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FULL ARTICLE

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Aqueous extract of berry (*Plinia jaboticaba*) byproduct modulates gut microbiota and maintains the balance on antioxidant defense system in rats

Juliana Kelly da Silva-Maia[1] | Angela Giovana Batista[1,2] | Luiz Claudio Correa[3] | Glaucia Carielo Lima[1,4] | Stanislau Bogusz Junior[5] | Mário Roberto Maróstica Junior[1]

[1]Faculty of Food Engineering, Department of Food and Nutrition, University of Campinas (UNICAMP), Campinas, Brazil

[2]Department of Food and Nutrition, Federal University of Santa Maria (UFSM, Campus Palmeira das Missões), Palmeira das Missões, Brazil

[3]Brazilian Agricultural Research Corporation, Embrapa Tropical Semi-arid, Petrolina, Brazil

[4]Faculty of Nutrition, Federal University of Goiás (UFG), Goiânia, Brazil

[5]São Carlos Institute of Chemistry (IQSC), University of São Paulo (USP), São Carlos, Brazil

Correspondence
Mário Roberto Maróstica Junior, Faculty of Food Engineering, Department of Food and Nutrition, University of Campinas (UNICAMP). Rua Monteiro Lobato, 80. Cidade Universitária. Campinas, SP 13083-862, Brazil.
Email: mmarosti@unicamp.br

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Abstract

Previous studies have assessed the properties of aqueous extracts, using byproducts such as jaboticaba peel. We have assessed potential antioxidant effects of jaboticaba extract (*Plinia jaboticaba*) (JAE = 50 g/L) in vitro and in vivo. Healthy Wistar rats received ad libitum JAE for either 15 or 49 days in vivo. Cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, gallic acid, rutin, myricetin, and quercetin were identified as the main polyphenols in JAE. Lipid peroxidation values in the serum and colon were similar throughout the groups. In addition, JAE did not disturb the antioxidant systems. JAE also altered gut microbiota, increasing since *Lactobacillus*, *Bifidobacterium* and *Enterobacteriaceae* counts. Bacterial metabolites were higher in the colon content of rats fed with JAE than in the control group. Given these results, under healthy conditions, JAE dietary supplementation could perform in vivo modulation of gut microbiota, without disturbing the antioxidant system.

Practical application

Jaboticaba (*Plinia jaboticaba*) peel is a rich and often-wasted source of bioactive compounds, such as polyphenols. Previous studies have shown that physiological benefits of this berry. The jaboticaba peel could contribute to antioxidant defense systems; it may also have an effect over gut microbiota related to polyphenols contents. Aqueous extraction may be a practical way of employing the bioactive compounds of jaboticaba peel; these compounds can be consumed daily and safely, and thus have attracted particular attention. This work showed positive impacts of jaboticaba peel treatments on microbiota and antioxidant defense systems, and could guide future clinical studies.

KEYWORDS

free radical, functional food, infusion, jaboticaba, *Myrtaceae*, short-chain fatty acids

1 | INTRODUCTION

One way to provide the body with bioactive compounds is the consumption of vegetables and herbal infusions (Farzaneh & Carvalho, 2015). Aqueous extracts have attracted particular attention because they can be consumed daily and safely, in contrast to harmful, organic solvent-based extracts, and require no further separations (Bhebhe, Füller, Chipurura, & Muchuweti, 2016). A

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; CAT, catalase; CFU, colony forming unit; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; FRAP, ferric reducing ability; GAE, gallic acid equivalent; GPx, glutathione peroxidase; GR, Glutathione reductase; JAE, aqueous extract of jaboticaba peel; JL, long-term treatment; JP, Jaboticaba peel; JS, short-term treatment; ORAC, oxygen radical absorbance capacity; PB, potassium phosphate buffer; SCFA, short-chain fatty acids; SD, standard deviation; SOD, superoxide dismutase activity; TBARS, test of thiobarbituric acid reactive substances; TE, trolox equivalent; TPC, total phenolic content.

mixture of bioactive compounds is present in plant-based extracts obtained via different solvents and extraction techniques, under different conditions (Lantano, Rinaldi, Cavazza, Barbanti, & Corradini, 2015). Phenolic compounds, a class of compounds which stands out due to its functional mechanisms, are one example (Pietta, 2000).

Phenolic compounds are bioactive dietary compounds with antioxidant and other beneficial actions (Pietta, 2000). They share a diphenylpropane ($C_6C_3C_6$) structure with varying degrees of hydroxylation, oxidation, and substitution (Pietta, 2000): different substitutions lead to different biological activity or bioavailability (Overall et al., 2017). The anti-radical activity of such compounds is linked to the inhibition of enzymes associated with reactive oxygen (ROS) production, direct quenching of free radicals, chelation of transition metals, or redox inactivation of ROS (Heim, Tagliaferro, & Bobilya, 2002).

ROS and nitrogen species are generated by exogenous factors, as well as normal metabolic processes. However, imbalances in the body's antioxidant defense system and free radical formation causes oxidative stress (Kehrer & Klotz, 2015); an excess of free radicals can damage a range of biomolecules, promoting diseases. On the other hand, research advances have indicated that reactive species also play a crucial role in normal physiological processes, including signaling pathways, growth factors response, immune response, and apoptotic elimination of damaged cells (Kurutas, 2016).

Additional attributes of phenolic compounds include modulating the gut microbiota and its metabolites, such as short-chain fatty acids (SCFA). Beneficial properties are attributed to SCFA, such as improvement of glucose tolerance, appetite control, reduction of gut absorption, and lipid synthesis on liver (Kasubuchi, Hasegawa, Hiramatsu, Ichimura, & Kimura, 2015). The gut microbiota plays a pivotal role in the biotransformation and bioavailability of both macronutrients and polyphenols. The resultant metabolites themselves are able to modulate the host microbiota, and their activity improves overall health (Wan, Ling, El-Nezami, & Wang, 2018).

