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Article

Chemical characterization, cytotoxicity, antimicrobial and antioxidant potential of *Justicia pectoralis* Jacq and *Croton jacobinensis* Baill extracts

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In this research, aqueous and ethanolic extracts from *Justicia pectoralis* Jacq and *Croton Jacobinensis* Baill were characterized. The UPLC-QTOF-MS^E analysis was performed on the extracts identified, predominantly, flavonoids, tannins and acids. The extracts did not indicate toxicity in human epithelial cells. *C. jacobinensis* presented a concentration of phenolics 60.5% higher than *J. pectoralis* in all scenarios evaluated and, for both samples, the hydroalcoholic extract at 70% exhibited the best efficiency in the extraction (14501.3 and 32521.5 mg GAE 100 g^{-1} for *J. pectoralis* and *C. jacobinensis*, respectively). The antioxidant activity presented a positive correlation with the concentration of phenolics, being 1.186,1 and 1.507,9 µM of Trolox for *J. pectoralis* and *C. jacobinensis* at 70% of ethanol; however, it was not verified statistical difference between the ethanolic solutions ($p < 0.05$). The antimicrobial activity of *J. pectoralis* extracts was highlighted once was the most effective against gram-positive bacteria. The results suggest that both *J. pectoralis* and *C. jacobinensis* extracts present the potential to be applied as natural additives due to their antioxidant and antimicrobial activity and safety. Thus, it is suggesting the development of studies that could investigate the interaction of these plant extracts with food matrices is required.

Keywords: Natural extracts. Bioactive compounds. Identification. Therapeutics.

INTRODUCTION

Natural plant extracts have been studied as food preservation additives, with the aim of using their activities against pathogenic microorganisms in food. The relevance of the use of these metabolites is related to the desire of food consumers for high-quality natural additives, without synthetic components (Calo *et al.*, 2015; Mak *et al.*, 2013). Plant extracts can be incorporated into the formulations used in the food industry, such as edible coatings (Lv *et al.*, 2011). In Brazil, some plant species have great potential for use as extracts given their simple cultivation and inexpensive production.

J. pectoralis is a plant popularly known in Brazil as chambá, chambabá, anador, or clover-cumaru (Kalyne *et al.*, 2017). Chambá was previouly reported as an alternative source of natural antimicrobial compounds in the function of the presence of bioactive compounds as coumarin (1,2 benzopyran) and umbelliferon. It was previously reported

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that aqueous extract of J. pectoralis was able to inhibit *Staphylococcus aureus* and *Escherichia coli* growth (Guimarães *et al.*, 2020). Phenolics compounds such as the flavonoids and tannins are markedly present in chambá extracts and may contribute to its antioxidant and anti-inflammatory activities (Chanfrau *et al.*, 2013). In addition, several species of the genus *Justicia* are used in popular medicine for the treatment of respiratory issues (Agra, Freitas, Barbosa-Filho, 2007), gastrointestinal issues (Corrêa, Alcântara, 2012), diabetes, and prostate diseases (Lizcano *et al.*, 2010).

C. jacobinensis is a plant popularly known as "black lobster" and presents several biological activities (Pontes *et al.*, 2011), including antibacterial and cytotoxic properties, acaricidal activities against *Tetranychus urticae,* anti-hypertensive property (Neves, Camara, 2011) antimalarial activity (Villamizar *et al.*, 2009) and antimycobacterial activities against *Mycobacterium tuberculosis* (Kulkarni *et al.*, 2013) and *Staphylococcus* (Liu *et al.*, 2014).

There is no reliable information regarding the use *J. pectoralis* and *C. jacobinensis* themselves in food preparations or processing. However, is undeniable the biological potential of both plants, which requires more studies to prospect their use in different research fields. Therefore, the purpose of this research was to investigate the antioxidant and antimicrobial activities of aqueous and hydroalcoholic extracts from leaves of *J. pectoralis* and *C. jacobinensis* to evaluate the potential of these for future application as natural food preservatives.

MATERIAL AND METHODS

Plant material

The *J. pectoralis* and *C. jacobinensis* leaves were manually collected by Dra. Leilanne Marcia in the Prisco Bezerra Herbarium (EAC) belonged to Federal University of Ceará (UFC) localized in Fortaleza (State of Ceará, Brazil). The identity of both plants was determined by comparison with a number of exsiccates (dried samples) deposited in EAC, which were 61.197 and 61.198 for *J. pectoralis* and *C. jacobinensis*, respectively. The plant material (fresh leaves) was collected with scissors in the morning from 8 to 10 am under the conditions of maximum (32°C) and minimum temperature (24°C) and 65% relative humidity, during the period from January to March. The leaves were washed with distilled water and dried in an air circulation oven (35 ± 3°C for 24 h for *J. pectoralis* and 48 h for *C. jacobinensis*). The dried material was stored using light protection at 25 ± 1°C until the extracts were prepared, which preparation was performed in triplicate.

