

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Antibiofilm activity of cashew juice pulp against *Staphylococcus aureus*, high performance liquid chromatography/diode array detection and gas chromatography-mass spectrometry analyses, and interference on antimicrobial drugs



Marcus V. Dias-Souza ^{a,b,*}, Renan M. dos Santos ^{b,c},
Ezequias P. de Siqueira ^d, Pedro H. Ferreira-Marçal ^{b,e,f}

^a Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

^b Integrated Pharmacology and Drug Interactions Research Group (GPqFAR), Belo Horizonte, MG, Brazil

^c Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

^d René Rachou Research Center/FIOCRUZ Minas, Belo Horizonte, MG, Brazil

^e Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil

^f University of Vale do Rio Doce, Governador Valadares, MG, Brazil

ARTICLE INFO

Article history:

Received 5 May 2016

Received in revised form

1 July 2016

Accepted 22 July 2016

Available online 3 November 2016

Keywords:

antimicrobial

cashew

pulp

Staphylococcus aureus

ABSTRACT

The epidemiology of *Staphylococcus aureus* infections has evolved in recent years, as this species is a major Gram-positive pathogen associated with healthcare services. The antimicrobial resistance of this species raises an urgent need for new treatment strategies. Fruits play important nutritional and economic roles in society, but their biological and pharmacological features are poorly explored when compared to nonedible parts of plants such as barks and leaves. In this study, we show that the cashew apple juice [cashew juice pulp (CJP)] extract is active against the planktonic cells of *S. aureus* strains, and for the first time, we show that CJP is also active against *S. aureus* biofilms. High performance liquid chromatography and gas chromatography-mass spectrometry analyses were conducted to prospect for polyphenols and free carbohydrates, respectively. Cashew apple juice, which is rich in nutrients, is widely consumed in Brazil; therefore, the quality attributes of CJPs were investigated. Samples were evaluated for pH, total titratable acidity, vitamin C levels, and total soluble solids. We also detected an antagonistic interference of CJP when it was combined with different antimicrobial drugs.

Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author. Microbiology Department, Biological Sciences Institute, Federal University of Minas Gerais, Avenida Antônio Carlos, 6627 Pampulha, Belo Horizonte, MG 31270-901, Brazil.

E-mail address: souzamv@ufmg.br (M.V. Dias-Souza).

<http://dx.doi.org/10.1016/j.jfda.2016.07.009>

1021-9498/Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The use of fruit pulps in the preparation of juices is popular worldwide. In the Brazilian market, despite the availability of liquid pulps, frozen pulps are largely used by the population especially during summer, and they are generally less expensive than liquid pulps [1]. Cashew apple juice pulp is widely available in Brazil and has an important economic role especially in the northeast region of the country, where the cashew tree (*Anacardium occidentale*) is mostly found. The cashew apple is a fibrous and juicy pseudo-fruit that contains several bioactive molecules such as polyphenols, tannins, anacardic acid, carotenoids, and vitamin C [2]. Anti-proliferative, antimicrobial, and anti-inflammatory activities have been suggested for cashew extracts obtained from the leaves and stem bark [3–5], making them worthy of study as candidates for potential use in antimicrobial and anticancer therapies.

Staphylococcus aureus is a commensal species of the human microbiota and is also an important pathogen involved in several infectious diseases including abscesses, osteomyelitis, endocarditis, and septic arthritis. This species may successfully persist within the host organism owing to virulence factors related to important features such as biofilm formation [6]. Biofilm formation is involved in the physiopathology of the aforementioned diseases, and also in infections caused by the use of implanted medical devices [6,7]. Biofilms are microbial colonies that can attach to biological tissues and abiotic surfaces, which often results in diseases. In biofilms, microorganisms grow surrounded by extracellular polymeric substances, which are generally composed of polysaccharides, proteins, nucleic acids, lipids, and channels for water and nutrients flow. This provides protection to microorganisms against pH extremes, desiccation, lack of nutrients, antimicrobial therapy, and the immune system [8].

