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Original Research Article

Antibacterial activity of *Butia odorata* Barb. Rodr. extracts

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ABSTRACT

Currently, synthetic preservatives are added to foods to increase their microbiological safety, but the demand for foods free of these agents is on the rise. *Butia odorata* Barb. Rodr. is a tree native to southern South America, with fruits rich in bioactive compounds. In this investigation, hexane (BHE) and methanol (BME) extracts of *B. odorata* fruit were evaluated for their antibacterial activity against three Gram-positive (*Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*) and three Gram-negative bacteria (*Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Pseudomonas aeruginosa*) by the agar diffusion method. Antibacterial activity of both *B. odorata* extracts was confirmed, with BHE showing inhibition zones significantly higher than BME, and with higher activity against Gram-negative bacteria. *E. coli* O157:H7 was the most sensitive microorganism, being inhibited at a minimum bactericidal concentration (MBC) of 3 mg.mL^{-1}. Of the Gram-positive bacteria, *S. aureus* was the most susceptible (MBC 11 mg.mL^{-1}). γ-Sitosterol was the major compound, constituting 22% of the total composition.

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1. Introduction

Currently, foodborne diseases (FBD) are a worldwide problem for human beings (WHO, 2015). In the developed countries, around 30% of the population is afflicted by some FBD annually (Loizzo et al., 2010). In the United States of America, it is estimated that 31 pathogens are responsible for 9.4 million annual cases of FBD. Of these, 39% are caused by bacteria of which non-typhoid *Salmonella* spp. and *L. monocytogenes* cause most deaths associated with food (Scallan et al., 2011). In 2014, 5251 FBD outbreaks were registered in the European Union, of which 20% were caused by the etiologic agent Salmonella and 16.1% by bacterial toxins, e.g. toxins produced by *Bacillus*, *Clostridium*, and *Staphylococcus* (EFSA/ECDC, 2015).

Microorganisms in food stuffs have been mainly controlled by the addition of synthetic preservatives. However, due to some undesirable properties such as carcinogenicity, toxicity, and teratogenicity, the use of these agents is limited (Faleiro, 2011). Consequently, an increasing number of studies have focused on the evaluation of natural compounds with a great potential as an alternative to synthetic preservatives. Among the natural antimicrobial agents investigated to date, plant compounds are particularly promising, being effective against various pathogenic bacteria (Weerakkody et al., 2010; Medina et al., 2011; Shen et al., 2014; Marques et al., 2015; Dannenberg et al., 2016).

In this context, species of the Arecaceae family are attractive from a chemical point of view (Silveira et al., 2005), and the *Butia* genus known as jelly palm belonging to this family is native to South America (Lorenzi et al., 2010). *Butia odorata* Barb. Rodr. fruits have a pale yellow to bright red color, a mean diameter of 1.7 to 4.2 cm (Hoffmann et al., 2014), and are characterized by high phenolic, carotenoid, and ascorbic acid contents (Fig. 1) (Beskow et al., 2015). However, despite its wide distribution, to our knowledge this is the first study in Brazil addressing the antibacterial activity of *B. odorata* for use in food preservation.

The purpose of this study was to produce *B. odorata*

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extracts with different polarities, test their antibacterial activity and chemically characterize the best-performing extract.

Fig. 1. Frozen *Butia odorata* Barb. Rodr. fruit prior to freeze-drying.

2. Experimental

2.1. Plant material

Butia odorata Barb. Rodr. fruit were harvested from a research orchard from a germplasm collection at the Centro Agropecuário da Palma, UFPel, Pelotas, Brazil (31º 52' 00'' S latitude, 52º 21' 24'' W Greenwich longitude and altitude of 13,24 m) in February 2015 (Fig. 2).

Fig. 2. Map indicating *Butia odorata* sampling site (Source Google maps).