Preparation of extracts

The extracts were made from infusions of dried leaves (with 0.84 mm of granulometry). The water extracts were obtained using distilled water at 90°C at the proportion 1:20 (dried leaves: water). The hydroalcoholic extracts (prepared in the concentration of 50 and 70% - v/v) were obtained with slight heating (70°C) in the same proportion used for water extract preparation. The extractions were sustained for 30 min and subsequently filtered. The material was freeze-dried using a Beta 1-8 LD plus (CHRIST, Germany), at -40°C and the pressure of 0.025 mbar. After freeze-drying process, the extracts were stored in polyethylene vessels, protected from light, and kept in a freezer (-18°C) until analysis.

Characterization of extracts

Chemical characterization

For chemical characterization, 10 mg of dried samples were solubilized in 10 mL of ultra-pure water. The solutions were homogenized in a USC 1400 bath ultrasound (Unique Indaiatuba, Brazil) for 5 minutes. After, the solutions were filtered in a 0.22µm Millipore filter, collected in vials e sent for analysis.

The analyses were performed in an Acquity UPLC (Waters) chromatographic system, coupled to a quadrupole/time of flight (QTOF, Waters). Chromatographic runs were performed on a Waters Acquity UPLC BEH (150 mm x 2.1 mm, 1.7 µm), fixed temperature 40 °C. The binary gradient elution system consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The UPLC elution

conditions were optimized as follows: linear gradient from 2 to 95% B (0-15 min), 100% B (15-17 min), 2% B (17.01), 2% (17.02-19.01 min), a flow of 0.4 mL min^{-1}, and a sample injection volume of 5 µL.

The chemical profile of samples was performed by coupling the Waters ACQUITY UPLC system to the QTOF mass spectrometer (Waters, Milford, MA, USA), with the electrospray ionization interface in negative ionization mode (ESI^-). The (ESI^-) mode was acquired in the rangeof 110-1180 Da, fixed source temperature at 120 °C, desolvation temperature 350 °C, desolvation gas flow of 500 L h^{-1}, 0.5 V extraction cone, 3 kV capillary voltage. Leucine enkephalin was used as lock mass. The MS mode used Xevo G2-XS QTOF. The spectrometer operated with MS^{and} centroid programming, using a tension ramp from 20 to 40 V. The instrument was controlled by Masslynx 4.1 software (Waters Corporation, USA).

Total polyphenols content (TPC)

For the quantification of polyphenosls, solutions with a concentration equal to 1 mg/mL were prepared from the solubilization of dried samples in distilled water. After complete homogenization, the solutions were stored under refrigeration until the analysis. The TPC was determined by the method described by Larrauri, Rupérez and Saura-Calixto (1997), which uses Folin-Ciocalteu reagent and gallic acid as the standard. Aliquots of 0.1 mL of the extracts, 0.5 mL of distilled water, 0.5 mL of the Folin-Ciocalteau reagent (1:3), 1.0 mL of 20% $NaCO_3$ solution, and 1.0 mL of distilled water were used for this analysis. After homogenization, the product was protected from light for 30 min and was read at 700 nm using a Shimadzu spectrophotometer (model UV-1800). The results were expressed as milligrams of gallic acid equivalent (GAE)/100 g of extract.

Total Antioxidant Activity

The same solutions prepared for polyphenols quantification were used to determine the total antioxidant activity of *J. pectoralis* and *C. jacobinensis*. The method was based on the reduction of the 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$), according to the technique reported by Re *et al.* (1999) and modified by Rufino *et al.* (2010). In the dark, an aliquot of 30 µL of each extract was mixed with 3 mL of the $ABTS^{\bullet+}$ radical and homogenized. After 6 min, the reads were made at 734 nm using the Shimadzu spectrophotometer. The results were expressed as TEAC - Antioxidant Capacity Equivalent to Trolox (2-carboxylic acid-6-hydroxy-2,5,7,8-tetramethylchroman) in micrometers per gram of extract.