Natural products from vegetable sources such as cashew extracts have been recognized as feasible alternatives to synthetic antimicrobials in clinical treatments. Microorganisms hardly develop resistance to phytochemicals, which generally act by unspecific mechanisms of action [9]. Because of the growing resistance of *S. aureus* to currently available antimicrobial drugs, there is an urgent need for new therapeutic options to treat staphylococcal diseases. Given that several plant foods are recognized for their benefits to human health, our group considered exploring fruit pulps for prospecting antimicrobial compounds.

The biological potentials of extracts obtained from different nonedible parts of the cashew tree have been described [5]; conversely, cashew juice extracts remain poorly investigated. This research aimed to investigate the antimicrobial potential of the cashew juice pulp (CJP). Also, we assessed the effects of the joint use of CJP and antimicrobial drugs. Here we show for the first time that CJP is active against planktonic cells and biofilms of clinical isolates of *S. aureus* strains. Moreover, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) analyses were conducted for prospecting phenols and free carbohydrates, respectively, on the pulps.

2. Materials and methods

2.1. Pulps samples

The pulps samples used in this study belong to a brand widely commercialized in Brazil. Samples were purchased in closed containers (10 units each) from local markets at Minas Gerais State (Brazil) and consisted of integral pulps packed in plastic bags. Products of the same batch code were assessed. All pulps were stored at -20°C until used and defrosted overnight in a refrigerator prior to analysis. Samples were kept in ice during the experiments.

2.2. Physicochemical analysis

Total soluble solids (TSS), total titratable acidity (TTA), and pH of the pulps were evaluated considering Brazilian standards of quality [1]. TSS were assessed by the refraction index using a handheld refractometer with temperature correction (20°C), and the results are expressed in $^{\circ}\text{Brix}$. TTA was determined by titration using a standard alkaline solution of 0.1 mol/L sodium hydroxide (NaOH) and ethanolic phenolphthalein (1%) as indicator, and expressed as a g of citric acid/kg of pulp fresh weight. The pH value was measured using a PM608 pH meter (Analion, Ribeirão Preto, Brazil). All assays were performed in triplicate.

2.3. Vitamin C quantitative detection

Vitamin C (ascorbic acid) content was determined by iodine titration as previously described [10]. To 25 g of pulp, 35 mL of starch–sulfuric acid solution was added and mixed. The resulting solution was titrated with standardized 0.1M iodine solution (protected from light exposure) while stirring until the first stable blue color was seen. This experiment was performed in triplicate at the day the pulps were opened and after 7 days, in order to access possible decays in ascorbic acid levels.

2.4. Polyphenols detection by HPLC

In order to analyze the polyphenols content, an extraction step was performed on the whole pulp as described by Zabi-dah et al [11], with modifications. A total of 10 mL of the samples was extracted with 20 mL of 80% methanol. This mixture was homogenized overnight in room temperature in the dark with magnetic stirring, and an aliquot of 1 mL of the supernatant was then filtered through a 0.22- μm PVDF filter (Millipore, Darmstadt, Germany) prior to the analysis. Using rutin as standard, the apparent flavonoid content of the pulps was determined using a SPD 20A Shimadzu High Performance Liquid Chromatography coupled to a diode array (HPLC/DAD) system. Samples of 20 μL were injected in a C18 column (Shim-pack ODS), and fractions were separated with gradient elution consisting in Milli-Q water (solvent A) and methanol (HPLC grade, solvent B) at a flow rate of 0.5 mL/min. The temperature was set at 20°C . The linear gradient mode was programmed as follows: 100% A and 0% B at the start, then to 10% A and 90% B at 20 minutes, remaining at 10% A and 90% B from 20 to 25

minutes, and falling back to 100% A and 0% B at 30 minutes. Polyphenols were detected at 240 and 254 nm.