An earlier work by da Silva et al. (2017) has shown that jaboticaba (*Plinia jaboticaba*) peel contains bioactive compounds, and that its aqueous extract has strong antioxidant capacity. Jaboticaba (*P. jaboticaba*) is a native Brazilian berry, which can also be found in other tropical regions (Wu, Long, & Kennelly, 2013). Its bioactive, mainly phenolic, compounds have been the objects research papers that highlight their various physiological actions (Wu et al., 2013). The tea-like, aqueous infusion of jaboticaba peel could be a way of enabling the consumption of this portion of the fruit, which is usually wasted when consumed fresh. A recently study has shown that the aqueous extract of jaboticaba peel could be an alternative to increase dietary the polyphenol content, since it has a total phenolic concentration of 150-mg gallic acid equivalent per 250-ml (8.5 oz) cup:, half the valor found, for example, in the green tea(da Silva et al., 2017). In view of the above, our aim was to investigate the effects of a bioactive aqueous extract of jaboticaba peel (JAE) bioactive aqueous extract on anti-radical defenses and gut microbiota of healthy rats.

2 | METHODS AND MATERIALS

2.1 | Jaboticaba and aqueous extract preparation

Ripe *P. jaboticaba* fruits, kindly donated by a commercial jaboticaba farmer from Lagoa Branca, Sao Paulo, Brazil (21°53′17″N, 47°2′59″W) were hand-picked in October 2013. The fruits were peeled, and the peels were oven-dried in an air circulation oven at 40°C (Marconi; Piracicaba, SP, Brazil), for 96 hr. Dried peels were ground (Marconi MA 630/1; Piracicaba, SP, Brazil), sieved (20 mesh), and stored in the dark at −20°C, until further use.

Prior to analysis, JAE was prepared by immersing 50 g of dried peel powder in 1,000 ml of boiling water (90–100°C), which was then manually mixed three times (0, 15, and 35 min) at room temperature (22 ± 2°C), before being vacuum-filtered through filter paper. For each analysis, extractions were carried out in triplicate.

2.1.1 | Spectrophotometric analyses of JAE's bioactive compounds and antioxidant capacity

Analysis of JAE's bioactive compounds and antioxidant capacity was performed according to detailed descriptions provided by previous studies (Batista et al., 2016; da Silva et al., 2017).

Total phenolic content (TPC) was expressed as gallic acid equivalents (mg GAE ml^{-1}) (Swain & Hillis, 1959). Diluted JAE samples (50 μl), distilled water (800 μl) and Folin–Ciocalteu reagent (50 μl) were mixed together and allowed to rest for 3 min, followed by the addition of a 20% (w/v) sodium carbonate solution (200 μl). The mixture was kept in the dark for 120 min. Absorbance was measured at 725 nm.

Total yellow flavonoids were expressed as catechin equivalents (mg CE ml^{-1}) (Zhishen, Mengcheng, & Jianming, 1999). Briefly, sample (100 μl), distilled water (500 μl) and 5% $NaNO_2$ solution (30μl) were mixed, then, 10% $AlCl_3$ solution (60 μl) was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 1 M NaOH solution (200 μl) and distilled water were added. The absorbance was measured at 510 nm.

Total monomeric anthocyanins were expressed as mg cyanidin 3-glucoside ml^{-1} (Wrolstad, 1976). Dilutions from the samples were carried out using 0.025 M potassium chloride buffer, pH = 1.0. Absorbances were measured at 510 and 700 nm. A second dilution was carried out for each sample, using 0.4 M sodium acetate buffer, pH 4.5, whose absorbance had the same wavelengths. Differences between the absorptions of the dilution systems were assessed using Equations (1) and (2) below:

$$A = \left[(A510\,\text{nm} - A700\,\text{nm})\ \text{pH} = 1.0\right] - \left[(A510\,\text{nm} - A700\,\text{nm})\ \text{pH} = 4.5\right] \quad (1)$$

Results were expressed as mg cyanidin 3-glucoside 100 ml^{-1}. Final results were calculated using Equation (2):

$$C\left(\text{mg } 100\,\text{ml}^{-1}\right) = A \cdot \text{MW} \cdot \text{DF} \cdot 100 \div \xi \cdot 1 \quad (2)$$

where ξ = molar absorptivity (26,900 mol/L), 1 = tray thickness (cm), MW = molecular weight of cyanidin 3-glucoside, and DF = dilution factor.

Assessment of total carotenoids was performed indicated in by Higby (1962) with results expressed in mg g^{-1}. Extraction was performed using isopropyl alcohol:hexane (1:3:1). Content was transferred to a 125-ml separatory funnel, where the volume was completed with distilled water. The mixture was left to rest for 30 min, followed by washing phases. The content was filtered through cotton sprayed with anhydrous sodium sulphate, into a 100-ml volumetric flask, to which 5-ml acetone were added, and then diluted with hexane (3–4 ml). Absorbances were measured at 450 nm.

The 2,2-diphenylpicrylhydrazyl (DPPH) assay results were expressed as IC_{50} and µmol Trolox equivalents (TE) ml^{-1} (Rufino et al., 2010). Extracts (33 µl) were added to a 1.3-ml DPPH methanolic solution (0.024 mg/ml) followed by shaking and incubation for 30 min, in the dark. Absorbance was measured at 515 nm.

Ferric reducing antioxidant power (FRAP) was expressed as µmol TE ml^{-1} (Benzie & Strain, 1996). The FRAP reagent was prepared in the dark, with 300 mmol/L acetate buffer (pH 3.6), and 10 mmol 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in a 40 mmol/L HCl and 20 mmol/L $FeCl_3$ solutions. Samples or standard solutions (20 µl), ultrapure water (60 µl), and the FRAP reagent (600 µl) were mixed together and kept in a water bath for 30 min at 37°C. After cooling to room temperature, absorbance of the samples and standards was measured at 595 nm.

Hydrophilic oxygen radical absorbance capacity (ORAC) was expressed as µmol TE ml^{-1}. Linear regression of the net area under the curve versus concentration was verified (Davalos, Gomez-Cordoves, & Bartolome, 2004). The reaction mixture consisted of extracts (20 µl), fluorescein (120 µl), and AAPH (60 µl). The reaction was carried out using 520 nm emission and 485 nm excitation filters. Incubation was conducted at 37°C. Fluorescence was recorded every 1 min, for 80 min.

