

## NON-COMMERCIALY EXPLORED *Campomanesia* spp. NATIVE FRUITS AS POTENTIAL SOURCE OF ANTIMICROBIAL COMPOUNDS

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### Abstract

*Campomanesia eugenioides*, *Campomanesia xanthocarpa* (Berg) and *Campomanesia xanthocarpa* var. *littoralis* comprises non-commercially explored native fruits from Brazil. Considering the scarcity of studies on the native fruits characterization from *Campomanesia* spp., the aim of the present study was to determine the antibacterial effects of *C. xanthocarpa* (Berg), *C. eugenioides* and *C. xanthocarpa* var. *littoralis* against 15 strains of food-borne pathogens. The antibacterial tests demonstrated a high antibacterial activity for some of the Gram-positive bacteria tested, i.e., *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, and *Staphylococcus aureus*. It is noteworthy that *C. xanthocarpa* var. *littoralis* fruit extract was the better against a more substantial number of bacteria. Finally, it was possible to report that phenolic and flavonoids compounds present in *C. eugenioides*, *C. xanthocarpa* and *C. xanthocarpa* var. *littoralis* could be strongly associated with the antibacterial activity observed.

**Keywords:** *Campomanesia eugenioides*, *Campomanesia xanthocarpa* var. *littoralis*, *Campomanesia xanthocarpa*, native fruit, antibacterial properties, bioactive compounds.

### 1. Introduction

Territorial extension, geographical position, soil and climatic conditions are conditions that makes Brazil as one of the world's largest fruit producers (BARROS et al., 2017). However, large production of few fruit species are taking over more area, and consequently, the population loses the chance to know native species and varying their diet. In this sense, several native species are not economically explored yet. These native species could offer rich and nutritious alternatives, that could be more widely used for *in natura* consumption or in the production of sweets, jams, juices and ice-creams (PEREIRA et al., 2013). Thus, several still little known species of fruit have been evaluated more recently as an alternative to traditional species (BRANDAO; ANJOS; BELL, 2017; PAZ et al., 2015).

The fruits belonging to the genus *Campomanesia* sp. Comprises several species that

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could be more explored (DONADO-PESTANA et al., 2018). The fruits are round and green in color when young and yellow and sweet when mature (VALLILO et al., 2008), and globose and smooth, measuring about 5.5 to 7 mm in diameter (LIMA et al., 2011). Some species such as *C. xanthocarpa* var. *littoralis* (D. Legrand) shows some different characteristic such as the green color attributed to the fruit when mature and their size (15 to 40 mm) (BIAVATTI et al., 2004). *Campomanesia xanthocarpa* (Berg) is one of the most popularly known and widespread species among the genus (BARBIERI et al., 2017). On the other hand, *C. eugenioides* is commonly confused with *C. xanthocarpa* because they have similar characteristics, but *C. eugenioides* is not so well known and studied. *Campomanesia* species are traditionally used in as traditional medicine for the treatment of intestinal disorders (MOURA-COSTA et al., 2012).

Currently, consumers have appreciated the availability of more natural and healthier foods, which may also bring some health benefit. In addition, with the development of bacteria resistant to many antibiotics, there is a growing interest in the study of antimicrobial substances from natural products. These factors have collaborated to increase interest in the search for natural products that have biological activities such as antimicrobial activity (SILVEIRA et al., 2014).

In the light of this observations, Cardoso et al. (2010) in their study with fruits of *C. pubescens* and *C. adamantium* found representative values of minimum inhibitory concentration for Gram-positive, Gram-negative bacteria, and also yeasts. This ability can be related with the presence of bioactive compounds in the fruits, such as phenolic compounds with proven antibacterial activities (CUSHNIE; LAMB, 2011; DRAGIŠIĆ MAKSIMOVIĆ et al., 2013). These compounds could be related with DNA molecule damage and with interferences on the reconstitution of damaged cell membranes (DRÖGE, 2002). Thus, considering the scarcity of studies on the native fruits characterization from *Campomanesia* spp., the aim of the present study was to determine the antibacterial effects of *C. xanthocarpa* (Berg), *C. eugenioides* and *C. xanthocarpa* var. *littoralis* against 15 strains of food-borne pathogens.

## **2. Material and Methods**

### **2.1. Location description and collection of samples**

Ripened *C. eugenioides* (*Campomanesia eugenioides*), *C. xanthocarpa* (*Campomanesia xanthocarpa* (Berg)) and *C. xanthocarpa* var. *littoralis* (*Campomanesia xanthocarpa* var. *littoralis* (D. Landrum)) fruits were collected in the West region of Santa Catarina State, Brazil, at latitude 27°14'2"S and longitude 52°1'40"W of Greenwich. The vouchers specimens

were deposited at the Herbário do Vale do Taquari (HVAT, Lageado, Rio Grande do Sul, Brazil) and at the Herbário Padre Balduino Rambo (HPBR, Erechim, Rio Grande do Sul, Brazil) as presented in Table 1. All the samples were collected when fully mature and were preselected considering the absence of visible injury, infections and color uniformity. Moreover, the fruits were freeze-dried with all the edible parts (skin, pulp, and seeds) and processed in a knife mill (Tecator, Knifetec 1095 model). The powders obtained were vacuum sealed in plastic bags and stored at a temperature of  $-18 \pm 0.2$  °C until analyzed.