2.5. Free carbohydrates detection by GC-MS

The presence of free carbohydrates on the pulp samples was determined with GC-MS using a modified method described by Monteiro et al [12]. The fruit juice contents were extracted as described in the HPLC subsection. The supernatant was concentrated (Speedvac, Thermo Fisher, Waltham, U.S.A.), and the residue was washed at least three times with methanol. The sample was then reduced with 1M aqueous sodium borohydride (NaBH_4 , 100 μL) and acetylated with acetic anhydride (100 μL). The excess reagent was removed by evaporation, and the sample was washed several times with methanol. The alditol acetates were extracted with ethyl acetate and water (1:1, v/v). The organic phase was concentrated in a nitrogen atmosphere, and then was recovered with HPLC grade ethyl acetate. Samples were analyzed in a QP 5050 A gas chromatograph coupled to a mass spectrometer equipped with a PTE-5-Supelco column, using helium as carrier gas. The column temperature was programmed to increase from 100°C (1 minute) to 200°C at a rate of 4°C/min, followed by 20°C/min to 300°C, and the column was maintained at this temperature for 5 minutes. Free carbohydrates were analyzed by comparing the relative retention times of sugar standards to sample peaks. The results were recorded and processed using Shimadzu Class 3.02 software.

2.6. Microbiological quality analysis

Microbiology tests consisted in the detection of *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Staphylococcus* sp., *Pseudomonas* sp., and yeasts and molds, as described previously by our group [1]. First, 25 g of the samples was placed in a sterile Erlenmeyer flask and homogenized with 225 mL of sterile 0.1% peptone water, and appropriate 1:10 dilutions of the resultant homogenate or the rinse fluid were prepared using peptone water. Aliquots of 0.1 mL were spread-plated on MacConkey agar for isolation of *E. coli*, *Salmonella*–*Shigella* agar for *Salmonella* sp. and *Shigella* sp., Cetrimide agar for *Pseudomonas* sp., Mannitol agar for *Staphylococcus* sp., and Sabouraud agar for yeasts and molds. For bacteria, the plates were incubated overnight at 37°C, and the number of colonies was counted. For yeasts and molds, the plates were incubated at 28°C for 5–7 days, and the number of colonies was counted.

2.7. CJP extract preparation for antimicrobial assays

CJP extracts were prepared as follows. The pulp content was freeze-dried, and the dry powder was exposed to a 70% ethanol solution. This system was maintained in magnetic stirring at maximum speed for 48 hours in room temperature. The content was then centrifuged (5000g, 20 minutes); the supernatant was partially concentrated in a vacuum process (4°C) and then freeze-dried. The final product was then weighed and stored at 4°C until used. A stock solution (4 mg/mL) was prepared using dimethyl sulfoxide, and serially diluted in phosphate-buffered saline buffer (pH 7.2) to perform antimicrobial assays.

2.8. β -Carotene antioxidant assay

The antioxidant assay was conducted with the CJP extract as described by Pratt [13], with modifications. β -Carotene (2 mg) was added to a boiling flask with linoleic acid (20 mg) and Tween 40 (100 mg), all dissolved in chloroform (10 mL). After the chloroform was removed at 40°C under vacuum, linoleic acid (40 mg), Tween 80 (400 mg), and oxygenated Milli-Q water (100 mL) were added to this system with vigorous shaking. Aliquots of 200 μL of the extract and 4.8 mL of the emulsion were mixed in open flasks, which were thermostated in a water bath at 50°C prior to and during measurements. Readings were taken in a spectrophotometer at 470 nm immediately, and with 15-minute, 30-minute, 45-minute, and 90-minute intervals. All determinations were performed in duplicate. The antioxidant potential of CJPs was calculated as previously described [13].

2.9. Antimicrobial assays: bacterial strains

Clinical isolates were obtained from the microorganism collection of the Microbiology Laboratory from University Vale do Rio Doce. They were isolated from indwelling catheters of hemodialysis patients. All strains were cultured in Brain Heart Infusion broth (Difco, Becton Dickinson, U.S.A.) prior to tests for identity confirmation with VITEK 2 system (version R04.02; bioMérieux, Marcy l'Etoile, France). Gram-positive identification cards were used according to the manufacturer's instructions.

