015 Crop	2014 Crop	2015 Crop
Sabará Fagan (SF)	87.63 ± 6.13 b A	58.62 ± 3.45 b B	77.49 ± 1.78 d A	71.63 ± 2.61 a B	74.52 ± 14.54 d e A	52.42 ± 3.58 a A	76.37 ± 2.45 c A	62.91 ± 4.31 b c B	70.21 ± 2.24 c A	78.29 ± 1.42 a B	52.70 ± 6.82 c A	51.28 ± 3.74 b A
Paulista Fagan (PF)	‡44.00% ± 5.80 c d A	81.02 ± 4.19 a B	97.54 ± 4.70 c d A	60.00 ± 7.47 b B	93.64 ± 1.22 b c A	52.17 ± 0.28 a B	109.46 ± 3.35 b A	59.48 ± 6.80 b c B	65.79 ± 1.65 c A	58.29 ± 4.99 b A	59.24 ± 6.74 b c A	50.23 ± 0.94 b c A
Sabará F.P (SFP)	‡41.88% ± 1.00 c d A	47.86 ± 2.68 b c B	119.93 ± 3.09 b A	32.72 ± 1.43 d B	84.6 ± 4.60 c d A	32.24 ± 0.72 c B	55.07 ± 2.59 c d A	50.62 ± 4.99 c A	47.97 ± 3.76 d A	38.00 ± 2.67 c B	48.22 ± 5.01 c A	57.63 ± 5.28 b A
Pintada F.P (PFP)	‡22.66% ± 2.12 e A	47.88 ± 8.66 b c B	197.23 ± 17.70 a A	13.71 ± 0.71 e B	232.20 ± 2.30 a A	29.73 ± 0.70 c B	150.76 ± 14.83 a A	25.86 ± 5.88 d B	108.56 ± 7.10 a A	39.84 ± 2.04 c B	79.72 ± 9.04 a A	38.89 ± 1.91 c B
Hibrida F.P (HFP)	‡48.23% ± 2.45 c A	58.85 ± 9.61 b B	112.3 ± 5.30 b c A	44.50 ± 2.03 c B	104.15 ± 7.62 b A	44.54 ± 0.86 b B	138.76 ± 12.53 a A	107.54 ± 9.57 a B	91.08 ± 4.56 b A	56.87 ± 3.55 b B	72.70 ± 5.68 a b A	75.39 ± 8.76 a A
Coroada F.P (CFP)	104.51 ± 6.14 a A	36.35 ± 2.16 c B	54.84 ± 6.06 e A	30.18 ± 2.47 d B	59.37 ± 0.82 e A	29.74 ± 1.3 c B	45.43 ± 3.02 d A	70.59 ± 6.91 b B	39.75 ± 2.77 d A	40.76 ± 3.94 c A	52.66 ± 1.75 c A	52.22 ± 0.47 b A
Doxorubicin	38.97% ± 1.35 d A	0.31 ± 0.10 d B	0.29 ± 0.03fA	0.17 ± 0.14fA	0.47 ± 0.03fA	0.44 ± 0.13 d A	§23.61% ± 2.17 e A	§15.98% ± 4.50 e A	§30.93% ± 1.67 e A	47.66 % ± 1.36 d B	16.96 ± 0.14 d A	0.13 ± 0.11 d B
Solvent / vehicle	‡0.00% ± 0.00 e A	§32.36% ± 4.04 e B	‡9.91% ± 2.67gA	§18.97% ± 1.01 e B	§17.72% ± 2.77gA	§21.29% ± 2.43 e A	‡0.00% ± 0.00fA	§7.51% ± 2.19f B	‡0.00% ± 0fA	§13.23% ± 4.02 e B	‡0.00% ± 0.00 e A	§4.97% ± 1.31 e B

IC_{50} average ± standard deviation n=3.
† Columns followed by the same capital letters did not differ statistically (comparison between crop seasons, $p < 0.05$), and rows followed by the same lower-case letters did not differ statistically (comparison among varieties, $p < 0.05$).
‡ Maximum percentage of growing inhibition in the concentration of 250 ug mL^{-1} of extract.
§ Maximum percentage of growing inhibition in the concentration of 0.6 ug mL^{-1} of Doxorubicin.

4. Conclusions

The phenolic compositions of the different jaboticaba varieties included a great variety of compounds, mainly flavonols and anthocyanins. The results obtained for tumor cell proliferation (DU-145 and MDA-MB-231 linages) are extremely promising, since all of the studied varieties from both crop seasons presented relevant results by in decreasing the cellular proliferation. The differences recorded between the years (2014 and 2015 crops) show the influence of edaphoclimatic factors on the biosynthesis of anthocyanin and nonanthocyanin phenolic compounds and consequently on the antiproliferative effects of the jaboticaba peel extracts.