All samples and standards were tested in triplicate using 96-well microplates. Absorbance and fluorescent intensity was measured using a BioTek Synergy HT microplate reader (Winooski, USA) coupled to the Gen5™ (version 2.0) data software program.

2.1.2 | High-performance liquid chromatography-diode array detection (HPLC-DAD) JAE analyses

JAE phenolic compounds were identified and quantified by HPLC (Agilent 1100 Series; Englewood, CO, USA) apparatus equipped with manual injection, a 20-µl sample loop, a ternary pump, and a diode array detector. Analysis was carried out as described in a previous work (Natividade, Corrêa, Souza, Pereira, & Lima, 2013). The extract was separated using a C18 column (150 × 4.6 mm, 3 µm, Phenomenex, Gemini NX; Torrance, CA, USA) protected by a C18 guard column (4.0 × 3.0 mm, Gemini NX) at 40°C. The mobile phase consisted of a 25 mmol/L potassium dihydrogen phosphate buffer (PB) prepared with ultrapure water (Elga Systems, Purelab Option Q; Woodridge, IL, USA) and pH adjusted to 2.05 using phosphoric acid (Fluka, Switzerland) as solvent A, methanol (HPLC grade, JT Baker; Phillipsburg, NJ, USA) as solvent B and acetonitrile (HPLC grade, JT Baker) as solvent C. The employed elution gradient was as follows: 0 min 100% A; 18 min 87.5% A, 2.5% B, 10.0% C; 30 min 83.5% A, 3.2% B, 13.3% C; 36 min 75.0% A, 5.0% B, 20.0% C; 48.5 min 65.0% A, 8.3% B, 26.7% C; 50 min 65.0% A, 8.3% B, 26.7% C and 65 min 100% A. JAE was filtered through a 0.45-µm membrane before injection. Flow rate was 0.6 ml/min. Injection volume was 20 µl. Data collection and analysis were carried out using Empore™ 2 software (Milford, CT, USA).

Phenolic compounds were identified by comparing their relative retention times (RT) and UV spectral data with those of identical standards at 520 nm (cyanidin 3-O-glucoside, delphinidin 3-O-glucoside), 220 nm (gallic acid), and 360 nm (rutin, myricetin, quercetin). Standard gallic acids were purchased from Chem Service (West Chester, PA, USA). Other standards were obtained from Extrasynthese (Genay, France).

Calibration curves, standard purities, calibration curve equations, coefficients of determination, detection limits, quantification limits, and assay repeatability through the coefficient of variation were described in a previous work (da Silva et al., 2015).

2.2 | In vivo experiment design

The in vivo experiment was approved by the local ethical committee (CEUA/UNICAMP #3199-1, Campinas, Brazil), and carried out in accordance with the guide for the care and use of experimental animals established by the National Council for the Controlling on Animal Testing (Brazil). Male Wistar rats (21 days old) were housed in individual cages under controlled temperature (22 ± 2°C) and inverted light–dark cycle (12/12 hr) conditions. The rats had ad libitum access to a commercial chow (Presence®, Evialis do Brasil Nutrição Animal; Ceará, Brazil). According to the manufacturer, the feed had approximately 23 g 100 g^{-1} proteins; 4,5 g 100 g^{-1} lipid; 63,5 g 100 g^{-1} carbohydrate, and 5 g 100 g^{-1} fiber. Its list of ingredients was: soybean meal, ground whole corn, extruded whole soybean, wheat bran, wheat flour, corn gluten meal, rice bran, meat and bone meal, fish meal, calcitic limestone, sodium chloride, iron sulfate, copper sulfate, manganese monoxide, zinc oxide, calcium iodate, cobalt sulfate, sodium selenite, vitamin A, vitamin D3, vitamin E, vitamin K, vitamin B1, vitamin B2, niacin, pantothenic acid, vitamin B6,folic acid, biotin, vitamin B12, choline chloride, lysine, methionine, threonine, propionic acid, silicon dioxide, urea formaldehyde. Water or JAE were also offered ad libitum. JAE was prepared every 2 days for the in vivo experiment.

After acclimatization for 1 week, the rats were randomly assigned to one of three groups: control (CT), JAE short-term (JS)

and JAE long-term (JL) (n = 8). Group CT had free access to water throughout the experiment. Group JS had water for 5 weeks, followed by free access to JAE, instead of water, for 2 weeks. Group JL received JAE instead of water throughout the experimental period (7 weeks). Diet and liquid intake were monitored every two days, and body weight once a week. The animals were anesthetized with ketamine chloride (Anasedan®) and xylazine chloride (Dopalen®) (Fortvale, Valinhos, SP, Brazil) (40 and 5 mg/kg, respectively) and euthanized by exsanguination through cardiac puncture. The serum was collected, centrifuged at 2,000 × g for 20 min, aliquoted and stored at −80°C, alongside colon tissue and colon fecal content.

Previous works have studied the jaboticaba peel through different experimental models (Alezandro, Granato, & Genovese, 2013; Batista et al., 2017; Plaza et al., 2016). However, data on jaboticaba's aqueous extract are scarce (Lenquiste et al., 2015). In this sense, the JAE concentration was determined through a pilot experiment, in which three different concentrations were tested (15, 25, and 50 g/L).

2.2.1 | Antioxidants and peroxidation analysis

Both serum and colon were analyzed. A colon homogenate was prepared in PB (pH 7.4), and the supernatant was assayed in search for antioxidant enzymatic activities, as described in a previous work (Da Silva et al., 2013). The protein concentration of tissue homogenates was determined via the Bradford method (Bradford, 1976).

Superoxide dismutase (SOD) activity was expressed as either U mg^{-1} protein or U ml^{-1} serum (Winterbourn, Hawkins, Brian, & Carrell, 1975). PB homogenates (100 µl) were added to 150-µl working solution (0.1 mmol hypoxanthine, 0.07 U xanthine xidase, and 0.6 mmol NTB in PB; 1:1:1 proportions). Kinetic reaction was monitored at 560 nm.