2.10. Minimal inhibitory concentration assay

The minimal inhibitory concentration (MIC) of the CJP extract was determined in untreated sterile 96-well polystyrene microtiter plates as described by the Clinical and Laboratory Standards Institute [14]. Bacterial cultures were prepared in Mueller–Hinton broth (Difco, Becton Dickinson, U.S.A.) in 1 McFarland scale by adjusting the optical density to 1 at 600 nm wavelength, and 100 μL was dispensed in the wells. Sequentially, the wells received the CJP extract serially diluted in final concentrations ranging from 1 mg/mL to 7.8 $\mu\text{g/mL}$, creating a final concentration of the bacterial inoculum equal to 0.5 McFarland scale. Plates were then incubated at 37°C overnight. A 0.1% resazurine solution was used for staining procedures. MIC was established as the lowest concentration in which resazurine staining had a negative result (no color modification from blue to pink) in all strains. The CJP extract was used as a negative control for resazurine staining (blue color indicated no bacterial growth, and pink color indicated bacterial survival). This assay was performed in triplicate.

2.11. Minimum bactericidal concentration assay

The minimum bactericidal concentration (MBC) of the CJP extract was determined in triplicate using the CLSI method [14]. Aliquots of 100 μL of each well in which resazurine staining result was negative (indicating bacterial death) were dispensed in Mueller–Hinton agar (Difco) plates and inoculated through spread plate technique. The CJP extract was used as a negative control. All plates were incubated overnight

at 37°C, and bacterial growth was observed. MBC was established as the lowest concentration that yielded no bacterial growth of all strains in agar plates.

2.12. Biofilm eradication

Biofilm formation was conducted in untreated sterile 96-well polystyrene microtiter plates as described [16], using aliquots (200 µL) of overnight bacterial cultures standardized at 0.5 McFarland scale turbidity and fresh Brain Heart Infusion broth as negative control. The minimal biofilm eradication concentration (MBEC) assay was carried out as follows. Aliquots of 100 µL of each CJP extract concentration were prepared in phosphate-buffered saline buffer and added in triplicate for each strain. Plates were then incubated overnight at 37°C. Then, 0.1% resazurine staining solution was used as described above. MBEC was established as the lowest concentration in which resazurine staining showed no color modification in all strains. This assay was performed in triplicate.

2.13. Interference of CJP on antimicrobial drugs

The possible interference of CJP extract on antimicrobial drugs was assessed in duplicate using the method standardized by our group [17]. Antimicrobial disks (10 µg meropenem, 10 µg ampicillin, 10 µg gentamicin, and 30 µg chloramphenicol; all were obtained from Sensifar, São Paulo, Brazil) were distributed in Mueller–Hinton agar (Difco) plates for performing an antimicrobial susceptibility assay [15]. Then, briefly, 10 µL of the CJP extract in the MBC was dispensed in each disk. Plates were incubated overnight at 37°C, and the inhibition zone mean diameter was compared with control plates (disks free of CJP extract). Synergism was considered if the inhibition zone mean diameter was at least 2 mm larger than the control, and antagonism was considered if the inhibition zone mean diameter was at least 2 mm shorter than the control, with statistical significance. If the inhibition zone mean diameters were larger or shorter than the control but no statistically significant difference was seen, this was described as tendency of synergism or antagonism [17].

2.14. Statistics

Mean and standard error were computed for triplicate determination. The data for pH, TSS (°Brix), TTA, ascorbic acid, as well as for CJP interference on antimicrobial drugs, was analyzed using Assistat for Windows. CJP interference on antimicrobial drugs was analyzed using Kruskal–Wallis and post hoc Student–Newman–Keuls test. Results were considered significant if $p < 0.05$.

3. Results

3.1. Physicochemical and vitamin C analyses

Regarding all tested parameters, only pH and TSS were out of the legislation permitted range (Table 1). TTA values are also

Table 1 – Physicochemical parameters and vitamin C levels.

Parameter	CJP analysis ^a	Reference value ^b
TSS	7 °Brix	10 °Brix
TTA	1.441 g/100 g	0.3 g/100 g
Vitamin C (1) ^c	1.9 mg/g	0.8 mg/g
Vitamin C (2) ^d	1.0 mg/g	
pH	2.8	4.6 ^e

CJP = cashew juice pulp; TSS = total soluble solids; TTA = total titratable acidity.

^a Average of results in triplicate.

^b Minimum reference values.

^c Vitamin C level when the fruit pulp was opened.

^d Vitamin C level 7 days after the fruit pulp was opened.