**Table 1.** *Campomanesia eugenioides*, *Campomanesia xanthocarpa* (Berg) and *Campomanesia xanthocarpa* var. *littoralis* (D. Landrum) studied in the present work.

Registry number	Popular name	Scientific name	Collection location
HVAT 2612	Guabiroba	<i>Campomanesia eugenioides</i>	Concórdia, SC, 27°18'46" South, 51°59'16" West, Brazil
HPBR 11579	Guabiroba do mato	<i>Campomanesia xanthocarpa</i> (Berg)	Concórdia, SC, 27°12'01" South, 52°01'58" West, Brazil.
HPBR 11580	Guabiroba verde	<i>Campomanesia xanthocarpa</i> var. <i>littoralis</i> (D. Landrum)	Concórdia, SC, 27° 14' 3" South, 52° 1' 43" West, Brazil.

Source: Herbário do Museu de Ciências Naturais da UNIVATES (HVAT, Lajeado, RS) and Herbário Padre Balduino Rambo da URI (HPBR, Erechim, RS).

## 2.2. Freeze dry

Initially, the whole fruits (about two kilos) were washed with distilled water and crushed in a mini-processor to obtain a homogeneous mass (pulp). The pulp was distributed in aluminum trays and frozen in a freezer at  $-18$  °C for 24 hours. Then, the samples were dehydrated in a lyophilizer (Liobrás LP810, SP, Brazil). The humidity was determined by gravimetry. The dry samples were processed in a knife mill (Tecator, model Knifetec 1095, Germany), vacuum packed and stored at  $-18$  °C until the extract preparation.

## 2.3. Extract preparation

The extract of each fruit species was prepared prepared with freeze-dried fruit and ethanol: water 80:20 (v/v) in a mass/solvent ratio of 1:5 to obtain the hydroalcoholic extract by exhaustive maceration with solvent exchange at 24, 48, 72 and 192 hours. The extraction was carried out under agitation in an orbital plate (Etica, 109-2-E model, São Paulo, Brazil) at room temperature without incidence of light. The extract was filtered and rota-evaporated ( $40 \pm 1$  °C) until dryness and dissolved in 100 mL of dimethyl sulfoxide (DMSO). All the extracts

were stored in the dark at  $4 \pm 1^\circ\text{C}$  until analyzed.

## **2.4. Evaluation of the antibacterial activity**

**2.4.1. Microorganisms.** Antibacterial activity was evaluated for the following strains: *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 19117, *Listeria monocytogenes* serotype 2 ATCC 19112, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Enteritidis ATCC 13076, *Salmonella* Typhimurium ATCC 14028, *Shigella sonnei* ATCC 25931, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 25923 and *Yersinia enterocolitica* ATCC 9610.

**2.4.2. Microorganism suspension standardization.** Bacterial cultures were grown on 5% sheep red blood agar. Following the initial incubation, 3-5 isolated colonies were suspended in 10 ml of tryptose soy broth (TSB) and incubated at  $35 \pm 2^\circ\text{C}$  for 2 to 6 hours, to obtain an actively growing culture. This inoculum was diluted in 0.9% saline solution to a concentration of approximately  $10^8$  CFU  $\text{mL}^{-1}$  comparable spectrophotometrically to 0.5 McFarland standard at 625nm. For the agar disc diffusion assay and the minimum inhibitory concentration determination, bacterial solutions of  $10^7$  CFU  $\text{mL}^{-1}$  were employed.

**2.4.3. Agar disc diffusion method.** The agar disc diffusion method was employed for the screening of antibacterial activities of the extracts of the fruit according to the Clinical and Laboratory Standards Institute (CLSI, 2019b). The test was performed in sterile Petri dishes (90 mm diameter) containing Mueller-Hinton agar medium (Difco, Sparks, USA). Briefly, the tested microorganism suspension was spread with a sterile swab on the MH agar. The extracts ( $100 \text{ mg mL}^{-1}$ ) absorbed on sterile paper discs (25  $\mu\text{L}$  per disc of 9 mm diameter and  $250 \text{ g m}^{-2}$ ) were placed on the surface of the media previously inoculated and then incubated at  $35 \pm 2^\circ\text{C}$  for 24 h. After the zone diameter of the inhibition was measured and expressed in mm. Commercial discs of ampicillin (10 mg per disc) and chloramphenicol (30 mg per disc) were used as positive controls and disks containing DMSO (25  $\mu\text{L}$ ) as a negative control. All tests were performed in triplicate. The inhibition was classified as strongly inhibitory, moderately inhibitory or not inhibitory according to Carović-Stanko et al. (2010). Minimum inhibitory concentrations were recorded for the extracts that showed positive antibacterial activity.