2.2. *B. odorata* extract preparation

Hexane and methanolic extracts from *B. odorata* fruit pulp (BHE and BME, respectively) were prepared according to the method proposed by Shen et al. (2014). A 500 mL Erlenmeyer flask was filled with 30 g of lyophilized *B. odorata* fruit pulp and 300 mL of hexane or methanol (Synth®). Next, the Erlenmeyer flask was placed on a shaker for 2 h (150 rpm) and then in an ultrasonic bath (48 A/15 min). After filtering the extract through filter paper and centrifuging for 20 min (6289 g), the supernatant was rotary-evaporated at 50 ºC to constant weight.

2.3. Cultivation conditions of target microorganisms

As target microorganisms, three Gram-positive (*S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, and *B. cereus* ATCC 11778) and three Gram-negative bacteria (*S.* Typhimurium ATCC 14028, *E. coli* O157:H7 NCTC 12900, and *P. aeruginosa* ATCC 15442) were used. At the beginning of the experiment, the strains stored at -80 ºC were cultured on Tryptic Soy agar (Acumedia®) with 0.6% yeast extract (Himedia®) (TSA-YE), and incubated at 37 ºC for 24 h.

2.4. Agar disc diffusion method

Inoculum of each target microorganism was first standardized at a concentration of 10^8 CFU.mL^{-1} and plated on Petri dishes containing Mueller-Hinton agar (MH, Difco®). Thereafter, sterile paper filter discs (6 mm) impregnated with 20 µL of each *B. odorata* extract were placed on the agar and the plates subsequently incubated at 37 ºC for 24 h. Standard streptomycin discs (10 µg) were used as a positive control and water was used in place of the *B. odorata* extracts as a negative control.

2.5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) was performed according to Weerakkody et al. (2010), with adaptations. *Butia odorata* extracts were tested at concentrations of 11, 10, 8, 7, 6, 4, and 3 mg.mL^{-1}. In test tubes containing 1 mL of MH broth, 10^5 CFU. mL^{-1} of the target bacterium was inoculated with *B. odorata* extract at the predetermined concentration, and incubated at 37 ºC/24 h under shaking (150 rpm). Two drops of Tween 80 (36 mg) (Vetec®) were added to the hexane extract to facilitate solubility. MH broth containing streptomycin (10 µg) inoculated with 10^5 CFU.mL^{-1} was used as a positive control. MH broth inoculated with 10^5 CFU.mL^{-1} without antibiotic was used as a negative control. MIC was defined as the lowest extract concentration at which no microbial growth in the culture broth was visually detectable.

After 24 h of incubation, 100 µL from tubes with no visible microbial growth were seeded on Petri dishes containing TSA-YE and incubated at 37 ºC/24 h. After this step, colonies were counted. MBC was defined as the lowest extract concentration at which 99.9% of the initially inoculated cells were killed.

2.6. Chemical characterization of *B. odorata* extracts by GC-MS

The extract that showed the best antibacterial activity in the agar diffusion method was selected for analysis by GC-MS. A sample was prepared with 500 µL of *B. odorata* extract (100 µL.mL^{-1} in HPLC grade chloroform) mixed with 50 µL of 1-nonanol (5 mg.mL^{-1} in HPLC grade chloroform) as internal standard. GC-MS analysis followed methodology proposed by Lisec et al. (2006). A Shimadzu GC-MS QP2010 Ultra device with an AOC 20i auto-injector and NIST 2011 mass spectral library was used. A 1 µL sample was injected at 60 ºC injector temperature and an injection split ratio of 1:35. Helium was used as carrier gas at a flow-rate of 2.04 mL.min^{-1} and linear speed as flow control mode. A Rtx-5MS capillary column (30 m×0.25 mm×0.25 µm) was used, and the GC oven temperature was programmed to operate at 60 ºC for 3 min, then increase to 300 ºC at 15 ºC per min, maintained for 1 min, increases to 320 ºC at of 5 ºC per min and maintain at this temperature for 6 min. For the mass spectrometric parameters, the temperatures of both the ion source and interface were 250 ºC. The mass range covered was from *m/z* 70 to 600 at a speed of 0.2 scans per second.