^e Limit reference value.

high, suggesting that nonmature cashew apples were used for the preparation of the frozen pulp [1]. Vitamin C levels were within the levels required by legislation both by the time of examination after opening the pulps, and after 7 days.

3.2. HPLC and GC-MS analyses

HPLC analysis confirmed the presence of phenolic compounds in the sample, as peaks were detected at 240 and 254 nm (Figures 1A and 1B). We did not perform further analysis to identify the polyphenols detected in this assays, because we aimed to provide evidence that may help to explain the antioxidant and antimicrobial activities observed in this study.

The results obtained with GC-MS analysis indicated the lack of free carbohydrates in the pulps. Soluble sugar content varies considerably within and among species depending on age, maturity, and environmental conditions. Immature fruits usually present this characteristic [1].

3.3. Microbiological analyses

The microbial quality of CJP was assessed using standard methods. No bacterial or fungal growth was detected in our assays. It is possible that the low pH values have influenced this result, once the environment was very acidic, and thus not favorable for microbial survival and multiplication.

3.4. Antimicrobial activity assays

To investigate the antimicrobial and antibiofilm potentials of the CJP extract, microdilution methods were used with overnight cultures of clinical isolates of *S. aureus* strains. The MIC value was lower than the MBC, and the MBEC was four times higher than the MBC (Table 2). Because of the difference in MIC and MBC values, we suggest that the CJP extract presented a bacteriostatic profile [18].

3.5. β-Carotene antioxidant assay

The antioxidant potential of CJP was superior to that of the control in all tested times (Figure 2). A decay of 47% on the antioxidant effect was observed at 30 minutes, 57% at 45

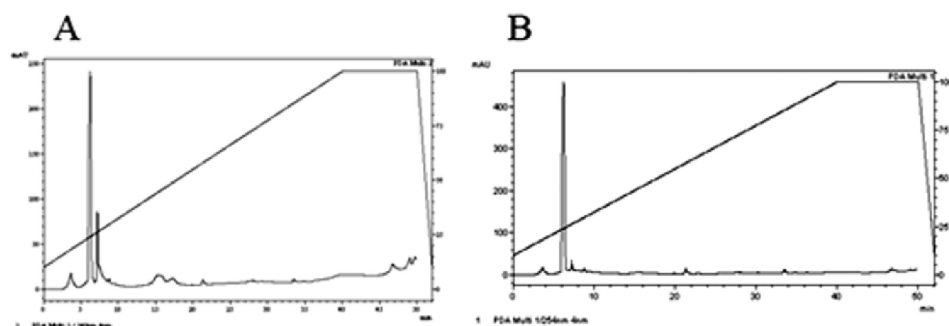


Figure 1 – High performance liquid chromatography (HPLC) chromatograms: (A) 240 nm; (B) 254 nm.

minutes, and 86% at 90 minutes. All the observations were statistically significant when compared to the control ($p < 0.05$).

3.6. Interference of CJP on the activity of antimicrobial drugs

CJP was prepared in its MBC concentration for this assay. The tested combinations resulted in statistically significant antagonism for meropenem, ampicillin, and gentamicin

($p < 0.05$, Table 3). When combined with chloramphenicol, the interference of the CJP extract was not statistically significant.

4. Discussion

Fruits are important components in human and animal nutrition; however, they are perishable and of variable availability, because of environmental influences associated to shifts of seasons [1]. Storage of fruits has limitations, because they are perishable and deterioration by microbial and environmental factors has a strong influence in shelf life. Thus, the preparation of stable pulps becomes an important way to improve the safe consumption of fruit juices at low costs [19].

4.1. Physicochemical analyses

Although our pulp samples were purchased locally, the selected brand is amidst the ones with the highest national sales. The results of physicochemical analysis suggested that the fruits used for preparation of the pulps we analyzed were immature. For instance, the pH of the pulps was lower than the limit established by legislation. This could be attributed to the action of citric acid, as indicated by the high values of TTA, which were superior to the legislation limits. Evaluation of pH in foods is important as it influences palatability and indicates possible use of fruits that are not in their best state for pulp preparation [20]. Although fungal species may grow at low pH, most known bacteria require neutral pH environment to grow. In this study, neither bacteria nor fungi could be detected by cultivation-dependent methods, and beyond the interference of the pH, the presence of polyphenols, indicated by HPLC, may have contributed to this result, as many molecules of this chemical group can present antimicrobial activity [16].