Glutathione peroxidase (GPx) (Flohe & Gunzler, 1984) was assessed from the oxidation of 10 mmol reduced glutathione by glutathione peroxidase coupled to the oxidation of 4 mmol NADPH by 1 U enzymatic activity of GR in the presence of 0.25 mmol H_2O_2. Kinetic reaction was monitored at 365 nm. Results were expressed in nmol NADPH consumed min^{-1} mg^{-1} protein (or nmol NADPH consumed min^{-1} ml^{-1} serum).

Glutathione reductase (GR) activity (Carlberg & Mannervik, 1985) was measured from PB homogenates, following the decrease in absorbance at 340 nm induced by 1 mmol oxidized glutathione in the presence of 0.1 mmol NADPH in phosphate buffer. The results were expressed as nmol NADPH consumed min^{-1} mg^{-1} protein (or nmol NADPH consumed min^{-1} ml^{-1} serum).

For measuring catalase (CAT) activity, the colon homogenate was prepared with 0.1 g/ml 50 mmol L^{-1} PB containing 1 mmol/L EDTA, pH 7.0, then centrifuged (10,000 × g, 4°C, 15 min). The supernatant was diluted in 25 mmol L^{-1} PB (pH 7.5, containing 1 mmol/L EDTA and 0.1% BSA) and added to a 96-well microplate containing 100 mmol L^{-1} PB (pH 7.0) and methanol. After 20 min on a microplate shaker, in the dark, H_2O_2 (10.58 mmol/L) was rapidly added to each well plate, followed by 7.8 mol/L KOH and 34.2 mmol/L purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) in 0.5 M hydrochloric acid, and then allowed to react for 10 min with continuous shaking, still in the dark. Finally, 65.3 mM potassium periodate in 0.5 NaOH was added to stop the reaction; absorbance was mesuared at 540 nm. Formaldehyde solution was used as a standard. Results were expressed as nmol of formaldehyde formation min^{-1} mg^{-1} protein at 25°C (or nmol of formaldehyde formation min^{-1} ml^{-1} serum), according to Johansson and Borg (1988), with the adaptations described above.

Lipid peroxidation was evaluated by the thiobarbituric acid reactive substances (TBARS) test according to Ohkawa, Ohishi, and Yagi (1979). Samples were macerated in liquid nitrogen and mixed with 8.1% sodium dodecyl sulfate (SDS) and working reagent (2-thiobarbituric acid—TBA, 5% acetic acid and 20% sodium hydroxide). The solution was allowed to react at 95°C for 60 min, then cooled in ice bath for 10 min, and centrifuged at 10,000 g for 10 min. The supernatant absorbance was mesuared at 532 nm. Results were expressed in nmol malondialdehyde (MDA) mg−1 tissue (or nmol MDA ml^{-1} serum).

2.2.2 | Hepatic enzymes

Hepatic function was evaluated by measuring alanine aminotransferase (ALT) and aspartate transaminase (AST) levels, using commercial kits (Sigma-Aldrich, St. Louis, MO, USA).

2.2.3 | Microbiota and SCFA in colon content

Gut microbiota and SCFA analyses of the colon content were performed according to an earlier study (da Silva, Cazarin, Bogusz Junior, Augusto, & Maróstica Junior, 2014). Serial dilutions of the cecum content were spread on agar plates appropriate for *Lactobacillus*, *Bifidobacterium*, *Enterobacteriaceae*, and total aerobes. After 24 or 48 hr, the number of colonies counted was expressed as log 10 colony forming units (CFU) g^{-1} (da Silva et al., 2014). The cecum content was prepared and contents (nmol butyric/acetic/propionic acid g^{-1} feces) were quantified by gas chromatography (Zhao, Nyman, & Jonsson, 2006).

2.3 | Statistical analyses

All analyses were carried out in triplicate and the data were expressed as means ± standard deviation (*SD*). GraphPad Prism 5.0 (GraphPad Software, Inc.; La Jolla, CA, USA) was used for all statistical analyses. The significance of the data was determined using one-way analysis of variance (ANOVA) and Tukey's post hoc test ($p < 0.05$), except for the analysis of liquid intake and body weight, where two-way ANOVA and Bonferroni's post hoc test were used ($p < 0.05$).

3 | RESULTS AND DISCUSSION

3.1 | In vitro experiment

A previous study has characterized the aqueous extract of jaboticaba peel (da Silva et al., 2017), showing that aqueous extraction is

TABLE 1 Bioactive compounds and antioxidant capacity in jaboticaba aqueous extract (JAE) and jaboticaba peel powder

Parameters	Per ml of JAE	Per g* of peel powder
Total Polyphenols (mg GAE)	1.32 ± 0.09	25.8 ± 1.36
Yellow Flavonoids (mg CE)	0.15 ± 0.002	3.03 ± 0.04
Total monomeric anthocyanins (mg C3G)	0.075 ± 0.001	1.50 ± 0.04
Total carotenoids (mg)	–	35.86 ± 5.13
FRAP (μmol TE)	10,842.4 ± 782.1	216.8 ± 15.65
DPPH# (μmol TE)	10,304.2 ± 1,162.6	203.8 ± 13.9
ORAC (μmol TE)	8,062.6 ± 1,341.3	161.3 ± 26.8

Note. GAE: gallic acid equivalent; CE: catechin equivalents; C3G: cyanindin 3-glucoside equivalents; TE: Trolox equivalent. ORAC linearity test: concentration range = 0.25–6.75 g/L, slope = 78.311, intercept = 86.204, R^2 = 0.9903. Results are expressed as means ± standard deviation (n = 3). *Extracted as JAE. #IC_{50} = 2.76 g/L.