These data can be used in future studies, mainly in the pharmaceutical field, for the development of drugs based on this fruit, and in the food industry, such as in studies on formulations with jaboticaba peels. Until now, jaboticaba peels have been only a byproduct. This work has demonstrated the high potential of this byproduct that, with further research, can be used as a raw material to produce various drugs and foods. Finally, this study also characterized the profile of phenolic compounds, both anthocyanins and nonanthocyanins, of some jaboticaba varieties that have not been studied until now.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments

The authors thank CNPq – National Coordination of Scientific and Technological Development (process number: 145652/2014-9), Brazil for the financial support of this study and CAPES – Coordination for the Improvement of Higher Education Personnel (CAPES Foundation, Brazil) – Finance Code 001 for providing the research laboratory and the PDSE scholarship (process number: 88881.135420/2016-01). We also thank Professor Hernandes Faustino de Carvalho for opening the doors of his laboratory and providing all necessary material for the biological tests.

References

[1] B.R.P. Cabral, P.M. de Oliveira, G.M. Gelfuso, T.S.C. Quintão, J.A. Chaker, M.G. O. Karnikowski, E.F. Gris, Improving stability of antioxidant compounds from Plinia cauliflora (jaboticaba) fruit peel extract by encapsulation in chitosan microparticles, J. Food Eng. 238 (2018) 195–201.

[2] K.O.P. Inada, T.B.R. Silva, L.A. Lobo, R.M.C.P. Domingues, D. Perrone, M. Monteiro, Bioaccessibility of phenolic compounds of jaboticaba (Plinia jaboticaba) peel and seed after simulated gastrointestinal digestion and gut microbiota fermentation, J. Funct. Foods 67 (2020), 103851. February.

[3] A.G. Tarone, P. Goupy, C. Ginies, M.R. Marostica, C. Dufour, Advanced characterization of polyphenols from Myrciaria jaboticaba peel and lipid protection in in vitro gastrointestinal digestion, Food Chem. 359 (2021), 129959. January.

[4] B.R. Albuquerque, C. Pereira, R.C. Calhelha, M. José Alves, R.M.V. Abreu, L. Barros, M. Oliveira, I. Ferreira, Jabuticaba residues (Myrciaria jaboticaba (Vell.) Berg) are rich sources of valuable compounds with bioactive properties, Food Chem. 309 (2020), 125735.

[5] F.A.N. Fernandes, T.V. Fonteles, S. Rodrigues, E.S. de Brito, B.K. Tiwari, Ultrasound-assisted extraction of anthocyanins and phenolics from jabuticaba (Myrciaria cauliflora) peel: kinetics and mathematical modeling, J. Food Sci. Technol. 57 (6) (2020) 2321–2328.

[6] A.V. Leite-Legatti, Â.G. Batista, N.R.V. Dragano, A.C. Marques, L.G. Malta, M. F. Riccio, M.N. Eberlin, A.R.T. Machado, L.B. de Carvalho-Silva, A.L.T.G. Ruiz, J. E. de Carvalho, G.M. Pastore, M.R. Maróstica, Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities, Food Res. Int. 49 (1) (2012) 596–603.

[7] B.R. Albuquerque, J. Pinela, L. Barros, M.B.P.P. Oliveira, I.C.F.R. Ferreira, Anthocyanin-rich extract of jabuticaba epicarp as a natural colorant: optimization of heat- and ultrasound-assisted extractions and application in a bakery product, Food Chem. 316 (2020), 126364. December 2019.

[8] R.A. Mentor-Marcel, G. Bobe, C. Sardo, L.S. Wang, C.T. Kuo, G. Stoner, N. H. Colburn, Plasma cytokines as potential response indicators to dietary freeze-

