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ORIGINAL ARTICLE

Antimicrobial effect of phenolic-rich jaboticaba peel aqueous extract on *Staphylococcus aureus* and *Escherichia coli*

Efeito antimicrobiano de extrato aquoso rico em polifenóis de casca de jabuticaba em Staphylococcus aureus e Escherichia coli

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Abstract

Jaboticaba peels are an important source of health-benefit and antimicrobial compounds. The present work aims to evaluate concentration of polyphenolics and the mode of action of aqueous extract from jaboticaba peels against *Staphylococcus aureus* and *Escherichia coli*. Jaboticaba peel extract showed minimum inhibitory concentration and minimum bactericidal concentration against *S. aureus* of 5.1 g L^{-1} and 10.1 g L^{-1}, respectively; meanwhile, against *E. coli* the parameters were 2.0 g L^{-1} and 3.4 g L^{-1}. Kinetics of viable cell counts indicated a bacteriolytic action against both bacteria and Scanning Electron Microscopy (SEM) showed that jaboticaba peel extract causes subtle morphological changes in bacterial cells. Concentration of total polyphenols in the extract was 1535.04±36.05 mg of gallic acid equivalent (GAE) mL^{-1}, monomeric anthocyanins was 14.52 ± 0.98 mg of cyanidin 3-glucoside mL^{-1}, condensed tannins was 0.49 ± 0.05 mg of epicatechin equivalent mL^{-1} and phenolic acids was 80.04 ± 4.11 mg of caffeic acid equivalent (CAE) mL^{-1}, which have demonstrated well-documented antibacterial activity. In conclusion, jaboticaba peel aqueous extract may be an interesting natural preservative to control Gram-positive and Gram-negative bacteria growth when interacting with the bacteria cell wall.

Keywords: *Myrciaria jaboticaba*; Biopreservation; Polyphenolics; Food residues.

Resumo

As cascas de jabuticaba são uma importante fonte de benefícios à saúde e compostos com atividade antimicrobiana. O presente trabalho tem como objetivo avaliar a concentração de polifenóis e o modo de ação do extrato aquoso da casca da jabuticaba contra *Staphylococcus aureus* e *Escherichia coli*. O extrato da casca da jabuticaba apresentou concentração inibitória mínima e concentração bactericida mínima contra *S. aureus* de 5,1 g L^{-1} e 10,1 g L^{-1}, respectivamente; já contra *E. coli*, esses parâmetros foram 2,0 g L^{-1} e 3,4 g L^{-1}. A cinética da contagem de células viáveis indica uma ação bacteriolítica contra ambas as bactérias e a microscopia eletrônica de varredura mostrou

que o extrato da casca da jabuticaba causa sutis alterações morfológicas nas células bacterianas. A concentração de polifenóis totais no extrato foi 1.535,04±36,05 mg ácido gálico equivalente mL^{-1}; antocianinas monoméricas, 14,52±0,98 mg cianidina 3-glicosídeo mL^{-1}; taninos condensados, 0,49 ± 0,05 mg equivalente epicatequina mL^{-1}, e ácidos fenólicos, 80,04±4,11 mg ácido cafeico equivalente mL^{-1}, compostos que já demonstraram atividade antibacteriana bem documentada. Em conclusão, o extrato aquoso da casca de jabuticaba mostra-se um bioconservante interessante para o controle de bactérias Gram-positivas e Gram-negativas, e interage com a parede celular bacteriana.

Palavras-chave: *Myrciaria jaboticaba*; Bioconservantes; Polifenóis; Resíduos de alimentos.