**2.4.4. Minimum inhibitory concentration (MIC): broth microdilution.** MICs were determined according to the method proposed by CLSI (CLSI, 2019a), with some modifications. In the

microplate, serial dilutions of the extracts were made directly to the wells in the first row until the eighth row with sterile DMSO, resulting in concentrations that varied from 100 mg mL<sup>-1</sup> to 0.75 mg mL<sup>-1</sup>. Ninety microliters of MHB, 10 µL of the diluted tested solution and 10 µL of the previously standardized microorganism suspension were added to each well, and the plate was incubated at 35 ± 2 °C for 24 h. It was assured the sterility control (MHB added of DMSO and without inoculum) and growth control (MHB added of DMSO and inoculum). Microbial growth was detected and confirmed with the addition of 20 µL of an aqueous solution of triphenyltetrazolium chloride (TTC, 0.5%, w/v), with one hour of additional incubation. All analyses were carried out in triplicate and expressed in mg mL<sup>-1</sup>.

*2.4.5. Minimum bactericidal concentration (MBC).* To determine the MBCs the method proposed by Celiktas et al. (CELIKTAS et al., 2007) was used, with some modifications. For this, 10 µL of the suspension in which was not microbial growth on the MIC assay were plated on TSA. The plates were incubated at 35 ± 2 °C for 24 h. The lowest concentration of the extract at which no growth of microorganisms was observed was considered the MBC. All tests were performed in triplicate and expressed in mg mL<sup>-1</sup>.

## **2.5. Statistical analysis**

The significance of the differences between the means of the samples was determined by analysis of variance (ANOVA) followed by Tukey's test (5 % significance). All statistical analyses were performed using the software STATISTICA version 13.3 (StatSoft Inc., Tulsa, OK, USA).

## **3. Results and Discussion**

The antibacterial activities of *C. eugenoides*, *C. xanthocarpa* (Berg), and *C. xanthocarpa* var. *littoralis* fruits extracts were evaluated against seven Gram-positive and eight Gram-negative bacterial strains. Considering a future use of the extracts of the fruit as a possible food additive, these microorganisms were chosen because they are considered some of the most common food-borne pathogens. Thus, the results of the agar diffusion assay, presented in Table 2, showed that the three extracts had no antibacterial activity against the eight species of Gram-negative bacteria tested. In accordance with Malanovic and Lohner (2016), Gram-negative bacteria present in their cell wall a lipopolysaccharide membrane that can interfere with some of these antibacterial substances blocking its activity.

Remarkably, all Gram-positive bacteria tested in the present study were susceptible to the three fruit extracts (Table 2). According to the classification of Carović-Stanko et al. (2010), the antibacterial activity can be classified in the following three categories: > 15 mm

zone of inhibition was strongly inhibitory; 10-15 mm zone of inhibition was moderately inhibitory, and <10 mm was not inhibitory. Taking this into account, *C. eugenioides* showed strong inhibitory effect against *Listeria monocytogenes* serotype 2 (Figure 1) and *Staphylococcus aureus* ATCC 6538 (Figure 2). Also, *C. xanthocarpa* (Berg) showed strong inhibitory effect against *S. aureus* ATCC 29213 (Figure 2), while *C. xanthocarpa* var. *littoralis* showed strong inhibitory effect against *Bacillus subtilis* (Figure 3), *L. monocytogenes* serotype 2 (Figure 1), *S. aureus* ATCC 6538 and *S. aureus* ATCC 25923 (Figure 2). The fruits extract moderately inhibited all other Gram-positive bacteria. Thus, *C. xanthocarpa* var. *littoralis* fruit extract demonstrated high inhibitory activity against a more substantial number of Gram-positive bacteria.

*Listeria monocytogenes* species is known to be potentially pathogenic to humans and animals, causing gastrointestinal disorders, septicemia, abortion and, in more serious cases, death. They grow in a very wide temperature range (from 1 to 45° C), which makes their control difficult (RYSER; DONNELLY, 2001). The growth of *S. aureus* in food also poses a great risk to human health because several strains of this species produce enterotoxins. Contaminations by *S. aureus* are among the main causes of foodborne diseases in the world (LANCETTE; BENNET, 2001). According to Ahmad, Mehmood and Mohammad (1998), fruits are popularly used as natural antimicrobials for the treatment of bacteriosis in Ayurvedic medicine. These authors reported zones of inhibition greater than 20 mm for Indian gooseberry, Haritaki and Bibhitaka, against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. Typhimurium* and *P. vulgaris*.