TABLE 2 Phenolic compounds by HPLC-DAD in jaboticaba aqueous extract (JAE)

Polyphenols			
		RT (min)	mg L^{-1}
Phenolic acid			
1	Gallic acid	11.40 ± 0.008	5.84 ± 0.43
Flavonols			
2	Quercetin	51.05 ± 0.015	0.10 ± 0.00
3	Myricetin	48.01 ± 0.004	0.10 ± 0.00
4	Rutin	43.19 ± 0.004	1.40 ± 0.17
Anthocyanins			
5	Delphinidin-3-*O*-glucoside	25.11 ± 0.015	2.29 ± 0.44
6	Cyanidin-3-*O*-glucoside	28.22 ± 0.023	52.53 ± 5.37

Note. Results are expressed as mean ± standard deviation (n = 3). RT = retention time.

able to remove polyphenols from the peel, mainly anthocyanin, resulting in a demonstrably strong antioxidant capacity. This extraction method could be easily applied to in vivo use since benefits of the jaboticaba peel have already been shown, such as positive effects on antioxidant defense, glycemic response, and lipid levels (Batista et al., 2017; Batista, Leite-Legatti, De Lima, Prado, & Maróstica Junior, 2014; Plaza et al., 2016). In this sense, this study confirmed the efficacy of aqueous extraction, using in vitro tests as a means of obtaining bioactive compounds from jaboticaba (*P. jaboticaba)* peel in order to evaluate the in vivo effects of the extract on anti-radical defenses and gut microbiota in healthy rats. Some of the bioactive compounds present in JAE, as determined by colorimetric assays are described in Table 1. The efficiency of aqueous extraction was not decreased using

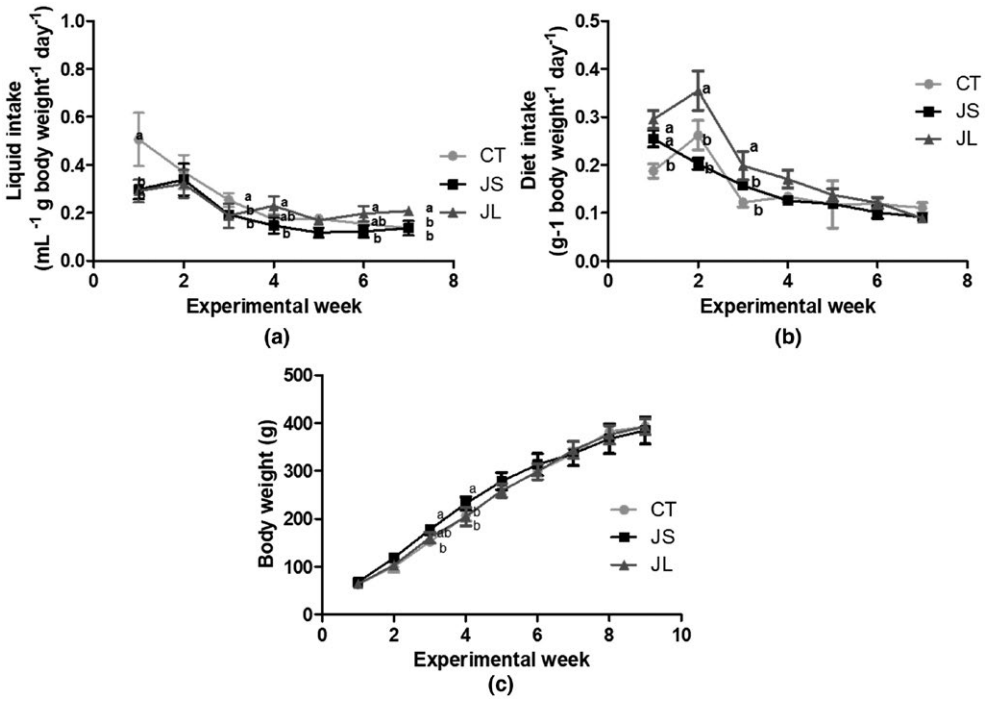

FIGURE 1 Consumption and body weight. (a) Liquid intake (ml^{-1} g body weight^{-1} day^{-1}) over nine experimental weeks, (b) Diet intake (g^{-1} g body weight^{-1} day^{-1}), and (c) Body weight (g^{-1}). Groups: CT = control; JS = short-term intake of jaboticaba aqueous extract; JL = long-term intake of jaboticaba aqueous extract. Results are expressed as the means ± standard deviation (n = 5–8). Different letters indicate $p \leq 0.05$ according to ANOVA and Bonferroni's test