Highlights

- The Jaboticaba peel aqueous extract acts in the exponential growth phase of *Staphylococcus aureus* and *Escherichia coli*
- The extract changes cell morphology
- The extract may be an interesting natural preservative

1 Introduction

Jaboticabas (*Myrciaria jaboticaba* (Vell.) O. Berg *and M. trunciflora* O. Berg) are Brazilian native fruits, whose peels present high concentrations of polyphenolics (Quatrin et al., 2019, 2020) with remarkable health benefits such as preventing liver steatosis and obesity (Lenquiste et al., 2019). The beneficial effects comprise the reduction of excessive body adiposity, adipocyte hypertrophy and hepatic lipid accumulation, as well as decreasing inflammation, hyperglycemia, glucose intolerance, insulin resistance, and hypercholesterolemia (Moura et al., 2021). Other health benefits include increasing HDL cholesterol, *i.e.*, having a positive effect on the cardiovascular system and antidiabetic properties (Wu et al., 2012; Lenquiste et al., 2012, 2015). Besides the well-documented human health-promoting effects of bioactive compounds obtained from jaboticabas, the antimicrobial activity of some polyphenols has been reported, representing an interesting tool to be used as natural preservatives in the food processing chain (Lacombe et al., 2010; Caxambú et al., 2016; Trojaike et al., 2019; Fleck et al., 2021).

Compounds from jaboticaba peels have shown interesting antimicrobial activity against important foodborne bacteria such as *Escherichia coli*, *Salmonella enterica*, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* (Hacke et al., 2016; Oliveira et al., 2018; Fleck et al., 2021). Fleck et al. (2021) recently observed that anti-staphylococcal activity of compounds extracted from jaboticaba peels are stable to pH changes, thermal treatments, and light exposure and this capacity is closely related to the concentration of monomeric anthocyanins and phenolic compounds. The major compounds in jaboticaba peels are consisted of tannins and anthocyanins, mainly cyanidin 3-glucoside, delphinidin-3-glucoside and trigalloylglucose (Quatrin et al., 2019). Gallic acid and ellagic acid were also found in high concentrations in jaboticaba peels (Lenquiste et al., 2015). However, a deeper understanding of the mode of action of jaboticaba peel extract on bacteria is essential to assist in the effective utilization of food applications.

Aiming at practical applications, utilization of aqueous extracts to obtain target compounds from food matrix is highly important, since water is a safe, abundant, cheap solvent and does not involve environmental impact as well as discussing common knowledge regarding the food chain actors (Sant'Anna et al., 2017; Fleck et al., 2021). So far, no data is found in current literature evaluating antimicrobial mode of action of an aqueous extract of this fruit residue. In this context, the present work aims to evaluate the antimicrobial mode of action of jaboticaba peel extract against *S. aureus* and *E. coli*, *i.e.*, important bacteria of public health concern.

2 Material and methods

2.1 Plant material

Non-fermented jaboticaba peels (*M. jaboticaba*), obtained in January 2019 from local industry (Vale do Taquari region, RS, Brazil) after juice extraction, were dried by lyophilization (Liotop, model L101, SP, Brazil), manually crushed in a crucible and porcelain pistil and passed them through the 5 mm sieves. Samples were storage at -18 °C in sealed plastic bags in the dark until analysis.

2.2 Chemicals

The reagents used for analysis were of analytical grade and obtained from the Sigma-Aldrich (St. Louis, MO, USA). Bacterial culture media were obtained from Himedia (Sumaré, SP, Brazil). Phenolic standards of gallic acid, rutin and epicatechin were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.3 Jaboticaba peel extract

Jaboticaba peel extracts were obtained as described previously (Caxambú et al., 2016; Trojaike et al., 2019; Fleck et al., 2021). Samples of 3 g of dried jaboticaba peels were kept in contact with 30 mL boiling distilled water for 10 min in sealed flask with constant agitation, when the samples were filtered on Whatman filter paper nº1 for falcon tubes with a lid under aseptic conditions.

2.4 Antimicrobial activity

S. aureus ATCC 25923 and *E. coli* ATCC 25922 were used as target bacteria. Antimicrobial activity was measured by four techniques: *i)* agar diffusion method; *ii)* The Minimum Inhibitory Concentration (MIC); *iii)* Minimum Bactericidal Concentration (MBC); and *iv)* direct contact of bacteria biomass with the extract.