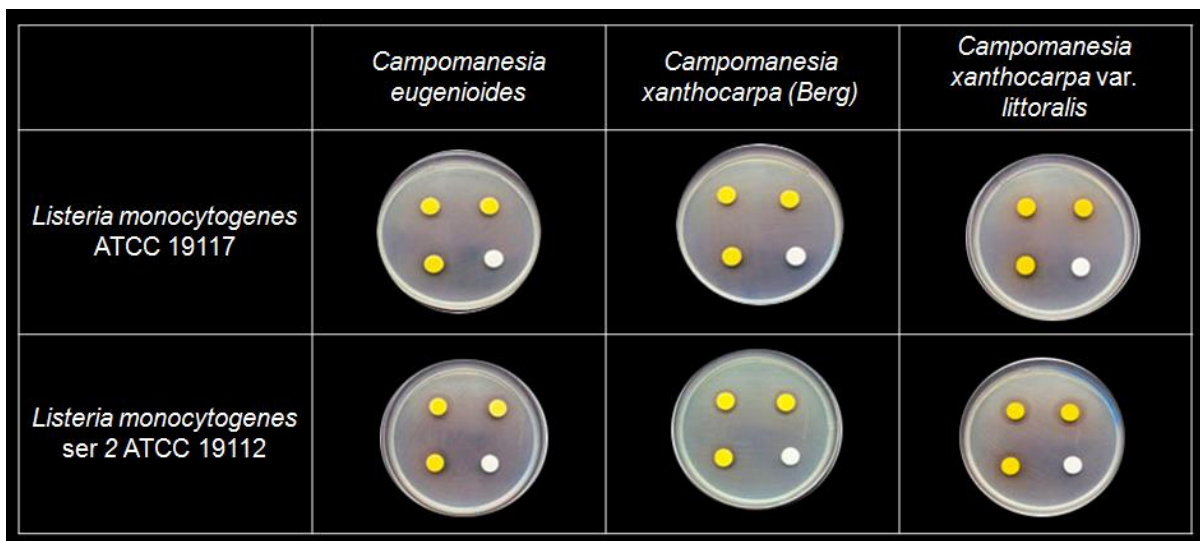
*C. eugenioides* and *C. xanthocarpa* var. *littoralis* showed moderate activity against *Bacillus cereus*, and these results did not differ significantly ( $p < 0.05$ ) from the zone of inhibition presented by the antibiotic ampicillin (14.7 mm) used as a positive control. Al-Zoreky (2009) reported antimicrobial activity of a methanolic extract of pomegranate peel against against *L. monocytogenes*, *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *Y. enterocolitica*, with inhibition halos ranging from 16 to 20 mm. Compatible values with those observed in the present work for the tested Gram-positive species. Among the Myrtaceae family, Boulekbache-Makhlouf, Slimani and Madani (2013) reported the antimicrobial activity of Tasmanian eucalyptus fruits against *S. aureus* (14.67 mm) and *B. subtilis* (11.5 mm) and Medina et al. (2011) reported the antimicrobial activity of the aqueous extract of yellow araçá and red araçá against *S. Enteritidis* (15.3 and 17 mm, respectively). The antimicrobial activity of Tasmanian eucalyptus fruits is equivalent to those reported for the *Campomanesia* species studied in the present work.

**Table 2.** Average diameter of inhibition zone  $\pm$  standard deviation (mm) of *Campomanesia eugenioides*, *Campomanesia xanthocarpa* (Berg) and *Campomanesia xanthocarpa* var. *littoralis* (D. Landrum) fruit extracts, positive and negative controls<sup>†</sup>.