Microorganisms and antimicrobial activity

E. coli (ATCC 25922), *S. enteritidis* (IAL 1132), *S. aureus* (ATCC 27664), and *L. monocytogenes* (ATCC 19115) were used to evaluate the antibacterial activity of the extracts of *J. pectoralis* and *C. jacobinensis*. This study was based on the plate microdilution method suggested by (MIC) and minimum bactericidal concentration (MBC) values. Water and ethanolic Branen and Davidson (2004) and Brandt *et al.* (2010), with adaptations. A method using 96-well microplates (MicrotestTM, Becton Dickinson and Co.) was used to determine the minimum inhibitory concentration extracts (50 and 70% - v/v) at 250 mg/mL were prepared similarly to previsouly described. The extracts were diluted in water to obtain different concentrations (0.5-100 mg/mL) and sterilized using 0.22 µm membrane filters. In each well, TSB broth, bacterial suspension (approximately 10^5 CFU/mL) and an aliquot of each dilution of the extracts studied were incubated with shaking at 35°C for 24 h. The test was also carried out in wells containing culture medium, distilled water, and bacterial suspension, as a negative control. A commercial antimicrobial agent (amikacin) and inoculum was used as a positive control. Optical density (OD) was read at 630 nm using the ELx 808 absorbance reader (BioTek instruments, Inc. Winooski, VT, USA). The final results were obtained by variation of the absorbance at different times (0 and 24 hours after incubation). Considering the MICs, the concentrations that presented differences between the readings were ≤ 0.05 nm. From the wells that indicated inhibitory activity (≤ 0.05 nm), 100 µL was inoculated into culture media and specific differential media were provided for each species: Mac Conkey Agar (Oxoid) for *E. coli*, Listeria Oxford Agar (Hi Media)

for *L. monocytogenes*, Baird-Parker Agar (BD) for *S. aureus*, and deoxycholate-lysine-xylose (XLD) agar for *S. eteritidis*. The plates were incubated at 35°C for 48 h, and MBC was considered the lowest test concentration at which a plate showed the absence of microbial growth. The results were expressed in milligrams per milliliter of dry extract.

Cytotoxicity Activity

Cytotoxicity activity tests were done with the extracts that presented the best antimicrobial activity, according to the method described by Mosmann (1983) using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide). Cytotoxicity was measured by exposing intestinal epithelial cells (approximately 20.000 cells of the IEC 6 line previously cultured) to 100 µL of the extract solutions with concentraton varying from 0.25 to 20 mg/mL. The fractions obtained were compared to positive (cell culture only in culture medium) and negative (*Clostridium difficile* cytotoxin A) controls, being the positive control considered 100% cell viability. The results of the absorbances obtained were submitted to Bonferroni's multiple comparison test and the results expressed as mean and standard deviation.

Statistical analysis

The results of total extractable polyphenols and total antioxidant activity were analyzed statistically by comparing the means by Variance Analysis (ANOVA) and Tukey's test, at the 5% level of significance, using the software Statistica 10.0. The data obtained for the antimicrobial activity was expressed through the minimum inhibitory and bactericidal concentrations.

RESULTS AND DISCUSSION

UPLC characterization

The UPLC results of the aqueous extract of *J. pectoralis* Jacq. showed the presence of twelve peaks (Figure 1A and Table I). For *C. jacobinences* extract, fourteen peaks were found (Figure 1B and Table II). The identification of the compounds present in each extract was made by comparison with results present in the literature database. Thus, for a higher confidence level, it was considered the maximum similarity with the mass calculated, fragments and empirical formula and, it was considered the minor error possible between the substances evaluated.