2.7. Statistical analysis

Data were submitted to an analysis of variance, one-way, (ANOVA) by Tukey test ($p \leq 0.05$) using STATISTICA software version 6.1 (StatSoft, France).

3. Results and Discussion

3.1. Antibacterial activity

The qualitative *in vitro* evaluation of the antibacterial activity of the two extracts (BHE and BME) by the agar disc diffusion method showed that all tested bacteria were inhibited by both extracts. However, a significant difference ($p \leq 0.05$) between the antibacterial activities of the extracts was observed in all bacteria with the exception of *L. monocytogenes*. BHE showed inhibition zones significantly larger than the BME, which is why the following experiments were carried out only with this extract (Table 1).

Table 1

Inhibition zones induced by *Butia odorata* fruit extracts in hexane (BHE) and methanol (BME) against six foodborne bacteria.

Microorganism	Zone diameter (mm)	
	BHE	BME
P. aeruginosa	64.0 ± 8.0 ^{a}	24.0 ± 1.0 ^{b}
S. Typhimurium	59.5 ± 4.5 ^{a}	24.0 ± 1.0 ^{b}
E. coli O157:H7	86.0 ± 2.0 ^{a}	24.0 ± 4.0 ^{b}
L. monocytogenes	30.5 ± 3.5 ^{a}	40.0 ± 4.0 ^{a}
S. aureus	45.0 ± 1.0 ^{a}	27.5 ± 1.5 ^{b}
B. cereus	42.0 ± 2.0 ^{a}	19.5 ± 2.5 ^{b}

Results expressed as means ± standard deviation. Letters "a" and "b" in the line represent significant difference ($p \leq 0.05$) between the extracts.

MIC values of BHE were 6 mg.mL^{-1} for *S. aureus*, 3 mg.mL^{-1} *E. coli* O157:H7, and 7 mg.mL^{-1} for *S.* Typhimurium and *P. aeruginosa*. *Listeria monocytogenes* and *B. cereus* were not inhibited even at the highest tested concentration (11 mg.mL^{-1}); however, there was a reduction of 2 log CFU.mL^{-1} for *L. monocytogenes* and 3 log CFU.mL^{-1} for *B. cereus*. The MBC of BHE was 11 mg.mL^{-1} for *S. aureus*, *P. aeruginosa* and *S.* Typhimurium, and 3 mg.mL^{-1} for *E. coli* O157:H7 (Table 2).

Table 2

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of hexane *Butia odorata* fruit extract on six foodborne bacteria.

Microorganism	MIC (mg.mL^{-1})	MBC (mg.mL^{-1})
P. aeruginosa	7	11
S. Typhimurium	7	11
E. coli O157:H7	3	3
L. monocytogenes	>11	>11
S. aureus	6	11
B. cereus	>11	>11

In general, Gram-negative bacteria such as *S.* Typhimurium, *E. coli* and *P. aeruginosa* were more susceptible to BHE than the Gram-positive bacteria like *S. aureus*, *L. monocytogenes* and *B. cereus*. This can be ascribed to the differences in the cell structure between Gram-positive and Gram-negative bacteria, resulting in a differentiated behavior when exposed to antimicrobial agents (Puupponen-Pimia et al., 2001). This finding is intriguing, since Gram-positive bacteria are usually more susceptible to natural antimicrobial agents such as extracts from herbs and spices (Shan et al., 2007) and essential oils (Chorianopoulos et al., 2004) than Gram-negative bacteria. This is due to the fact that the cell structure of the outer membrane of Gram-negative bacteria is composed of lipopolysaccharides, providing protection against several agents (Tortora et al., 2012).

The effectiveness of an antimicrobial compound depends on the type of microorganism and the microbial species and strain. This was verified in this study, since the MBC for *E. coli* was lower (3 mg.mL^{-1}) than for the other Gram-negative bacteria tested [*S.* Typhimurium and *P. aeruginosa* (11 mg.mL^{-1})]. Likewise, the MBC for *S. aureus* was 11 mg.mL^{-1}, whereas the other Gram-positive bacteria *B. cereus* and *L. monocytogenes* were not inhibited even at the highest tested concentration (11 mg.mL^{-1}). However, the reduction in the cellular concentration of these two microorganisms (from 3 log CFU.mL^{-1} for *B. cereus* and 2 log CFU.mL^{-1} for *L. monocytogenes*) using 11 mg.mL^{-1} of BHE was noteworthy.