	<i>C. eugenioides</i>	<i>C. xanthocarpa</i> (Berg)	<i>C. xanthocarpa</i> var. <i>littoralis</i>	Amp	Chlo	DMSO
<b>Gram (+)</b>						
<i>Bacillus cereus</i> ATCC 11778	14.0 $\pm$ 0.0 <sup>c</sup>	13.0 $\pm$ 0.0 <sup>d</sup>	15.0 $\pm$ 0.0 <sup>b</sup>	14.7 $\pm$ 0.6 <sup>bc</sup>	30.0 $\pm$ 0.0 <sup>a</sup>	-
<i>Bacillus subtilis</i> ATCC 6633	14.0 $\pm$ 0.0 <sup>c</sup>	13.7 $\pm$ 0.6 <sup>c</sup>	16.0 $\pm$ 0.0 <sup>b</sup>	30.0 $\pm$ 0.0 <sup>a</sup>	31.0 $\pm$ 1.1 <sup>a</sup>	-
<i>Listeria monocytogenes</i> ATCC 19117	12.3 $\pm$ 0.6 <sup>c</sup>	12.0 $\pm$ 0.0 <sup>c</sup>	12.7 $\pm$ 0.5 <sup>c</sup>	31.7 $\pm$ 0.6 <sup>a</sup>	28.0 $\pm$ 1.1 <sup>b</sup>	-
<i>Listeria monocytogenes</i> ser 2 ATCC 19112	15.3 $\pm$ 0.6 <sup>c</sup>	14.3 $\pm$ 0.6 <sup>c</sup>	15.7 $\pm$ 0.5 <sup>c</sup>	39.3 $\pm$ 0.6 <sup>a</sup>	27.0 $\pm$ 0.6 <sup>b</sup>	-
<i>Staphylococcus aureus</i> ATCC 29213	14.0 $\pm$ 0.0 <sup>e</sup>	18.3 $\pm$ 0.6 <sup>c</sup>	15.0 $\pm$ 0.0 <sup>d</sup>	22.7 $\pm$ 0.6 <sup>b</sup>	25.0 $\pm$ 0.0 <sup>a</sup>	-
<i>Staphylococcus aureus</i> ATCC 6538	19.3 $\pm$ 0.6 <sup>c</sup>	13.3 $\pm$ 0.6 <sup>e</sup>	16.0 $\pm$ 0.0 <sup>d</sup>	42.7 $\pm$ 0.6 <sup>a</sup>	29.0 $\pm$ 0.6 <sup>b</sup>	-
<i>Staphylococcus aureus</i> ATCC 25923	15.0 $\pm$ 0.0 <sup>d</sup>	13.5 $\pm$ 0.5 <sup>e</sup>	16.8 $\pm$ 0.2 <sup>c</sup>	32.7 $\pm$ 0.6 <sup>a</sup>	29.0 $\pm$ 1.0 <sup>b</sup>	-
<b>Gram (-)</b>						
<i>Enterobacter aerogenes</i> ATCC 13048	-	-	-	-	27.0 $\pm$ 1.0	-
<i>Escherichia coli</i> ATCC 25922	-	-	-	20.0 $\pm$ 0.0 <sup>b</sup>	29.0 $\pm$ 1.0 <sup>a</sup>	-
<i>Proteus vulgaris</i> ATCC 13315	-	-	-	30.7 $\pm$ 0.6 <sup>a</sup>	27.0 $\pm$ 1.1 <sup>b</sup>	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	-	-	-	-	-	-
<i>Salmonella</i> Enteritidis ATCC 13076	-	-	-	25.7 $\pm$ 0.6 <sup>b</sup>	29.0 $\pm$ 0.5 <sup>a</sup>	-
<i>Salmonella</i> Typhimurium ATCC 14028	-	-	-	26.0 $\pm$ 0.0 <sup>b</sup>	27.0 $\pm$ 0.6 <sup>a</sup>	-
<i>Shigella sonnei</i> ATCC 25931	-	-	-	23.0 $\pm$ 0.0 <sup>b</sup>	29.0 $\pm$ 1.0 <sup>a</sup>	-
<i>Yersinia enterocolitica</i> ATCC 9610	-	-	-	24.0 $\pm$ 0.0 <sup>b</sup>	33.0 $\pm$ 0.6 <sup>a</sup>	-

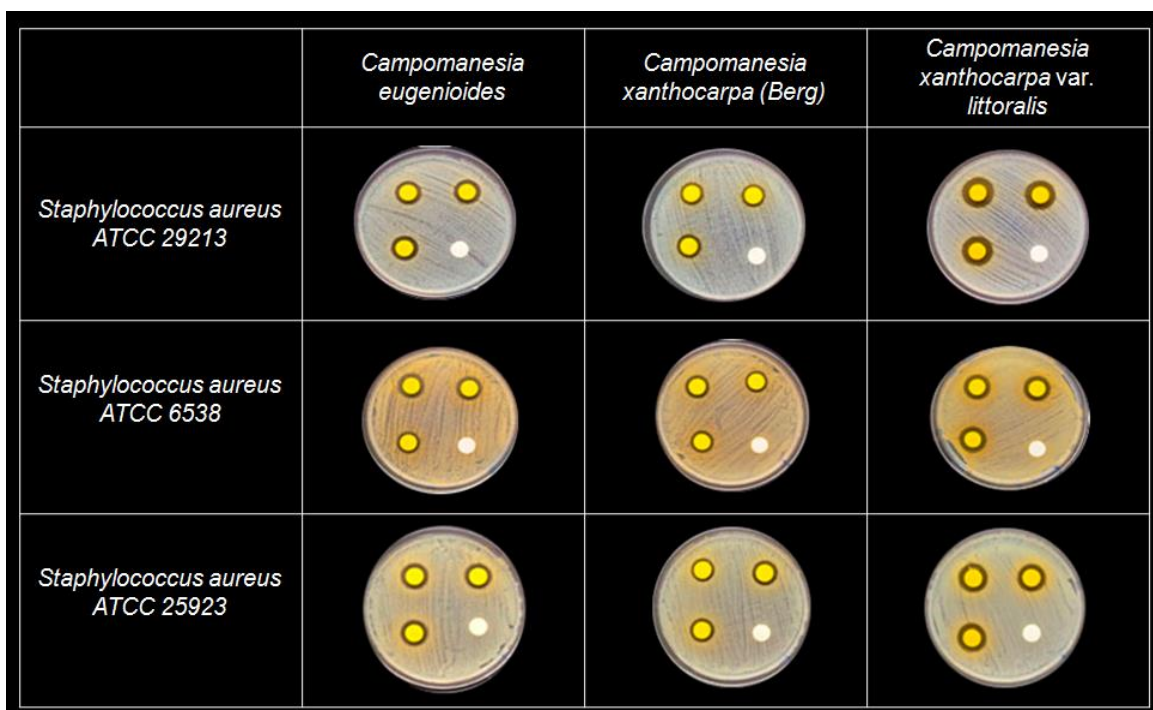
<sup>†</sup> Results presented as mean  $\pm$  SD, n=3. The same letters in the same line indicate no significant difference (P < 0.05). (-) – Without inhibition zone. Amp – ampicillin; Chlo – chloramphenicol; DMSO – dimethyl sulfoxide. **Source:** The authors (2020).