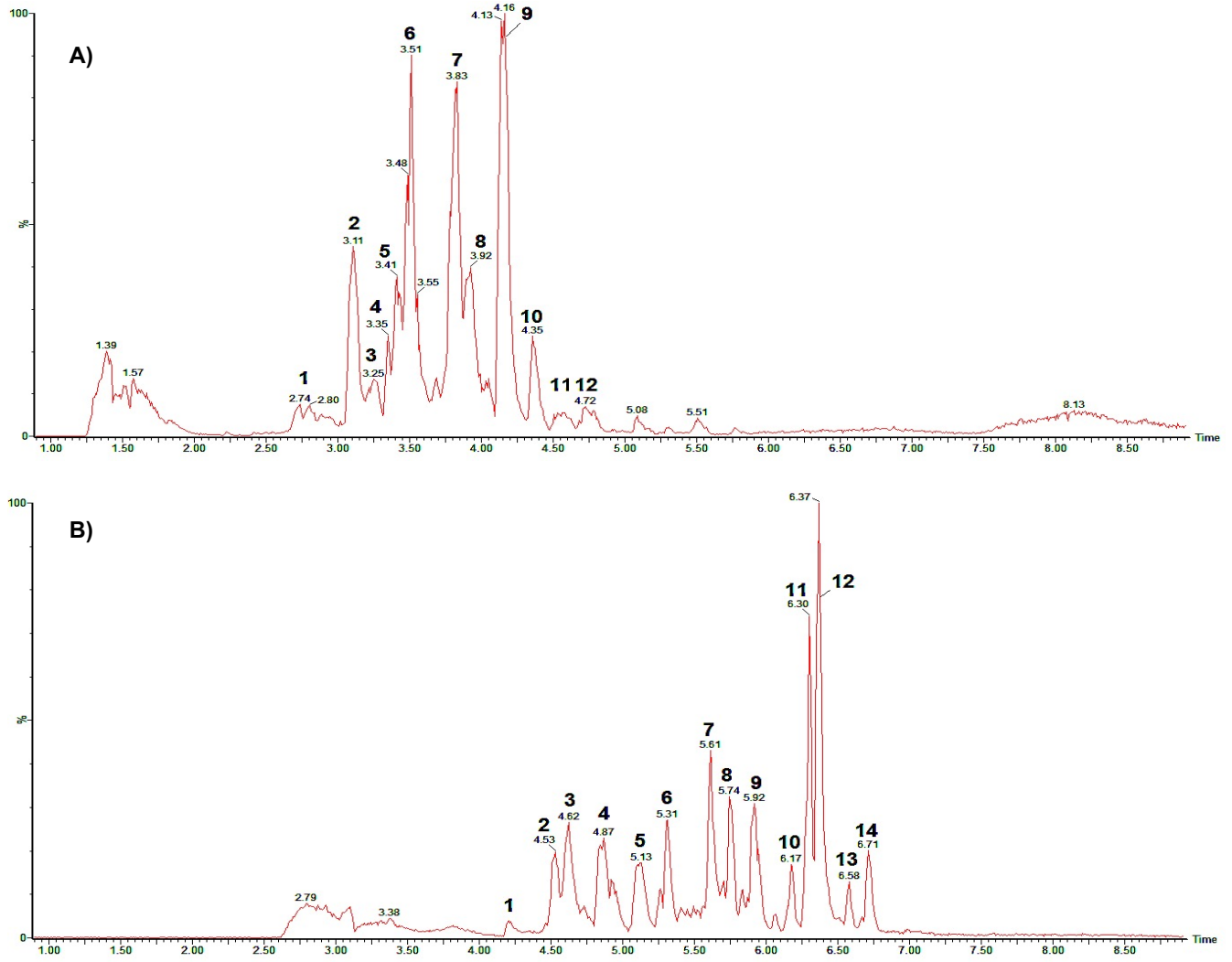

FIGURE 1 - Chromatogram of (A) Justicia pectoralis Jacq and (B) Croton jacobinences Baill extracts by UPLC-QToF-MSE extract .

TABLE I - Compounds identified in the hydroalcoholic extract of *Justicia pectoralis* Jacq.

Peak	T_R (min)	$[M-H]^-$ Observed	$[M-H]^-$ Calculated	Ion fragments (MS/MS)	Empirical Formula	Error	Reference
1	2,74	503,1406	503,1401	341,0875; 315,1050; 179,0343; 135,0444	$C_{21}H_{27}O_{14}$	1,0	(Spínola; Castilho, 2017)
2	3,11	341,0881	341,0873	179,0325; 135,0416	$C_{15}H_{17}O_9$	2,3	(Spínola; Castilho, 2017)
3	3,25	355,0658	355,0665	179,0370; 135,0440	$C_{15}H_{15}O_{10}$	-2,0	(Bresciani et al., 2017)
4	3,35	313,0923	313,0923	137,0607	$C_{14}H_{17}O_8$	0,0	(Vennila; Rajangam, 2015)
5	3,41	329,0973	329,0973	135,0333; 72,9945	$C_{14}H_{17}O_9$	0,0	-

TABLE I - Compounds identified in the hydroalcoholic extract of *Justicia pectoralis* Jacq.

Peak	T_R (min)	$[M-H]^-$ Observed	$[M-H]^-$ Calculated	Ion fragments (MS/MS)	Empirical Formula	Error	Reference
6	3,51	325,0916	325,0923	163,0361; 119,0514	$C_{15}H_{17}O_8$	-2,2	(Simirgiotis et al., 2017)
7	3,82	327,1068	327,1080	165,0520	$C_{15}H_{19}O_8$	-3,7	(Simirgiotis et al., 2017)
8	3,92	325,0909	325,0923	163,0358; 119,0483	$C_{15}H_{17}O_8$	-4,3	(Simirgiotis et al., 2017)
9	4,13	651,1984	651,1984	-	$C_{23}H_{39}O_{21}$	0,0	-
10	4,35	503,2480	503,2492	429,1721; 161,0344	$C_{24}H_{39}O_{11}$	-2,4	-
11	4,57	771,2230	771,2254	637,2074; 327,1073	$C_{23}H_{47}O_{28}$	-2,4	-
12	5,08	709,3126	709,3130	223,0609; 165,0626	$C_{28}H_{53}O_{20}$	-0,6	-

TABLE II - Compounds identified in the hydroalcoholic extract of *Croton jacobinences* Baill.