The agar diffusion method was performed using a 0.85% (w/v) sodium chloride solution with final concentration of 10^7 colony forming unit per milliliter (CFU mL^{-1}) of each strain. They were spread evenly onto Plate Count Agar, left drying in laminar flow hood for 2 min, when aliquots of 20 µL of the extract (diluted sequentially in distillate water) were applied. Plates were incubated at 37 °C for 24 h, when inhibition halo zones were verified (Fleck et al., 2021). Results were expressed as activity units per milliliter (AU mL^{-1}), which represent the reciprocal of the lowest dilution with halo zone

The MIC and MBC were evaluated by keeping 10^7 CFU mL^{-1} of the bacteria in contact with two-fold serial dilution of the jaboticaba extract for 24 h at 37 °C, when the absorbance at 630 nm was evaluated (National Committee for Clinical Laboratory Standards, 2005). Control tests were performed using sodium chloride solution. The last jaboticaba dilution to present turbidity was considered to be the MIC. MBC was performed by adding a 20 µL aliquot of the tubes that did not show microbial growth in the MIC tests onto PCA and incubated at 37 °C for 24 h to verify viable cell growth (Motta et al., 2007). Results were expressed as gram per liter (g L^{-1}), considering the total polyphenol content into the extract (Lenquiste et al., 2012), as described in section 2.7.

The direct contact of the extract with the bacteria was evaluated as described by Lappe et al. (2009). Pellets of 10^7 CFU of each bacterium were dissolved with 1 mL of the jaboticaba peel extract and kept at 37 °C for 90 min. The system was then centrifuged at 4,000 *g* for 10 min and the pellet washed twice with saline solution before analysis of the viable cell count was performed.

2.5 Growth kinetics

Aliquots of 2 mL of jaboticaba peel extract were added to 20 mL (final concentration of 40 UA mL^{-1} and 20 UA mL^{-1}, for *S. aureus* and *E. coli*, respectively) of overnight culture (obtained by growing the respective bacteria at 37 °C in a shaker at 100 cycles/min) of 10^7 CFU mL^{-1} after 2 h of growth at 37 °C (Motta et al., 2007). Saline solution was used as a control. Cultivation was conducted for 11 h, and every 90 min, performing the dilution of the culture medium when necessary to comply with the readings with the Lambert-Beer Law.

2.6 Scanning electronic microscopy (SEM)

Cells from the direct contact tests were fixed with 2.5% (v/v) glutaraldehyde, 2% (v/v) formaldehyde in 0.12 mol L^{-1} phosphate buffer for 10 days and then post-fixed in 2% (w/v) osmium tetroxide in the same buffer for 45 min. The samples were dehydrated in a graded series of acetone (30 to 100%) and incorporated in Araldite Durcupan for 72 h at 60 °C. Thin sections cut in microtome (UPC-20, Leica) were mounted in grids, covered with collodion film and post-dyed with 2% uranyl acetate in Reynold's lead citrate. The preparations were observed with a JEOL JEM 1200ExII electron microscope (JEOL, Tokyo, Japan) operating at 120 kV.

2.7 Phenolic compounds

Total phenolic content (TPC), monomeric anthocyanins, condensed tannins (CT) and phenolic acids (PA) were quantified by spectrometric methods following previous literature and recently published procedures (Sant'Anna et al., 2017; Fleck et al., 2021).

Briefly, TPC was measured by the reaction of extract with Folin-Ciocalteau reagent and sodium carbonate saturated solution and absorbance of the reaction mixture at 765 nm was measured, using a gallic acid curve to express results as mg gallic acid equivalent per milliliter of extract (mg GAE mL^{-1}). MA were determined using the pH differential method, by measuring the absorbance of diluted samples in potassium chloride buffer pH 1.0 and sodium carbonate buffer pH 4.5 at 520 and 700 nm. The units for extracted MA were expressed as mg of cyanidin 3-glucoside per milliliter (mg C3G mL^{-1}). CT was analyzed by the reaction of extracts with vanillin solution and further analysis of system's absorbance at 500 nm and results were calculated and expressed as mg epicatechin equivalents (mg of ECE mL^{-1}). PA was measured by the thoroughly mix of the extract with acidified ethanol and distilled water and absorbance measuring at 310 nm and the results were expressed as mg of caffeic acid equivalent (CAE) per milliliter of extract (mg CAE mL^{-1}).