**Figure 1.** Inhibition zone of *C. eugenoides*, *C. xanthocarpa* (Berg), and *C. xanthocarpa* var. *littoralis* fruit extracts against *Listeria monocytogenes* ATCC 19117 and *Listeria monocytogenes* ser 2 ATCC 19112. The white disk represents the negative control with DMSO.



Source: The authors (2020).

**Figure 2.** Inhibition zone of *C. eugenoides*, *C. xanthocarpa* (Berg), and *C. xanthocarpa* var. *littoralis* fruit extracts against *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* ATCC 25923. The white disk represents the negative control with DMSO.

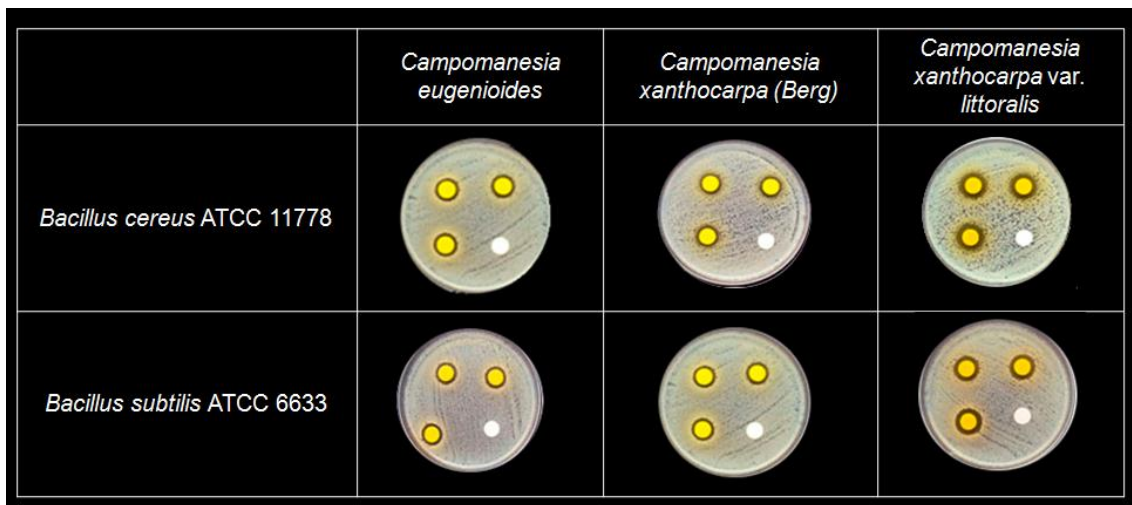


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rc: The authors (2020).



**Figure 3.** Inhibition zone of *C. eugenioides*, *C. xanthocarpa* (Berg), and *C. xanthocarpa* var. *littoralis* fruit extracts against *Bacillus cereus* and *Bacillus subtilis*. The white disk represents the negative control with DMSO.