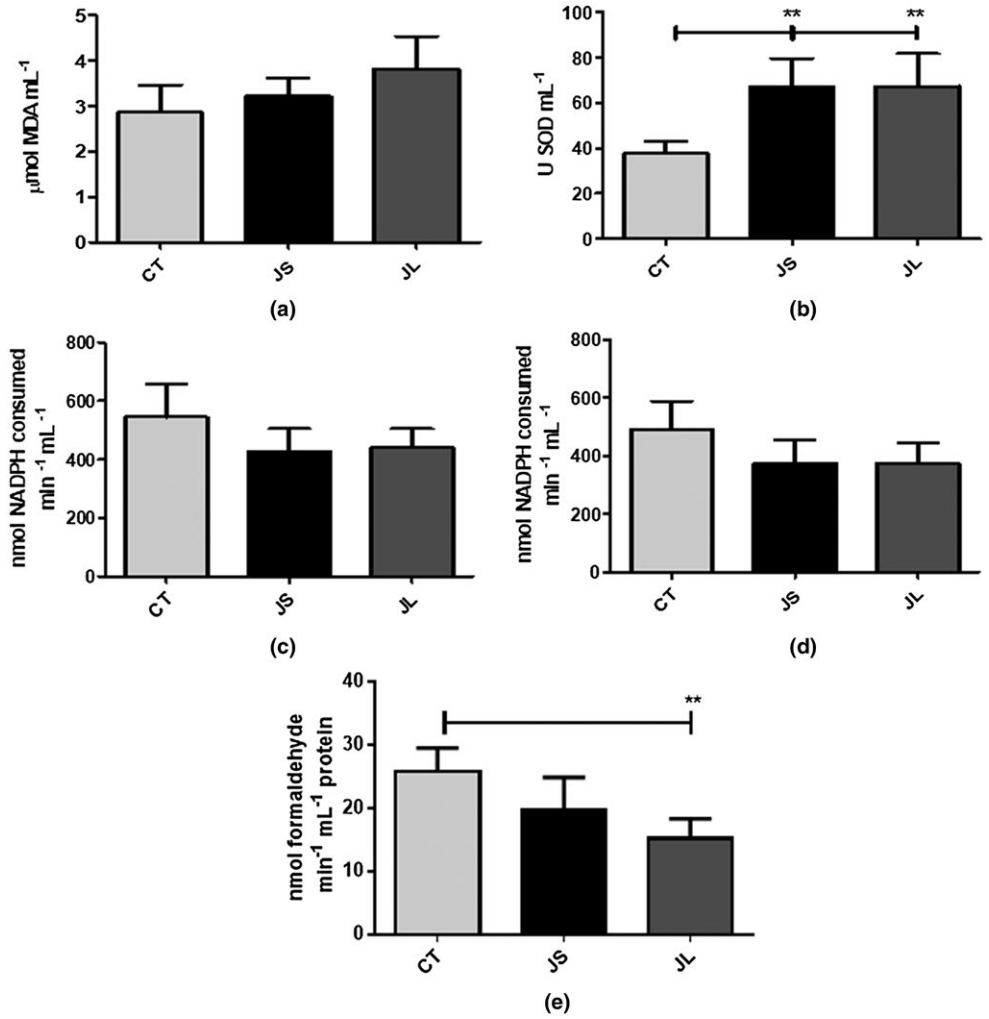

FIGURE 2 Antioxidant status in serum. (a) Thiobarbituric acid reactive substances—TBARS expressed in µmol malondialdehyde (MDA) ml^{-1}, (b) Superoxide dismutase—SOD expressed in U SOD ml^{-1}, (c) Glutathione reductase—GR expressed in nmol NADPH consumed min^{-1} ml^{-1}, (d) Glutathione peroxidase—GPx expressed in nmol NADPH consumed min^{-1} ml^{-1}, and (e) Catalase—CAT expressed in nmol formaldehyde min^{-1} ml^{-1}. Groups: CT = control; JS = short-term intake of jaboticaba aqueous extract; JL = long-term intake of jaboticaba aqueous extract. Results are expressed as the means ± standard deviation (n = 5–8). *$p \leq 0.05$; **$p \leq 0.01$ according to ANOVA and Tukey's test

jaboticaba peel powder at higher concentration than that used previously (da Silva et al., 2017), since results (per g, Table 1) were similar to those found at a lower concentration (25 g/L) (da Silva et al., 2017). Moreover, as expected, the concentration of bioactive compounds per milliliter of extract was superior in the 50 g/L JAE (da Silva et al., 2017).

TPC and DPPH in JAE were higher than other work with aqueous extracts of 20 different plants (Wong, Leong, & William Koh, 2006). TPC and yellow flavonoids values found in a study on the aqueous extract of blackberries by Celant, Braga, Vorpagel, and Salibe (2016) were approximately 2.5 and 3 times lower, respectively, than for JAE, in this study. Carotenoids contribute to the lipophilic antioxidant effect of the food matrix. JAE has shown higher total carotenoids than mango peel powder (Ajila, Aalami, Leelavathi, & Rao, 2010). The IC_{50} value is inversely proportional to the antioxidant potential. JAE values were similar to those found by other authors in the peel of 13 citrus fruits (Ghasemi, Ghasemi, & Ebrahimzadeh, 2009). Therefore, these findings show that the extraction method used in the present study was efficient, and, in addition, showed high antioxidant capacity.

JAE has shown higher anti-radical potential in comparison to the methanol extract of *Vaccinium uliginosum* L. berry peels from 16 distinct geographic regions according to ORAC, FRAP, and DPPH assays (Su et al., 2016). Therefore, extraction using water as a solvent by the infusion method is an effective and environmentally friendly technique to obtain an extract with high antioxidant capacity from *P. jaboticaba* peel. This method could be used by future studies, combined with different approaches.

Recently, the interest in byproducts has been increasing, due to the economic and social interest of the agri-food industry in fully utilization of raw materials, and also due to the bioactive compounds present in such products (Peschel et al., 2006). Authors have