Of the Gram-negative bacteria tested, *Escherichia coli* O157:H7 was the most susceptible bacteria to the *B. odorata* extract (MBC 3 mg.mL^{-1}). This result is remarkable, considering that this *E. coli* serotype is an

Table 3

List of compounds tentatively identified by GC-MS in hexane *Butia odorata* extract (BHE).

Retention time (RT)	Kovats retention index*	Compound	Molecular weight	Relative quantitation (%)
3.1	428	2-Hexenal	98	0.3
3.4	455	1,3-Dimethylbenzene	106	1.4
8.5	939	4,7-Dimethyl-2,3-dihydro-1H-indene	146	0.3
8.8	968	1,2,3,4,5-Pentamethylbenzene	148	0.2
9.8	1072	Phenylbenzene	154	0.8
9.9	1088	*n*-Alkyl-naphthalene	156	0.3
11.4	1270	2,2-Dimethylphenylbenzene	182	0.1
11.6	1285	3,3-Dimethylphenylbenzene	182	0.6
11.7	1296	*n,n*-Dimethyl-1,1-biphenyl	182	0.6
12.1	1347	7-Ethyl-1,4-dimethylazulene	184	0.1
13.1	1491	Anthracene	178	0.2
13.8	1600	Methyl hexadecanoate	270	0.1
13.9	1605	2-Methylanthracene	192	0.1
13.9	1612	9-Methylanthracene	192	0.1
14.0	1618	(*E*)-Hexadec-9-enoic acid	254	0.1
14.1	1639	Hexadecanoic acid	256	5.3
15.2	1816	(9*Z*,12*Z*)-Octadeca-9,12-dienoic acid	280	1.8
15.4	1841	Ethyl (9*E*,12*E*)-octadeca-9,12-dienoate	308	0.6
17.3	2190F	1,3-Dihydroxypropan-2-yl hexadecanoate	330	2.1
18.8	2485	(*E*)-Octadec-9-enal	266	2.0
18.9	2502	Cyclopentadecanol	226	4.9
19.8	2687	(*E*)-Hexadec-9-enal	238	2.0
19.9	2708	Octadecanal	268	**3.3**
21.4	3012	Campesterol	400	**3.1**
21.9	3105	Obtusifoliol	426	1.1
22.1	3139	γ-Sitosterol	414	**22.0**
22.7	3254	Cycloartenol	426	**9.7**
23.1	3343	24-Methylenecycloartanol	440	**9.8**
		Total		**73**

*Kovats index was calculated based on the comparison to a fatty acid methyl ester homologous series (C_8- C_{24}) using GC-MS solution software.

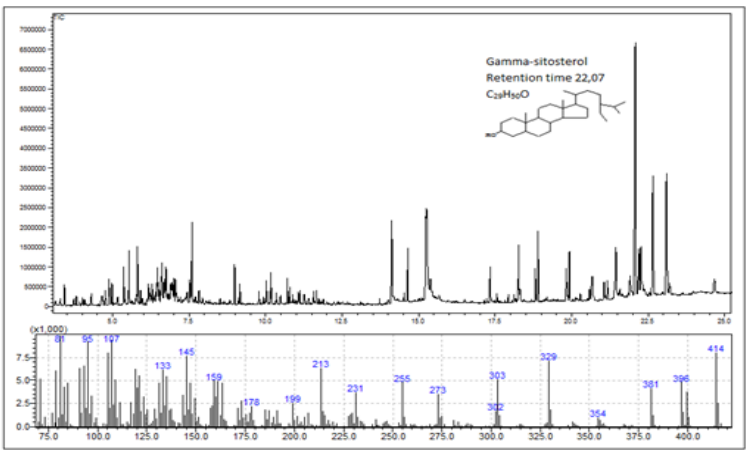

Fig. 3. Chromatogram of hexane extract from *Butia odorata* (BHE) and mass spectra fragmentation profile of γ-sitosterol.

important cause of FBD outbreaks (Rangel et al., 2005), aggravated by a high severity degree and low infectious dose (Farrokh et al., 2013).