The TSS level of CJP was lower than the rate established by legislation, what also suggests that the fruits were used at an immature stage for pulp preparation [1]. This might result in poor palatability of the juice prepared with such pulps, mostly because of the high concentration of tannins. Adequate levels of TSS in fruit pulps, and the stability of °Brix values as time flows, indicate the maturity of fruits used for pulp preparation. Considering that sugars are the main constituents of TSS, it is possible that the lack of sugars detected in CJPs can be attributed to the stage of maturation of the fruits, or to the

Table 2 – Antimicrobial activity of the CJP extract.

Parameter	Results (µg/mL)
MIC	15.6
MBC	125
MBEC	500

Results are related to all tested strains.
CJP = cashew juice pulp; MBC = minimum bactericidal concentration; MBEC = minimum biofilm eradication concentration; MIC = minimum inhibitory concentration.

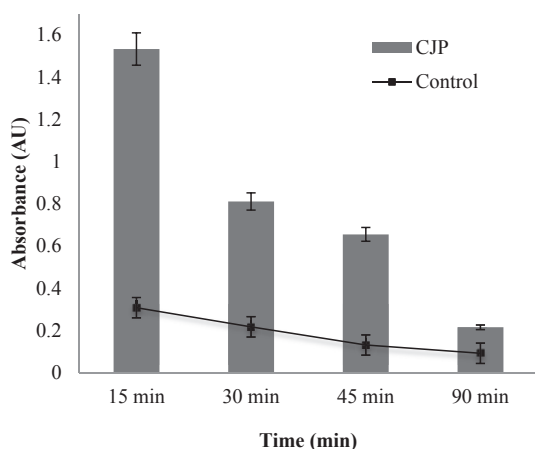


Figure 2 – Antioxidant assay. Absorbance reads are presented for each time for CJP and control, with standard deviation bars. AU = arbitrary units; CJP = cashew juice pulp.

Table 3 – Interference of CJP in the activity of different antimicrobial drugs.

Isolate	MER	MER + CJP	AMP	AMP + CJP	CHLOR	CHLOR + CJP	GEN	GEN + CJP
<i>S. aureus</i> 1	22	28 ^a	11	0 ^b	23	20 ^c	14	0 ^b
<i>S. aureus</i> 2	22	17 ^b	10	0 ^b	21	21	18	15 ^b
<i>S. aureus</i> 3	25	20 ^b	10	0 ^b	22	16 ^c	15	0 ^b
<i>S. aureus</i> 4	21	15 ^b	7	0 ^b	19	16 ^c	16	13 ^b
<i>S. aureus</i> 5	26	21 ^b	11	0 ^b	23	17 ^c	15	12 ^b
<i>S. aureus</i> 6	22	26 ^a	8	0 ^b	21	17 ^c	20	23 ^a
<i>S. aureus</i> 7	23	15 ^b	10	0 ^b	22	19 ^c	16	13 ^b
<i>S. aureus</i> 8	23	16 ^b	10	0 ^b	21	18 ^c	18	0 ^b
<i>S. aureus</i> 9	25	0 ^b	11	0 ^b	20	17 ^c	16	0 ^b
<i>S. aureus</i> 10	21	14 ^b	12	0 ^b	18	16 ^c	16	0 ^b

AMP = ampicillin; CHLOR = chloramphenicol; GEN = gentamicin; MER = meropenem; +CJP = addition of the cashew apple fruit pulp extract to the antimicrobial disk in its minimum bactericidal concentration.

^a Synergism tendency (no statistical significance).

^b Statistically significant antagonism.

^c Antagonism tendency (no statistical significance).

utilization of sugars by fermenting organisms [20]. This observation was further confirmed by GC-MS analysis, in which free carbohydrates were not detected.