dried black raspberries in colorectal cancer patients, Nutr. Cancer 64 (6) (2012) 820–825.
[9] G.D. Stoner, L.-S. Wang, B.C. Casto, Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries, Carcinogenesis 29 (9) (2008) 1665–1674.
[10] L. Marzocchella, M. Fantini, M. Benvenuto, L. Masuelli, I. Tresoldi, A. Modesti, R. Bei, Dietary flavonoids: molecular mechanisms of action as anti- inflammatory agents, Recent Pat. Inflamm. Allergy Drug Discov. 5 (3) (2011) 200–220.
[11] W.-H. Wang, Y.-C. Tyan, Z.-S. Chen, C.-G. Lin, M.-H. Yang, S.-S. Yuan, W.C. Tsai, Evaluation of the antioxidant activity and antiproliferative effect of the jaboticaba (Myrciaria cauliflora) seed extracts in oral carcinoma cells, Biomed. Res. Int. 2014 (2014) 1–7.
[12] S.H. Hassanpour, M. Dehghani, Review of cancer from perspective of molecular, J. Cancer Res. Pract. 4 (4) (2017) 127–129.
[13] A. Barsouk, S.A. Padala, A. Vakiti, A. Mohammed, K. Saginala, K.C. Thandra, P. Rawla, A. Barsouk, Epidemiology, staging and management of prostate cancer, Med. Sci. 8 (3) (2020) 28.
[14] E. Heer, A. Harper, N. Escandor, H. Sung, V. McCormack, M.M. Fidler-Benaoudia, Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study, Lancet Glob. Health 8 (8) (2020) e1027–e1037.
[15] I.B. Abubakar, A.N. Ukwuani-Kwaja, F.S. Olayiwola, I. Malami, A. Muhammad, S. J. Ahmed, Q.O. Nurudeen, M.B. Falana, An inventory of medicinal plants used for treatment of cancer in Kwara and Lagos state, Nigeria, Eur. J. Integr. Med. 34 (2020), 101062. Feb;34(November 2019).
[16] M.S. Butler, The role of natural product chemistry in drug discovery, J. Nat. Prod. 67 (12) (2004) 2141–2153.
[17] X. Hou, J. Lu, J. Weng, L. Ji, Z. Shan, J. Liu, et al., Impact of Waist Circumference and Body Mass Index on Risk of Cardiometabolic Disorder and Cardiovascular Disease in Chinese Adults: A National Diabetes and Metabolic Disorders Survey, in: S.B. Votruba (Ed.), PLoS One, 8, 2013, e57319.
[18] J. Fang, Some anthocyanins could be efficiently absorbed across the gastrointestinal mucosa: extensive presystemic metabolism reduces apparent bioavailability, J. Agric. Food Chem. 62 (18) (2014) 3904–3911.
[19] T.T. Fung, S.E. Chiuve, W.C. Willett, S.E. Hankinson, F.B. Hu, M.D. Holmes, Intake of specific fruits and vegetables in relation to risk of estrogen receptor-negative breast cancer among postmenopausal women, Breast Cancer Res. Treat. 138 (3) (2013) 925–930.
[20] L.L. Teixeira, G.R. Costa, F.A. Dörr, T.P. Ong, E. Pinto, F.M. Lajolo, N. Hassimotto, Potential antiproliferative activity of polyphenol metabolites against human breast cancer cells and their urine excretion pattern in healthy subjects following acute intake of a polyphenol-rich juice of grumixama (Eugenia brasiliensis Lam.), Food Funct. 8 (6) (2017) 2266–2274.
[21] J. Dai, R.J. Mumper, Plant phenolics: extraction, analysis and their antioxidant and anticancer properties, Molecules 15 (10) (2010) 7313–7352.
[22] Lima V.L.A.G. De, Melo, E.A. Pinheiro, I.O. Guerra NB, Antioxidant capacity of anthocyanins from acerola genotypes, Ciência e Tecnol Aliment 31 (1) (2011) 86–92.
[23] G.S. Vieira, A.S.F. Marques, M.T.C. Machado, V.M. Silva, M.D. Hubinger, Determination of anthocyanins and non-anthocyanin polyphenols by ultra performance liquid chromatography/electrospray ionization mass spectrometry (UPLC/ESI–MS) in jussara (Euterpe edulis) extracts, J. Food Sci. Technol. 54 (7) (2017) 2135–2144.
[24] N. Castillo-Muñoz, M. Fernández-González, S. Gómez-Alonso, E. García-Romero, I. Hermosín-Gutiérrez, Red-color related phenolic composition of Garnacha Tintorera (Vitis vinifera L.) grapes and red wines, J. Agric. Food Chem. 57 (17) (2009) 7883–7891.
[25] M. Paludo, R. Colombo, J. Teixeira, I. Hermosín-Gutiérrez, C. Ballus, H. Godoy, Optimizing the extraction of anthocyanins from the peel and phenolic compounds from the seed of jabuticaba fruits (Myrciaria jabuticaba (Vell.) O. Berg) with ternary mixture experimental designs, J. Braz. Chem. Soc. 30 (7) (2019) 1506–1515.