Among the Gram-positive bacteria tested here, *S. aureus* was the most susceptible to the *B. odorata* extract, which is particularly outstanding, since poisoning caused by this microorganism is one of the most common causes of FBD (Bennett, 2005). In addition, this bacterium can form biofilms, making it a persistent contamination source in food processing environments (Herrera et al., 2007; Gutiérrez et al., 2012).

The antimicrobial activity of around 1340 plants has been confirmed (Tajkarimi et al., 2010) and in recent years, numerous studies were conducted to assess the antimicrobial activity of fruit extracts (Singh et al., 2016; Paunović et al., 2017). Some of these studies addressed native fruits from Brazil; however, to date there are no reports in the literature on the evaluation of the antibacterial activity of *B. odorata*, a fruit with wide distribution in South America. The ethanolic extract of *Paullinia cupana* Mart. for example, was found to be active against Gram-positive bacteria such as *S. aureus* (MIC=64 μg.mL^{-1}) and Gram-negative bacteria such as *P. aeruginosa* and *E. coli* (MIC=16 and 32 μg.mL^{-1}, respectively), at concentrations between 16 and 128 μg.mL^{-1} (Basile et al., 2005). On the other hand, Medina et al. (2011) evaluated extracts of *Psidium cattleianum* Sabine and confirmed their antimicrobial activity against *S. Enteritidis*, which varied with the extraction solvent (in acetone or water) and fruit type (red or yellow). Additionally, the antimicrobial activity of a commercial

Euterpe oleracea Martius extract against *L. innocua*, with a MIC of 10 $g.L^{-1}$ was described by Belda-Galbis et al. (2015).

3.2. Chemical characterization

GC-MS analyses identified twenty-eight compounds in BHE, representing 73% of the total composition (Table 3). The major component present in the BHE was γ-sitosterol (22%) (Fig. 3), followed by 24-methylenecycloartanol (9.8%), cycloartenol (9.7%), hexadecanoic acid (5.3%), cyclopentadecanol (4.9%), octadecanal (3.3%), and campesterol (3.1%).

γ-Sitosterol, as a phytosterol, was the major compound present in the BHE (22%). Similarly, γ-sitosterol was also one of the main components of the fruit extract of *Hylocereus undatus*, representing 19.3% of the total composition. In *Hylocereus polyrhizus* extract, γ-sitosterol represented 9.3% (Luo et al., 2014). However, only β-sitosterol was detected in *Euterpe oleracea* Martius (0.44 $mg.g^{-1}$) (Schauss et al., 2006).

Phytosterols are one of main components of plant cell membranes and contribute to membrane stability and functionality (Devaraj and Jialal, 2006; Marangoni and Poli, 2010; Dziedzic et al., 2016; Liao et al., 2014). Although this was the predominant found in the Butia extract, further studies are needed to determine whether γ-sitosterol or other compounds present in smaller amounts were responsible for the antibacterial activity, or if there is a synergistic contribution from the many compounds present in the extract.

4. Concluding remarks

Hexane (BHE) and methanol extracts (BME) of *Butia odorata* Barb. Rodr. fruit showed antibacterial activity against all pathogenic bacteria tested (*S. aureus*, *L. monocytogenes*, *B. cereus*, *S.* Typhimurium, *E. coli* and *P. aeruginosa*); however, the activity of the hexane extract was superior to the methanol extract. BHE contained γ-sitosterol as a major component and showed potential to be used as an alternative to synthetic preservatives to increase shelf life and safety of foods, requiring further research to assess its behavior in food systems and the antibacterial action mechanism of the extract.

Conflict of interest

The authors declare that there is no conflict of interest.

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