3 Results and discussion

The results of antibacterial activity of jaboticaba peel aqueous extract against *S. aureus* and *E. coli* are shown in Table 1. Results showed that the extract could present the capability to inhibit both Gram-positive and Gram-negative bacteria. The aqueous extract showed activity of 400 AU mL^{-1} and 200 AU mL^{-1} against *S. aureus* and *E. coli*, respectively. MIC and MBC against *S. aureus* were found to be 5.1 g L^{-1} and 10.1 g L^{-1}, respectively; meanwhile, against *E. coli*, 2.0 g L^{-1} and 3.4 g L^{-1}, respectively. Similar results against the same bacteria were recently observed for jaboticaba extracts (Fleck et al., 2021), thus reinforcing the observed anti-bacterial activity of phenolic and anthocyanin fractions. These results are in agreement with Lacombe et al. (2010) who observed that phenolics from cranberry presented MIC and MBC values of 2.7 g L^{-1} and 14.8 mg L^{-1} against *E. coli* respectively; and those results from Diniz-Silva et al. (2017), who observed that anthocyanin-rich blueberry extract showed MIC value of 500 mg mL^{-1} against *S. aureus*. MIC values of 5 mg mL^{-1}, were reported for cyanidin-3-O-glucoside against both *E. coli* and *S. aureus* strains derived from food and ATCC collection (Li et al., 2022). The MIC of anthocyanins extracted from *Cinclidotus fontinaloides* against *E. coli* was 10 mg mL^{-1} (Yayintas et al., 2017) and from wine pomace was 9% (Tseng & Zhao, 2012). Moreover,

MIC from grape pomace extract against strains of *S. aureus* ranged from 3.000 to 600 mg mL^{-1}, depending on the source of bacteria (from ATCC collection to different clinical isolates) (Sanhueza et al., 2017).

Table 1. Antibacterial activity of jaboticaba peel extract against *S. aureus* and *E. coli.*

Bacteria	Antimicrobial activity (AU mL^{-1})	MIC (g L^{-1})	MBC (g L^{-1})	Reduction of viable counts (log UFC mL^{-1})
S. aureus	400 ± 0	5.1 ± 0.2	10.1 ± 0.4	4.0 ± 0.4
E. coli	200 ± 0	2.0 ± 0.1	3.4 ± 0.3	3.2 ± 0.5

AU: activity units; MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration.

It is not uncommon Gram-negative bacteria to be more susceptible to anthocyanin-rich extracts from berries than Gram-positive bacteria (Park et al., 2011; Demirbas et al., 2017; Fleck et al., 2021). The antimicrobial activity of fruits that contain anthocyanins is likely caused by multiple mechanisms and synergisms, as they contain several compounds, including anthocyanins, weak organic acids, phenolic acids and their mixtures in different chemical forms (Cisowska et al., 2011).

The effect of jaboticaba peel extract on the evaluated bacteria is presented in Figure 1. The addition of the antibacterial extract to the suspension of *S. aureus* cells after 2 h growth resulted in a reduction in the viable cell counts (Figure 1A) as well as the turbidity of the medium (Figure 1B) over the 11 h of culture. The results of the turbidity of the culture medium against *S. aureus* showed a short period of adaptation, followed by exponential growth and reaching the stationary phase at the end of 11 h cultivation; whereas OD values remained almost constant for treated cells (Figure 1A). This indicates that the addition of jaboticaba peel extract suppressed this exponential phase of bacterial growth. Similar results were found for *E. coli* exposed to the antimicrobial extract (Figure 1C and 1D).