[26] C.J. Sarneckis, R.G. Dambergs, P. Jones, M. Mercurio, M.J. Herderich, P. Smith, Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis, Aust. J. Grape Wine Res. 12 (1) (2006) 39–49.
[27] L.P.G. Rebello, E.S. Lago-Vanzela, M.T. Barcia, A.M. Ramos, P.C. Stringheta, R. Da-Silva, N. Castillo-Muñoz, S. Gómez-Alonso, I. Hermosín-Gutiérrez, Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubea×IAC 1398-21) using HPLC–DAD–ESI-MS/MS, Food Res. Int. 54 (1) (2013) 354–366.
[28] E.S. Lago-Vanzela, R. Da-Silva, E. Gomes, E. García-Romero, I. Hermosín-Gutiérrez, Phenolic composition of the edible parts (flesh and peel) of Bordô grape (Vitis labrusca) using HPLC–DAD–ESI-MS/MS, J. Agric. Food Chem. 59 (24) (2011) 13136–13146.
[29] E.S. Lago-Vanzela, R. Da-Silva, E. Gomes, E. García-Romero, I. Hermosín-Gutiérrez, Phenolic composition of the Brazilian seedless table grape varieties BRS Clara and BRS Morena, J. Agric. Food Chem. 59 (15) (2011) 8314–8323.
[30] S. Kamiloglu, G. Sari, T. Ozdal, E. Capanoglu, Guidelines for cell viability assays, Food Front. 1 (3) (2020) 332–349.
[31] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, J. Immunol. Methods 89 (2) (1986) 271–277.
[32] N. Neves, A. de, P.C. Stringheta, S. Gómez-Alonso, I. Hermosín-Gutiérrez, Flavonols and ellagic acid derivatives in peels of different species of jabuticaba (Plinia spp.) identified by HPLC-DAD-ESI/MSn, Food Chem. 252 (2018) 61–71.
[33] J.K. da Silva, Â.G. Batista, C.B.B. Cazarin, A.P. Dionísio, E.S. de Brito, A.T. B. Marques, M.R. Maróstica Junior, Functional tea from a Brazilian berry: overview of the bioactives compounds, LWT – Food Sci. Technol. 76 (2017) 292–298.
[34] K.O.P. Inada, A.A. Oliveira, T.B. Revorêdo, A.B.N. Martins, E.C.Q. Lacerda, A. S. Freire, B.F. Braz, R.E. Santelli, A.G. Torres, D. Perrone, M.C. Monteiro, Screening of the chemical composition and occurring antioxidants in jabuticaba (Myrciaria jaboticaba) and jussara (Euterpe edulis) fruits and their fractions, J. Funct. Foods 17 (2015) 422–433.
[35] N. de Andrade Neves, P. César Stringheta, I. Ferreira da Silva, E. García-Romero, S. Gómez-Alonso, I. Hermosín-Gutiérrez, Identification and quantification of phenolic composition from different species of Jabuticaba (Plinia spp.) by HPLC-DAD-ESI/MSn, Food Chem. 355 (2021), 129605.
[36] S.-B. Wu, K. Dastmalchi, C. Long, E.J. Kennelly, Metabolite profiling of jaboticaba (Myrciaria cauliflora) and other dark-colored fruit juices, J. Agric. Food Chem. 60 (30) (2012) 7513–7525.
[37] M.R. Alezandro, P. Dubé, Y. Desjardins, F.M. Lajolo, M.I. Genovese, Comparative study of chemical and phenolic compositions of two species of jaboticaba: Myrciaria jaboticaba (Vell.) Berg and Myrciaria cauliflora (Mart.) O. Berg, Food Res. Int. 54 (1) (2013) 468–477.
[38] M. Plaza, Â.G. Batista, C.B. Cazarin, M. Sandahl, C. Turner, E. Östman, M. R. Maróstica Júnior, Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: a pilot clinical study, Food Chem. 211 (2016) 185–197.
[39] R. Huang, C. Xu, An overview of the perception and mitigation of astringency associated with phenolic compounds, Compr. Rev. Food Sci. Food Saf. 20 (1) (2021) 1036–1074.
[40] J.L. Espadas, E. Castaño, M.L. Marina, L.C. Rodríguez, M. Plaza, Phenolic compounds increase their concentration in Carica papaya leaves under drought stress, Acta Physiol. Plant 41 (11) (2019) 180.
[41] A. Sharma, B. Shahzad, A. Rehman, R. Bhardwaj, M. Landi, B. Zheng, Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress, Molecules 24 (13) (2019) 2452.
[42] P. Mendonça de Assis, R. Cypriano Dutra, C. Amarante, M. Afonso Miranda Chaves, C. Moreira, M. Brandão, N. Raposo, Plinia cauliflora (Mart.) Kausel: toxicological assays, biological activities, and elemental analysis of organic compounds, Nat. Prod. Res. 35 (10) (2019) 1727–1731.