Peak	T_R (min)	$[M-H]^-$ Observed	$[M-H]^-$ Calculated	Ion fragments (MS/MS)	Empirical Formula	Error	Reference
1	4,21	191,0195	191,0192	111,0121	$C_6H_7O_7$	1,6	(Jiménez-Sánchez et al., 2015)
2	4,53	371,0613	371,0614	209,0265; 191,0194	$C_{15}H_{15}O_{11}$	-0,3	(Ruiz et al., 2013)
3	4,62	371,0612	371,0614	209,0256; 191,0215	$C_{15}H_{15}O_{11}$	-0,5	(Ruiz et al., 2013)
4	4,87	463,0892	463,0877	301,0330; 300,0279	$C_{21}H_{19}O_{12}$	3,2	Padrão
5	5,13	593,1479	593,1506	285,0401	$C_{27}H_{29}O_{15}$	-4,6	(Navarro et al., 2017)
6	5,31	291,1600	291,1596	273,1513	$C_{17}H_{23}O_4$	1,4	-
7	5,61	331,1904	331,1909	201,1284; 71,0183	$C_{20}H_{27}O_4$	-1,5	(Xu; Liu; Liang, 2018)
8	5,74	357,1326	357,1338	-	$C_{20}H_{21}O_6$	-3,4	(Xu; Liu; Liang, 2018)
9	5,92	347,1846	347,1858	329,0634; 314,0413; 299,0165	$C_{20}H_{27}O_5$	-3,5	(Xu; Liu; Liang, 2018)
10	6,17	339,1216	339,1232	295,1371; 116,0312;109,0322	$C_{20}H_{19}O_5$	-4,7	(Xu; Liu; Liang, 2018)
11	6,30	315,1949	315,1960	225,1668	$C_{20}H_{27}O_3$	-3,5	(Xu; Liu; Liang, 2018)
12	6,37	325,1441	325,1440	281,1558; 265,1228; 235,1138	$C_{20}H_{21}O_4$	0,3	(Xu; Liu; Liang, 2018)
13	6,58	329,1761	329,1753	285,1899; 116,9326	$C_{20}H_{25}O_4$	2,4	(Xu; Liu; Liang, 2018)
14	6,71	327,1598	327,598	283,1722; 163,1791	$C_{20}H_{23}O_4$	0,6	(Xu; Liu; Liang, 2018)

Regarding the compounds found, derivatives of caffeic acid (caffeic acid dihexoside, caffeic acid hexoside and caffeic acid glucuronide) are reported in the literature associated with antioxidant and anti-inflammatory potential. In addition, they have antimicrobial activity (Moosavi *et al*. 2017). The substances p-Coumaroil glucoside and p-Coumaroil galactoside are derived from phenolic compounds with antioxidant activity as described by Tang *et al.* (2001).

The literature shows several classes of diterpenes associated with the molecular formulas of the compounds referring to peaks 7 to 14 (Xu, Liu, Liang, 2018). However, due to the large number of isomers, it was not possible to establish the identification of each substance and they were tentatively identified as diterpenes. Citric acid is known in the literature as a natural antioxidant (Ryan *et al*., 2019) and has an antimicrobial effect by modifying the pH of the medium. Diterpenes are reported with various biological activities, with larvicide (Geris *et al*., 2008), insecticide (Viegas Júnior, 2003) and antimicrobial (Sá Firmino *et al*., 2019). Isoquecitrin has a positive effect on osteogenesis (Li *et al*., 2019), antibacterial activity (Yun, Woo, Lee, 2018) and antioxidant (Silva *et al*., 2009). Kaempferol-O-rutinoside is reported in the literature with a hepatoprotective effect (Wang, Tang, Zhang, 2015), in addition to other phenolic compounds have antioxidant and anti-inflammatory activity (Ma *et al*., 2019).

Polyphenol content and antioxidant activity

It is possible to verify in Table III that, compared to water, the hydroalcoholic solutions presented more efficiency on the recovery of phenolics and, in all scenarios evaluated, *C. jacobinensis* Baill. presented the highest concentration of these compounds. Generally, the incrase of the medium polarity favors the solubization of different phytochemicals, such as the phenolics Between the hydroalcoholic. Thus, as was expected, the hydroalcoholic solution of *C. jacobinensis* prepared at 70% (v/v) was the most effective on the extraction of phenolics (32.521,5 mg GAE.100g^{-1}), being statistically different from those prepared with 50% (v/v) (31.384,7 mg GAE.100g^{-1}) and with water (24.908,9 GAE.100g^{-1}) with a significance of 5%.

TABLE III - Total polyphenols content (TPC) and antioxidant activity of *J. Pectoralis* Jacq and *C. jacobinensis* Baill. extracts.

Extraction method	Total extractable polyphenols expressed (mg GAE 100g^{-1})		Antioxidant activity (µM of Trolox g^{-1})	
	J. Pectoralis **Jacq.**	*C. jacobinensis* **Baill.**	*J. Pectoralis* **Jacq.**	*C. jacobinensis* **Baill.**
Aqueous	8.436,1^{cB} ± 105,3	24.908,9^{cA} ± 227,7	367.5^{cB} ± 0,80	967.8^{bA} ± 13.69
50% ethanol	12.111,9^{bB} ± 406,5	31.384,7^{bA} ± 250,4	1.146,8^{bB} ± 83,85	1.486,4^{aA} ± 2.34
70% ethanol	14.501,3^{aB} ± 416,8	32.521,5^{aA} ± 219,9	1.186,1^{aB} ± 54,65	1.507,9^{aA} ± 34.96

Means with equal letters, in the same column and line, did not differ among themselves at the 5% level of significance for the Tukey test.