**Source:** The authors (2020).

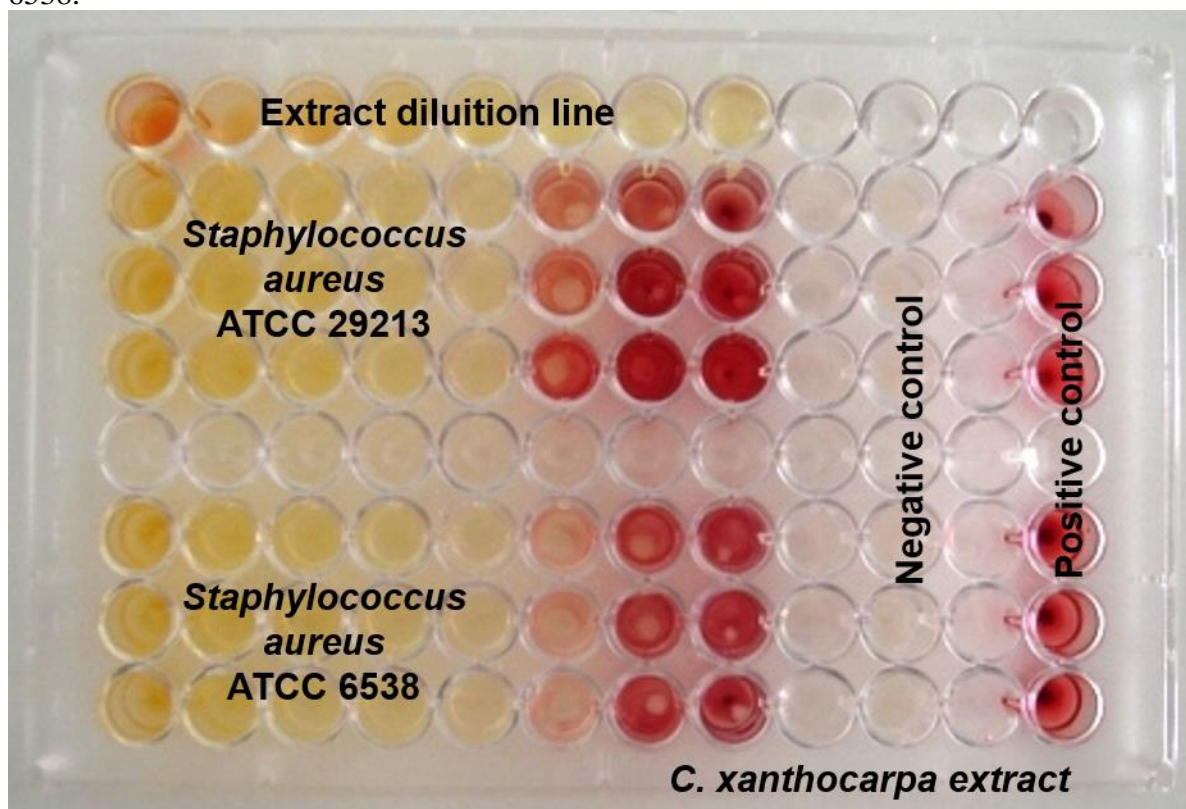
Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined for the bacteria species that showed susceptibility in the disk diffusion method, and these results are shown in Table 3. In general, all fruit extracts showed good antibacterial activity against all species of bacteria tested, with emphasis on the MBC of *C. eugenioides* against *Bacillus cereus* and *B. subtilis*; *C. xanthocarpa* (Berg) against *S. aureus* ATCC 29213; and *C. xanthocarpa* var. *littoralis* against *B. subtilis* and *L. monocytogenes* serotype 2. Figure 4 shows the microdilution plate for the MIC test of *C. xanthocarpa* against *S. aureus* ATCC 29213 and *S. aureus* ATCC 6538.

**Table 3.** Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) ( $\text{mg mL}^{-1}$ ) of *Campomanesia eugenioides*, *Campomanesia xanthocarpa* (Berg) and *Campomanesia xanthocarpa* var. *littoralis* (D. Landrum) fruit extracts <sup>a</sup>.

	<i>C. eugenioides</i>		<i>C. xanthocarpa</i> (Berg)		<i>C. xanthocarpa</i> var. <i>littoralis</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Bacillus cereus</i>	0.62	0.62	0.62	10.00	0.31	1.25
<i>Bacillus subtilis</i>	0.62	0.62	0.62	10.00	0.31	0.62
<i>Listeria monocytogenes</i>	1.25	2.50	0.62	1.25	0.62	1.25
<i>Listeria monocytogenes</i> sor 2	1.25	2.50	0.62	1.25	0.62	0.62
<i>Staphylococcus aureus</i> ATCC 29213	1.25	1.25	0.62	0.62	0.31	1.25
<i>Staphylococcus aureus</i> ATCC 6538	1.25	1.25	0.62	1.25	0.31	1.25
<i>Staphylococcus aureus</i> ATCC 25923	1.25	1.25	0.62	2.50	0.31	1.25

<sup>a</sup> Results presented as modal value, n=3. **Source:** The authors (2020).

**Figure 4.** Result of the minimum inhibitory concentration (MIC) of the extract of *C. xanthocarpa* in DMSO against *Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* ATCC 6538.