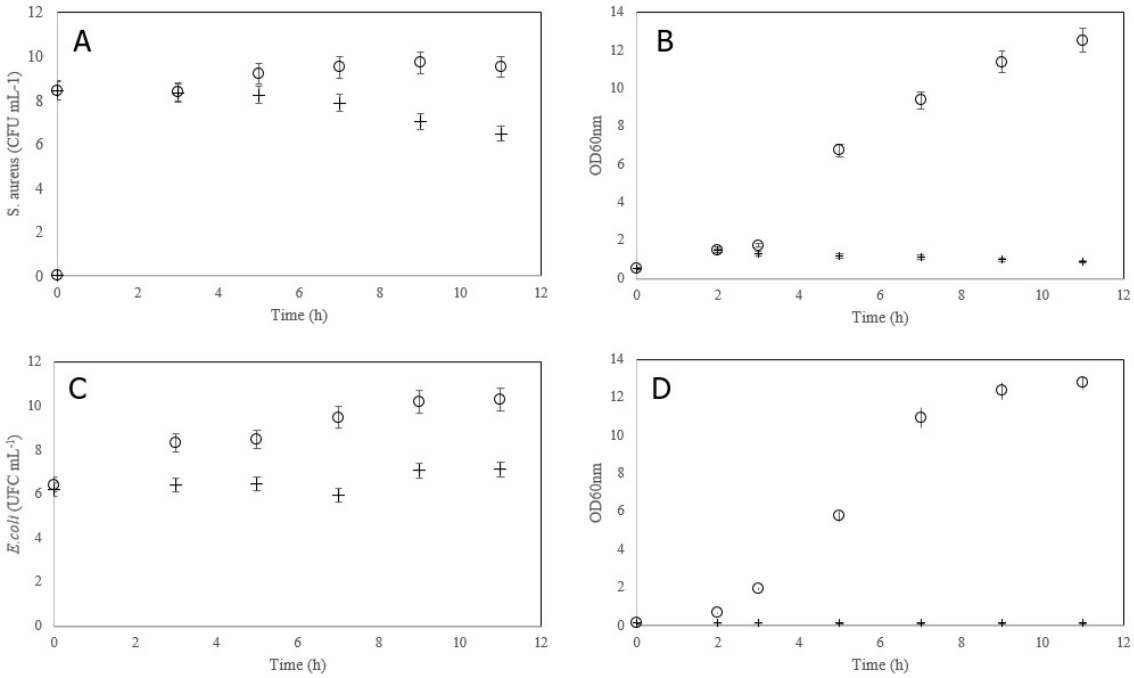

Figure 1. Kinetic growth of viable cells (A and C) and absorbance at 600 nm (B and D) of *Staphylococcus aureus* and *Escherichia coli*, respectively. In the Figure, circles (o) are the control and plus signs (+) are the treated samples with their respective standard deviation bars.

Park et al. (2011) observed that growth of *S. enterica* sv. Enteritidis and *L. monocytogenes* were also inhibited due to the presence of blueberry and muscadine extracts (phenolic concentration of 46.25 ppm and 24 ppm, respectively) and that the Gram-negative bacteria were more susceptible than Gram-positive. Similar results were found by Diarra et al. (2020) when evaluated the effect of phenolic-rich extract from American cranberry against different strains of *Listeria*. The results of this study (Figure 1) indicated a possible bacteriolytic action of the compounds in the extract on the pathogenic bacteria (Motta et al., 2007; Li et al., 2022). Lacombe et al. (2010) observed that phenolics and anthocyanins from cranberry inactivate *E. coli* through disintegration of the outer membrane. The antimicrobial activity of phenolic compounds against Gram-negative bacteria may be due to the chelating of divalent cations in their outer membranes (Nohynek et al., 2006). In Gram-positive bacteria, Diarra et al. (2020) observed that anti-listeria activity of polyphenolics is associated with increased permeability of bacterial membranes. Furthermore, it is believed that tannins can react with cell wall components inhibiting the biosynthesis of peptidoglycan (Jones et al., 1994).

In order to obtain additional insight on the mode of action of the jaboticaba peel extract, SEM analyzes of *S. aureus* and *E. coli* were carried out and the results are summarized in Figure 2. The results for untreated *S. aureus* indicated perfectly spherical cocci in the shape of a bunch of grapes, typical of staphylococci bacteria (Figure 2A). Although the differences are subtle, some treated cells appeared as irregularly shaped cocci (Figure 2B). The effect of the jaboticaba peel extract on *E. coli* is shown in Figure 2C and 2D. A typical rod-shaped morphology was observed for *E. coli* samples, while treated cells appeared more agglomerated (Figure 2D), which could be associated with changes in the cell surface.