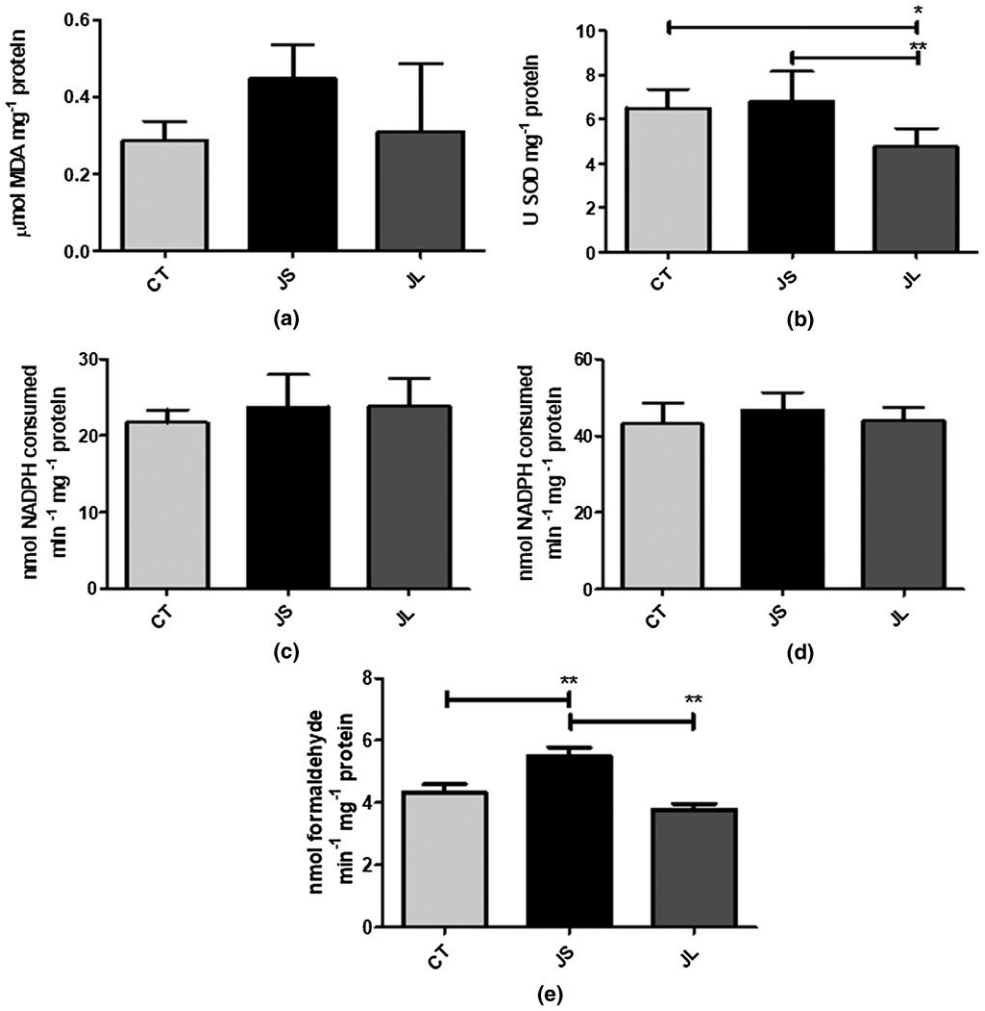

FIGURE 3 Antioxidant status in colon. (a) Thiobarbituric acid reactive substances—TBARS expressed in µmol malondialdehyde (MDA) mg^{-1} protein, (b) Superoxide dismutase—SOD expressed in U SOD mg^{-1} protein, (c) Glutathione reductase—GR expressed in nmol NADPH consumed mg^{-1} protein, (d) Glutathione peroxidase—GPx expressed in nmol NADPH consumed min^{-1} mg^{-1} protein, and (e) Catalase—CAT expressed in nmol formaldehyde min^{-1} mg^{-1} protein. Groups: CT = control; JS = short-term intake of jaboticaba aqueous extract; JL = long-term intake of jaboticaba aqueous extract. Results are expressed as the means ± standard deviation (n = 5–8). *$p \leq 0.05$; **$p \leq 0.01$ according to ANOVA and Tukey's test

shown that the peel has greater concentrations of antioxidants than other plant parts, which is attributed to its role in plants as a defense mechanism against insect pests, pathogens, and UV radiation (Elfalleh et al., 2012). According HPLC-DAD assessment of phenolic compounds in JAE (Table 2), the major compounds in the jaboticaba peel were two anthocyanins, namely cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside. In addition, other phenolic compounds were identified in significant concentrations, including gallic acid, rutin, myricetin, and quercetin, in agreement with the findings of Inada et al. (2015). These results establish the compounds profile of this extract, which could guide prospective studies and technological application.

3.2 | In vivo experiment

Natural extracts rich in bioactive compounds, such as anthocyanins, have been previously explored as dietary supplements or ingredients in the food industry, and their promotion is associated with health claims (Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015). This experiment found that the extract was well accepted by the rats. They have drunk about 39.5 ± 5.3 ml of JAE per day, and polyphenols intake was approximately 1.3 ± 0.2 mg GAE per day. The consumption of JAE and water were similar in the control group and the JS groups, respectively. Although the JS rats had a lower ingestion (since they received no treatment in the first three weeks), their body composition was unaffected by the treatment, as shown in the growth curve. Therefore, JAE had no influence on weight gain throughout the evaluated period, an expected result, considering the animals were healthy from the start (Figure 1).

Oxidative stress contributes to many health disorders by impairing a range of biomolecules (Kehrer & Klotz, 2015). Despite this, reactive species have a crucial role in normal physiological processes (Weidinger & Kozlov, 2015). The TBARS assay, an indicative of oxidative stress, showed no tissue or serum difference in either treated or

nontreated groups (Figures 2 and 3). Future investigations of JAE antioxidant effects using stress stimulus rather than health conditions could better predict the effect of JAE on lipid peroxidation status. Another limitation is the scarce knowledge of the bioavailability of phenolic compounds found in aqueous extract of jaboticaba peel.

Nonenzymatic antioxidants, such as dietary components, can act in the endogenous antioxidant defense system by directly scavenging free radicals that, for example, modify the activity of enzymes (Kurutas, 2016). Both JAE treatments increased serum SOD activity by about 75% (Figures 2 and 3), whereas the JL treatment lowered serum CAT activity, while elevating colon CAT activity in the same group by approximately 50% (Figure 2). Neither serum nor gut tissue activity of the other measured antioxidant enzymes (GR and GPx) were significantly different among the groups (Figures 2 and 3). In vivo, a complex and integrated network of antioxidant metabolites and enzymes act together to prevent oxidative damage. Furthermore, critics of the indiscriminate use of antioxidants have raised the question that oxidative species are important to physiologically essential signaling pathways and the role of the antioxidant enzymes is paradoxical (Kurutas, 2016). All things considered, our findings indicated that JAE intake had a balanced impact on the activity of antioxidant enzymes, considering the healthy (physiological) conditions of the model.

The gut is a natural habitat for a large and dynamic bacterial community. This microbiota has a role in the host physiology and pathology and its composition can be modulated by many factors, such as dietary components (Wang, Yao, Lv, Ling, & Li, 2017). Even nonnutritive compounds, like polyphenols, can interact with gut microbiota and avoid dysbiosis, a disequilibrium of the resident bacterial community (Wan et al., 2018). In this experiment, the jaboticaba peel extract was able to modulate the commensal gut microbiota, as evidenced by from the enterobacteria and bifidobacteria proliferation in both treatment protocols, and by the increase in *Lactobacillus* genus after short-term treatment. However, the total amount of aerobic bacteria was not affected (Figure 4). Among their diverse health benefits, *Lactobacillus* and *Bifidobacterium* spp. have been linked to inhibitory action against harmful bacteria, probably due to pH reduction in the intestinal environment (Likotrafiti & Rhoades, 2015). *Lactobacillus* spp. and *Escherichia coli* are among the few gut bacteria currently related to the metabolism of polyphenols (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013). In vitro and in vivo studies by Tzounis et al. (2008) suggested that treatment with a purified flavonol enhanced the growth of *E. coli*, as observed in this study, while *Lactobacillus* spp. was unaffected. Conversely, resveratrol promoted fecal *Lactobacillus* counts in an experimental model (Larrosa et al., 2009). Curcumin is a polyphenol that has been shown to interact with gut microbiota, mechanisms involved were discussed in a recent work (Shen & Ji, 2018).Prospective studies can go deeper and evaluate the relationship between JAE, or anthocyanin specifically, and gut microbiota.