**Source:** The authors (2020).

For hexanic extracts of the fruits of *C. pubescens* and *C. adamantium*, Cardoso et al. (2010) reported minimal inhibitory concentrations in the range of 5 to 20  $\mu\text{g mL}^{-1}$  for four species of bacteria (*S. aureus*, *P. aeruginosa*, *E. coli* and *Salmonella* Setúbal) and two yeasts (*Saccharomyces cerevisiae* and *Candida albicans*). The authors observed lower MIC values for the bacterium *S. aureus* (15  $\mu\text{g mL}^{-1}$ ) compared to the results described in the present study. They also present, for *P. aeruginosa*, MIC of 15  $\mu\text{g mL}^{-1}$  and for *E. coli* MIC of 20  $\mu\text{g mL}^{-1}$ , bacteria that were not susceptible to the extracts of the fruits of this study by the agar diffusion technique. Therefore, it is evident that the genus *Campomanesia* presents antimicrobial properties against potentially pathogenic bacteria to humans.

Cavanagh, Hipwell and Wilkinson (2003) observed strong antimicrobial activity of raspberry, cranberry, blackberry and currant against *E. faecalis*, *E. coli*, *P. aeruginosa*, *S. Enteritidis*, *S. Typhimurium*, *S. sonnei* and *S. aureus*. It can be seen, therefore, that fruits of different species (including those of the genus *Campomanesia*) have antimicrobial capacity and could be used as adjuvants in treatments of bacteriosis caused by the consumption of contaminated food.

Regarding other fruits of the Myrtaceae family, studies on Serrana guava are reported. Basile et al (1997) presented MIC for *S. aureus* (8  $\mu\text{g mL}^{-1}$ ), *P. aeruginosa* (1  $\mu\text{g mL}^{-1}$ ), *E*

*coli* ( $1 \mu\text{g mL}^{-1}$ ) while in relation to MBC the maximum concentration of the extract used was not effective ( $1000 \mu\text{g mL}^{-1}$ ). Vuotto et al. (2000) observed for *S. aureus* MIC of  $64 \text{ mg L}^{-1}$  and MBC not detected, for *E. coli* MIC of  $4 \text{ mg L}^{-1}$  and MBC of  $32 \text{ mg L}^{-1}$ , for *P. vulgaris* MIC of  $8 \text{ mg L}^{-1}$  and MBC of  $64 \text{ mg L}^{-1}$ , for *P. aeruginosa* MIC of  $1 \text{ mg L}^{-1}$  and MBC of  $8 \text{ mg L}^{-1}$  and for *E. aerogenes* MIC of  $2 \text{ mg L}^{-1}$  and MBC of  $16 \text{ mg L}^{-1}$ . In the light of these observations, according to Cardoso et al. (2010), the extraction protocol could be improved using other extraction solvents to obtain better results against Gram-negative bacteria.

The antimicrobial activity shown by fruits is mainly due to the presence of secondary metabolites in its composition, such as phenolic compounds, among them the flavonoids that have antimicrobial activity reported in the literature (HARBORNE; WILLIAMS, 2000; CUSHNIE; LAMB, 2005; 2011). Finally, the results obtained in our study, lead us to believe that the phenolic and flavonoids compounds from *C. eugenioides*, *C. xanthocarpa* (Berg) and *C. xanthocarpa* var. *littoralis* are primarily responsible by the antibacterial activity observed. The data reported depicting the potential use of these three *Campomanesia* species as antibacterial agents, and the role they may play in their future application fields aiming to generate a functional food.

#### **4. Final Considerations**

This study will provide important scientific data supporting the application of valuable fruits from three edible *Campomanesia* species for producing bioactive ingredients and natural preservatives for food products. *C. eugenioides*, *C. xanthocarpa* (Berg), and *C. xanthocarpa* var. *littoralis* showed distinct antimicrobial characteristics. All the three extracts had no antibacterial activity against the eight species of Gram-negative bacteria tested. Nevertheless, all Gram-positive bacteria tested were susceptible to the three fruit extracts, where *C. xanthocarpa* var. *littoralis* fruit extract demonstrated high inhibitory activity against a more substantial number of Gram-positive bacteria. We concluded that the phenolic and flavonoids compounds of *C. eugenioides*, *C. xanthocarpa* (Berg) and *C. xanthocarpa* var. *littoralis* are primarily responsible by the antibacterial activity detected. Thus, our results contribute to the discovery of the potential application of these native *Campomanesia* Brazilian fruits, as a natural product with antibacterial properties.

#### **Conflicts of interest statement**

Authors declare no conflicts of interest in this manuscript.

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### **Author's contributions**

All authors contributed extensively to the work presented in this manuscript. S.V. and S.M.S.: Designed and performed research, wrote and revised the manuscript. A.C.J.: Contributed to the development of experimental part and reviewed the manuscript. N.F.: Helped in design research and discussion. J.C.B.: performed the species identification. All authors read and approved the final manuscript.

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