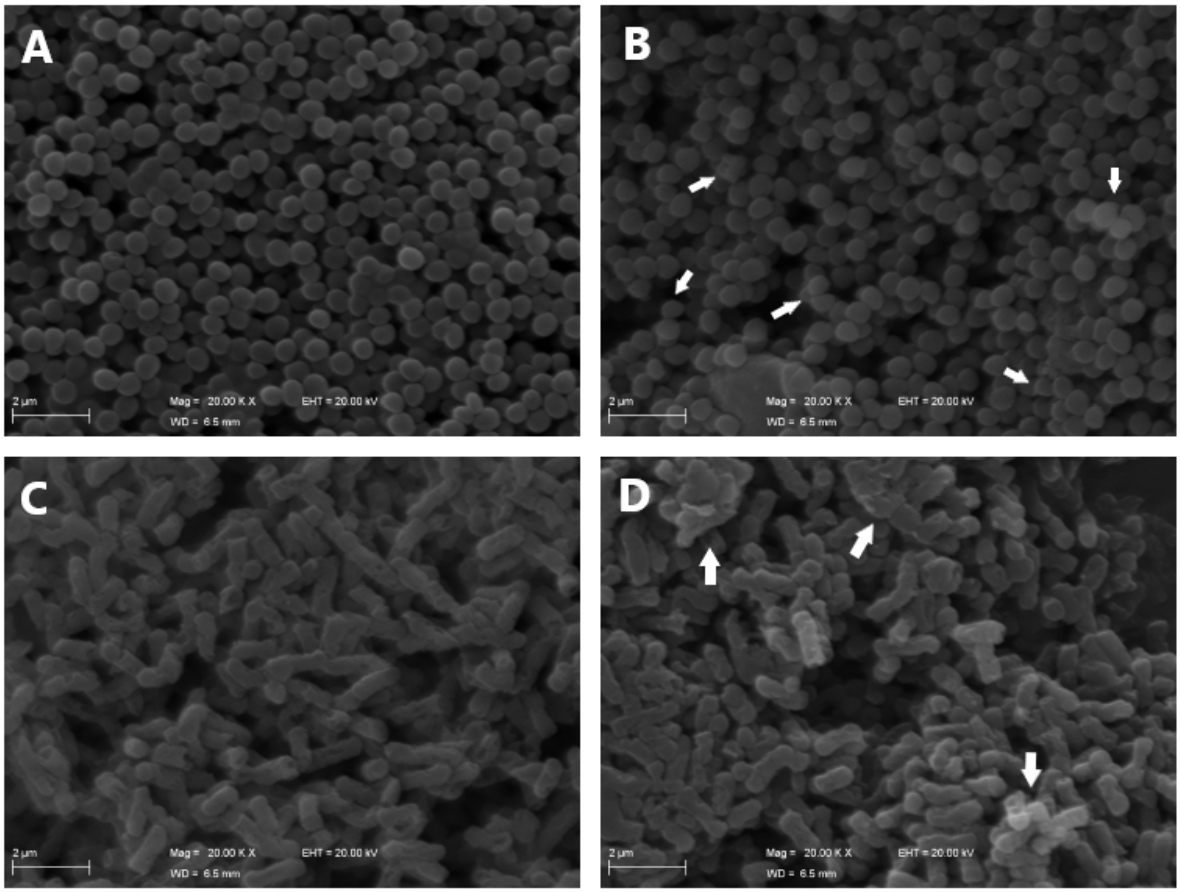

Figure 2. MEV imagens for control (A) and treated (B) *S.aures* samples; and control (C) and treated (D) E.coli samples.