Regarding to *J. pectoralis* Jacq., the ethanolic solution at 70% (v/v) (equal to 14.501,3 GAE.100g-1) presented an extraction efficience 16 and 42% higher than that prepared with 50% of ethanol (12.111,9 mg GAE.100g^{-1}) and water (8.436,1 mg GAE.100g^{-1}), respectivetly. In the same situation, for *C. jacobinensis* Baill., the values were 3.5 and 23.4%. Considering the average of the concentrations obtained for each solvent the amount of phenolics present in *C. jacobinensis* Baill. was 60.5% superior to that present in *J. pectoralis* Jacq ($p>0.05$).

Current research involves an evaluation of different solvents and methods of extracting bioactive compounds from plants (Feng *et al.*, 2020). Artega *et al.* (2011) compared the effects of the use of water, ethanol, and ethyl acetate as

solvents on the extraction of phenolics from *J. pectoralis*. The authors pointed out ethanol as the best extractant on the recovery of the polyphenols, being 42 and 66% more efficient than water and ethyl acetate, respectivetely. Agra, Freitas and Barbosa-Filho (2007) found higher levels of phenolics in the ethanolic extract of *J. pectoralis* with an average value of 23.795 mg AGE/100 g. This value was higher than the one reported in this research for that plant, and this may be function of the differences of the cultivar characteristics, as well as in the process extraction itself.

Previously, it was highlighted the considerable amount of phenolics presented by *C. jacobinensis* Baill; however, there is a lack of information regarding its phytochemical characterization in the literature. Thus, the amount of phenolic found in other species of *Croton* was compared to the data found in this research. da Silva *et al.* (2019) evaluated different phytochemicals in hydroalcoholic extracts of *C. floribundus* and *C. urucurana* and found a phenolic concentration equal to 9.141 and 7.997 mg AGE/100g, respectively. An average of 2.602 mg AGE/100g for the aqueous extract of *C. cajucara* Bentda was reported by da Silva *et al.* (2013). For comparision, the aqueous extracted produced in this research presented a concentration of phenolics 89.55% higher than that point out by the authors. Dall'Acqua *et al.* (2021) evaluated the use of methanol, water, dichloromethane, ethyl acetate and water in the extraction of phenolics present in the *C. hirtus* L'Hér. The author found concentrations equal to 1.796, 2.424, 2.251 and 2.238 997 mg AGE/100g, which were expressively lower than the average obtained in this research for *C. jacobinensis* Baill (29.604,3 mg AGE/100g). According to Dutta, Dey and Chaudhuru (2013), the leaves of *Croton ssp* are rich in phytochemicals (e.g., phenolic compounds) that possess several bioactive properties, meaning that the plant has the potential to be used as a therapeutic agent. According to the authors, the phenolic compounds present anti-inflammatory, vasodilator, and antioxidant actions and can prevent several diseases, like cancer and heart complications. The total phenolic content and the antioxidant activity could depend on the solvent used for extraction.

It is possible to verify in Table III that the average antioxidant activity was 900 and 1.320,7 μM of Trolox g^{-1} for *J. pectoralis* Jacq and *C. jacobinensis* Baill, respectively. It was found a positive correlation between the phenolics concentration and the antioxidant activity; thus, the hydroalcoholic extracts presented a higher antioxidant activity compared to aqueous extract, for both species evaluated. Between the species, the *C. jacobinensis* Baill. exhibited the higher antioxidant activity in all scenarios evaluated, however the extracts prepared with 50 and 70% of ethanol did not present a statistical difference ($P>0.05$).

The information regarding the antioxidant characterization of *J. pectoralis* species is limited. For the aqueous infusions prepared with the leaf of *J. pectoralis*, Lizcano *et al.* (2010) found an antioxidant activity of 18.8 and 89.7 μM of Trolox g-1 and employing $ABTS^{\bullet+}$ and the oxygen radical absorbance capacity (ORAC) methods, respectively. These results were 95 and 75.6%, respectively, lower than that presented by the aqueous extract evaluated in this research. *Croton spp* are considered a source of antioxidant compounds, being reported in the literature several studies demonstrating their free radical scavenging capacity in a dose-dependent way (da Silva *et al*., 2019; Suresh Reddy *et al*., 2015; Baqueiro-Peña, Guerrero-Beltrán, 2017). For *C. jacobinensis*, Keerthana, Kalaivani and Sumathy (2013) reported that the ethanolic extract of *C. bonplandianum* leaves has good antioxidant capacity after using the DPPH radical capture method.