Gut microbiota can modulate host energy and lipid metabolism in rats. In this study, JAE significantly increased acetate

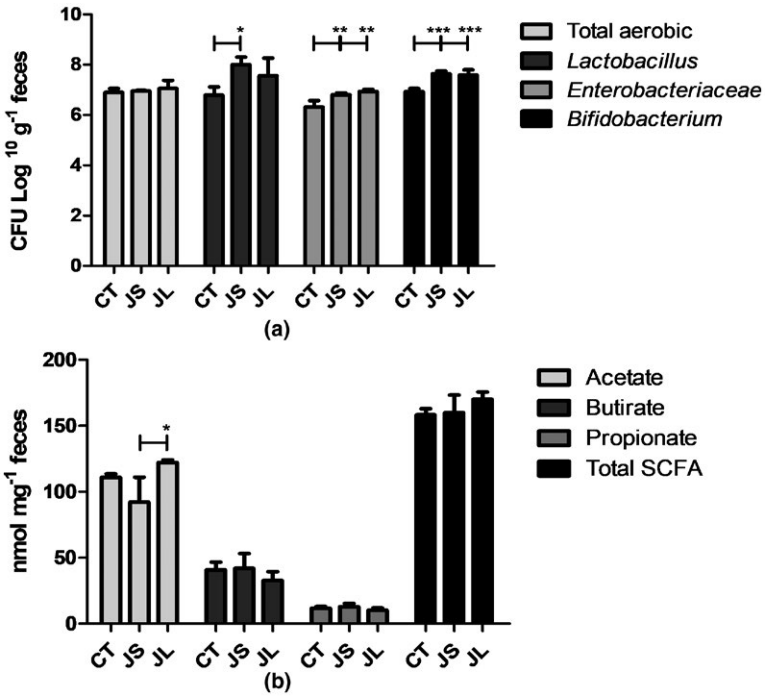

FIGURE 4 Effect on the gut microbiota. (a) Microbiota colonies expressed in colony formation unit (CFU) $\log^{10}$ g^{-1} feces, (b) Short-chain fatty acids production expressed in nmol mg^{-1} feces. Groups: CT = control; JS = short-term intake of jaboticaba aqueous extract; JL = long-term intake of jaboticaba aqueous extract. Results are expressed as the means ± standard deviation (n = 5–8). *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$ according to ANOVA and Tukey's test

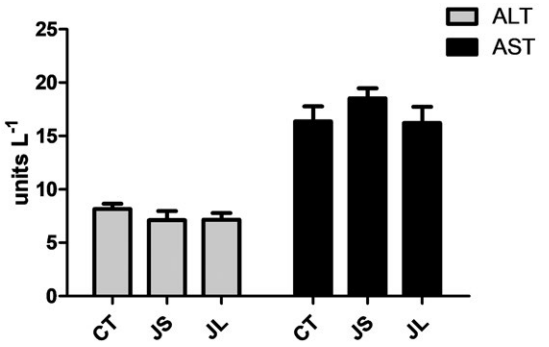

FIGURE 5 Hepatic toxicity. Alanine aminotransferase—ALT and aspartate transaminase—AST in serum expressed in Units L^{-1}. Groups: CT = control; short-term intake of JS = jaboticaba aqueous extract; JL = long-term intake of jaboticaba aqueous extract. Results are expressed as the means ± standard deviation (n = 5–8). ***$p \leq 0.001$ according to ANOVA and Tukey's test

production in the colon of long-term treated rats, but propionate, butyrate and total SCFA had no change (Figure 4). Literature has shown in vivo the potential of acetate to improve glucose tolerance and appetite control Kasubuchi et al. (2015). In this context, the potential of jaboticaba peel extract to ameliorate some consequences of diet-induced obesity is evident in the fact that it increases acetate production (Lenquiste et al., 2015). Previous studies have already attributed the prevention of weight gain and insulin sensitivity to the intake of raw jaboticaba peel, added to diets in obesity models (Batista et al., 2017; Lenquiste et al., 2015). This is another aspect that could be further explored in studies with the JAE.

JAE can be considered safe for consumption. As previous mentioned, treatments did not affect rats' body weight and lipid peroxidation. Furthermore, JAE administration did not increase ALT and AST, demonstrating that it is not toxic to the liver (Figure 5). The liver plays a major role in body's metabolism system. Increased levels of endogenous hepatic enzymes are indicative of damage and/or tissue overload (Batista et al., 2014). Therefore, these data suggest that JAE is a suitable source of natural antioxidants, encouraging further research with it, especially given the toxicity of many of the herbal preparations described in the literature (Teschke, Wolff, Frenzel, & Schulze, 2014). In any case, our results need corroboration; further investigation needed so other parameters can be assessed.

4 | CONCLUSION

Besides investigating new bioactive sources and the integral use of plant materials, it is worthwhile to consider their potential market applications. In this work, we assumed that aqueous extraction, such as an ordinary tea, is a practical way of employing bioactive compounds of jaboticaba peel than previously studied ones. In vitro and in vivo assays showed a favorable action on gut microbiota of rats, with no change of hepatic enzymes and no imbalance in serum and gut antioxidant status, confirming this study's hypothesis. Results showed significantly precocious effects on healthy subjects, possibly paving the way to preclinical studies involving different diseases, as well as and other clinical experiments.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

ORCID

Juliana Kelly da Silva-Maia http://orcid.org/0000-0002-7970-534X

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