The uneven cell surface may suggest that some interactions between the jaboticaba peel compounds and bacterial cells take place. Once the membrane has been penetrated, smaller phenolic compounds can enter the cell and disrupt metabolism (Kwon et al., 2007). Membrane irregularities and cellular damage were observed in *V. parahaemolyticus* treated with blueberry extract (Sun et al., 2018), which presents bioactive compounds such as anthocyanins, flavonoids, phenolic compounds and proanthocyanidins. *S. aureus* cells treated with sugarcane bagasse extract, containing high concentration of phenolic compounds, showed wrinkling and surface irregularities, with fragmentation, adherence and aggregation of damaged cells (Zhao et al., 2015). Anthocyanins may also be able to enter the inner membrane and decrease the activity of alkaline phosphatase, ATPase and superoxide dismutase in *S. aureus*, thus inhibiting bacterial growth by preventing the transfer of information and energy or controlling the pathogen's self-protection capacity (Sun et al., 2018). For Gram-negative bacteria, Lacombe et al. (2010) observed that phenolics and cranberry anthocyanins inactivate *E. coli* through disintegration of the outer membrane. These results were reinforced by Doughari et al. (2012) who observed that anthocyanin extract from *Curtisia dentata* inhibits *E. coli* cells by destroying the cell wall of the bacteria. Additionally, anthocyanin extract presents great anti-toxic activity and inhibits the expression of the *vtx1* and *vtx2* genes in *E. coli* (Doughari et al., 2012).

Results from total polyphenolic compounds, anthocyanins, condensed tannins and phenolic acids in jaboticaba peel aqueous extract are shown in Table 2. Total polyphenol content was 1535.04 ± 36.05 mg GAE mL^{-1}, meanwhile monomeric anthocyanin concentration was 14.52 ± 0.98 mg C3G mL^{-1}, condensed tannin concentration was 0.49 ± 0.05 as mg EE mL^{-1} and phenolic acid concentration was 80.04 ± 4.11 mg CAE mL^{-1}. Similar results were published before for jaboticaba pomace and rinds (Gurak et al., 2014; Fleck et al., 2021). Thus, aqueous extracts from jaboticaba residue showed to be an anthocyanin-rich extract and presented interesting amounts of phenolic acids and tannins.

Table 2. Concentration of polyphenolics in jaboticaba peel extract

Polyphenolics	Concentration
Total polyphenolics	1,535.04 ± 36.05 mg GAE mL^{-1}
Monomeric anthocyanins	14.52 ± 0.98 mg C3G mL^{-1}
Condensed tannins	0.49 ± 0.05 mg EE mL^{-1}
Phenolic acids	80.04 ± 4.11 mg CAE mL^{-1}.

Major extractable polyphenolics from *M. jaboticaba* peel are anthocyanins, and delphinidin-3-O-glucoside and cyanidin-3-O-glucoside are the two major anthocyanins reported in jaboticaba fruits (Lenquiste et al., 2015; Quatrin et al., 2019). Li et al. (2022) observed that cyanidin-3-O-glucoside acts on membrane integrity of *E. coli* and *S. aureus* strains by decreasing intracellular ATP concentration and intracellular pH. Ellagic and gallic acids are important phenolic acids within jaboticaba peels (Quatrin et al., 2019, 2020). Ellagic acid showed to be essential for the inhibition of Gram-positive and Gram-negative food-borne bacteria (Rosas-Burgos et al., 2017), promoting coccoid bacterial morphology of *Helicobacter pylori* (De et al., 2018). Gallic acids change bacterial hydrophobicity due to their electrophilicity that interact with the bacterial surface components (Rosas-Burgos et al., 2017). Hydroxycinnamic derivative and tannins found within jaboticaba peel matrix are generally antibacterial and are less polar than the corresponding hydroxybenzoic acids, due to their propanoid side chain, and this property might facilitate the transport of these molecules through the cell membrane (Borges et al., 2013).

4 Conclusion

Jaboticaba peel extracts are anthocyanin-rich infusion that present interesting anti-bacterial activity against *S. aureus* and *E. coli.* The natural agent acted in the exponential growth phase of both bacteria, resulting in a

reduction in the viable cell count, indicating a possible bacteriolytic action. Results from the SEM showed that jaboticaba peel extract induced changes in cell morphology. In conclusion, jaboticaba peel aqueous extract showed to be an interesting natural preservative to control Gram-positive and Gram-negative bacteria growth when interacting with the bacteria cell wall.

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