The research on natural plant antioxidants has been applied in tests for pharmaceutical and food grade additives, due to its therapeutic and nutritional properties. Regarding the food industry, these substances from plants are considered alternatives to the substitution of synthetic antioxidants, such as butylhydroxyanisole and butylhydroxytoluene, which can cause adverse health effects (Rameshkumar, Sivasudha, 2012). Thus, the preliminary results indicate both *J. pectoralis* and *C. jacobinensis* Baill as potential natural antioxidants.

Antimicrobial activity

It is possible to evaluate in Table IV that all plant materials showed activity against the bacterial strains tested. The aqueous and 50% ethanolic extracts from *J.*

pectoralis leaves showed antimicrobial activity against *S. aureus*, *L. monocytogenes*, *S. enteritidis*, and *E. coli*. The ethanolic extract (70% v/v) did not show antibacterial activity for *E. coli*. The extracts of *J. pectoralis* were more effective against gram-positive bacteria. The *C. jacobinensis* leaf extracts showed action against the Gram-positive bacteria, *S. aureus* and *L. monocytogenes* (Table IV). Only the hydroalcoholic extract (70% v/v) presented inhibitory and bactericidal action against the two gram-negative bacteria tested.

TABLE IV - Antimicrobial activity of extracts of *J. pectoralis* Jacq and *C. jacobinensis* Baill

Microorganism		*J. pectoralis* Jacq			*C. jacobinensis* Baill		
Aqueous		Ethanol 50%	Ethanol 70%	Aqueous	Ethanol 50%	Ethanol 70%	
S. aureus	CIM	1	15	5	0,5	1	1,5
	CBM	1	15	15	1	2	1,5
L. monocytogenes	CIM	10	15	25	8	6,25	3
	CBM	10	15	35	12,5	25	6
S. enteritidis	CIM	25	25	45	-	25	25
	CBM	25	25	-	-	25	25
E. coli	CIM	25	40	-	-	-	25
	CBM	50	50	-	-	-	30

MIC = minimal inhibitory concentration; CBM = minimum bactericidal concentration. Values in (mg / mL).

The antimicrobial action is associated with the specific characteristics of the extract to be used as an antimicrobial agent: its composition will determine the performance of this product as a preservation additive in food. The particular aspects of the antimicrobial agent to be applied in the food may limit its action as a preservative. Some metabolites with antimicrobial activity have hydrophobic properties that can prevent dissolution of the extract in water, limiting their use as food additives. The concentrations required for inhibition/inactivation of microorganisms are intrinsically linked to the specific targets of the antimicrobial agent because they depend on the structure of the target microbial cell and the specific genetic machinery of each species of microorganism (Furtado *et al.*, 2015).

The aqueous extracts provided greater antimicrobial activity (1 and 10 mg/L MIC, respectively) against *S. aureus* and *L. monocytogenes*. These bacteria are considered to be more sensitive to antimicrobial agents due to the constitution of their cell walls. These are mostly of peptideoglycan, which facilitates the permeability of these substances (Singh *et al.*, 2011). Higher extract concentrations were required for cell growth inhibition and death of gram-negative bacteria. The same concentration for MIC and MBC (25 mg/mL) was observed for the aqueous extracts and for 50% ethanolic extract for *S. enteritidis* (Table IV).

The aqueous extract was more efficient than the ethanolic one (at 50% v/v) for *E. coli*. The gram-negative bacteria are more resistant to antimicrobials than gram-positive ones, because the cell wall of gram-negative microorganisms has an outer membrane made of lipopolysaccharides and lipoproteins. In addition, there are flow pumps that actively remove harmful molecules from the cells, thereby further increasing tolerance to antimicrobial treatment. However, some authors have found different behavior when *J. pectoralis* extract is

used against gram-negative bacteria. Furtado *et al.* (2015) *Justicia pectoralis, Cymbopogon citratus frente a cepas de bactérias gram-positivas e gram-negativas. Trata-se de uma pesquisa de caráter experimental, quantitativo e observacional. O material botânico (folhas* studied the antimicrobial activity of an aqueous extract of *J. pectoralis* made by agar diffusion and verified that the extract was not efficient against *E. coli* and *Klebsiella pneumoniae*, both Gram-negative. This is justified by the fact that different techniques were used to determine the antimicrobial activity, different methods were used to obtain the extracts, and the plants originated in different countries. The hydroalcoholic extract (50% ethanol v/v) was only effective for *S. enteritidis*. Singh *et al.* (2011) evaluated the antimicrobial potential of aqueous, methanolic, and petroleum ether extracts of *C. bonplandianum* at concentrations of 50-125 mg/mL. These were obtained using the agar diffusion method and were used against *Bacillus* (now *Paenibacillus) macerans*, *S. aureus*, *Pseudomonas aeruginosa*, and *Pseudomonas striata*. The authors verified that the aqueous extract had action only against the bacterium *P. aeruginosa* (37.5% inhibition at 100 mg/mL). The methanolic extract presented the best antimicrobial potential, being ineffective only for *S. aureus.*

Citotoxicity test

For the cytotoxicity test, it was evaluated the aqueous extract of *J. pectoralis* and the hydroalcoholic extract at 70% (v/v) of *C. jacobinensis.* According to Figure 2, it is possible to state that both extracts did not present cytotoxic effects for the IEC 6 culture from direct comparison with negative control (cytotoxin A), which, as expect, presented a notable reduction in the cell population. It was observed a proportional increase in the cell viability with the increase of the concentrations. At higher concentration (20 mg/mL) it was verified an increase of 40 and 10% in the cell culture growth, for the *J. pectoralis* and *C. jacobinensis*, respectively. This result means that the extracts stimulate the growth of the cells and implies the safety of both extracts.

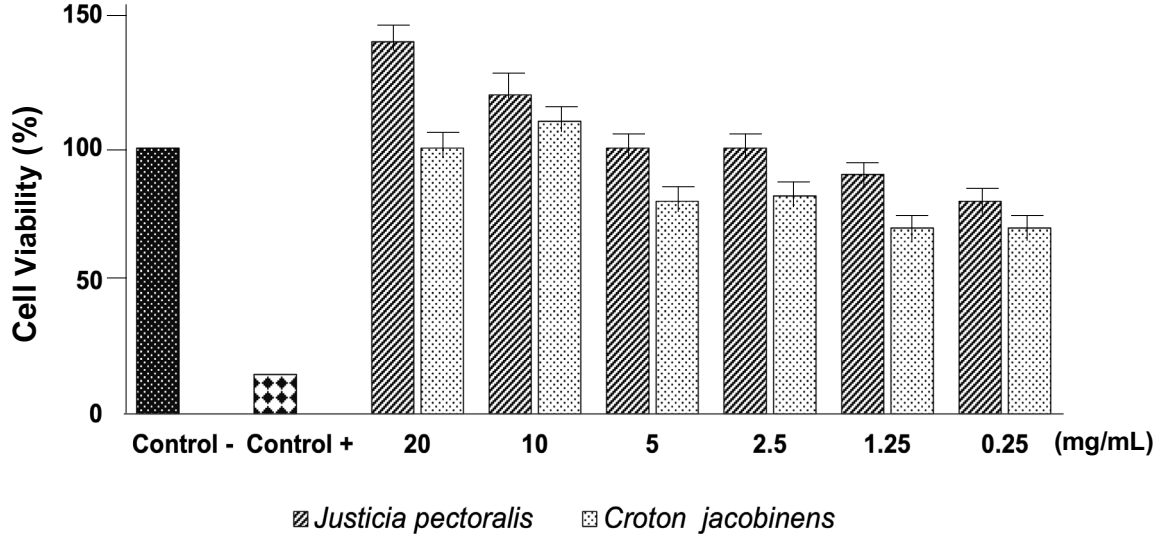

FIGURE 2 - *In vitro* cytotoxicity of aqueous extract of Justicia pectoralis Jacq and hydroalcoholic extract (70% v/v) of Croton jacobinences Baill.

Similar to this research, Nunes *et al.* (2018) did not found cytotoxic effects in normal peripheral blood mononuclear cells treated with *J. pectoralis* extract usinf a concetration of 100 µg/mL. On the other hand, the same extract reduced the viability of neoplastic cell lines, demonstrating its potential on cancer treatment. According to Brighenti *et al.* (2014), the identification and separation of the active fraction from the crude

extract of each plant may reduce the cytotoxicity potential of the concentrations studied. The authors also emphasize that the use of MTT has methodological limitations, requiring more accurate studies with animal models. In this case, studying the toxicity of *J. pectoralis* microcapsules using the Zebrafish method, Guimarães *et al.* (2020) did not report any sedative effect or impairment in the locomotion in the animals tested with the sample in different concentrations. Also employing the Zebrafish method, Ramos *et al.* (2009) evaluated the cytotoxicity of different plant extracts used in Northeastern medicide, between them several species of *Croton*. Regarding *C. jacobinensis*, the authors pointed the extracts prepared with the leaves using ethanol and hexane did not present toxicity for the animals. On the other hand, the ethanolic extract prepared with stem presented elevated toxicity. In general, even being different methods to evaluate the toxicity of natural samples, the results reported by the authors may collaborate to state the safety of the extracts produced with both studied plants.

CONCLUSIONS

The 70% ethanolic extract proved to be more effective for the presentation of higher amounts of bioactive compounds, as polyphenol compounds and presented higher antioxidant activity. The organic extracts studied showed antimicrobial activity against *S. aureus*, *L. monocytogenes*, *S. enteritidis*, and *E. coli*. The *J. pectoralis* and *C. jacobinensis* extracts showed potential for use in food products, justified by the indicated antioxidant and antimicrobial properties and by the non-toxicity to human cells of the extracts from both plants.

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