4.2. Chemical analyses

In this study, the presence of polyphenols on CJP was detected by HPLC analysis, and the presence of vitamin C was confirmed by iodine titration. Vitamin C levels remained beyond the minimum values required by Brazilian law [21], even 7 days after the packages were opened. The antioxidant potential of CJP can be attributed mostly to the presence of both compounds. There are evidences that a high intake of foods rich in natural antioxidants including vitamins and phenolic compounds such as flavonoids, is an effective way of reducing risks of diseases such as cancers and stroke [22]. Flavonoids are the most studied natural phenolic compounds and have been detected in extracts prepared with different parts of the cashew tree [16,17,23]. Flavonoids from the cashew pulp presented higher antioxidant activity when compared to extracts from other edible and nonedible parts of the cashew tree [23].

4.3. Antimicrobial and antibiofilm potentials of CJP

The antimicrobial activity of the CJP extract against all tested clinical isolates of *S. aureus* was observed at 15.6 µg/mL. For the first time, we describe that CJP may eradicate biofilms of *S. aureus* strains at 500 µg/mL. Most staphylococcal diseases are related to biofilm formation; thus, investigations on the antibiofilm potential of antimicrobial candidates such as the CJP extract are relevant. We do recognize that the MIC, MBC and MBEC values we observed (Table 2) are somehow high for natural products. Possible explanations for this observation include the efficiency of the extraction of antimicrobial phytochemicals present in CJP. Moreover, tests were conducted with a crude extract; thus, some interference on the antimicrobial activity due to the interactions of multiple phytochemicals is expected [24]. Finally, the presence of nutritive compounds in CJP may have supported bacterial growth and impaired the antimicrobial activity of the extract [25].

Nevertheless, the CJP extract was more potent than the extracts obtained from parts of the cashew tree. A previous work of our group demonstrated that MIC of the stem bark extract for *S. aureus* strains was of 15 mg/mL, and biofilm eradication was achieved with 30 mg/mL [16]. Vivek et al [26] also observed that the cashew apple juice is effective against *S. aureus* and *Streptococcus mutans* strains, as inhibition zones were observed in an agar diffusion test. Curiously, the concentration in which this effect was observed is not mentioned in their work. Moreover, different organic fractions of the cashew leaves extract were effective for *S. aureus* at concentrations ranging from 0.313 mg/mL to 1.25 mg/mL [27]. Thus, the CJP extract seems to be a better option for prospecting antimicrobial compounds against *S. aureus* strains when compared to extracts obtained from nonedible parts of the cashew tree.

4.4. Interference of CJP on antimicrobial drugs

As the CJP extract presented antimicrobial activity, and polyphenols were detected in the pulp, we hypothesized that the CJP extract would present synergism when interacting with synthetic antimicrobial drugs with different mechanisms of action. Such interactions studies are important considering that the administration of oral dosage forms is often performed by patients using juices, mainly to make them more palatable [28], what can influence therapeutic outcomes [29].

We detected antagonistic interactions when CJP was combined with the tested drugs, and no statistically significant synergic effect was observed in these strains. It is possible that the variety of chemical compounds in the CJP extract have impaired the activity of antimicrobial drugs, as drug complexation may happen with phytochemicals. One might ask if such interactions might be influencing disk diffusion. This hypothesis was disregarded as some synergism tendency was detected in this study (Table 3) and in other studies developed by our group using this methodology [16,22,30].

Interestingly, the cashew tree stem bark extract also presented antagonistic interactions when combined with synthetic drugs [17]. Our group provided evidence of the antagonistic effects of flavonoids combined with

antimicrobial drugs against *S. aureus* and *E. coli* strains [22]. However, we also detected synergism when flavonoids were combined with antimicrobials against *P. aeruginosa* [30].

5. Conclusion

Antimicrobial, antibiofilm, and antioxidant activities of CJP were demonstrated, and antagonistic interactions were detected when it was combined to antimicrobial drugs. Further *in vivo* studies are necessary to completely demonstrate that this combination can influence antimicrobial efficacy as it is administered orally, given the poor representativeness of *in vitro* models concerning hepatic metabolism and the immune system behavior. For safety reasons, pharmaceutical manufacturers and prescribers should keep advising patients to administer oral medication with water, to avoid possible and generally hardly predictable side effects, due to drug–phytomolecules interactions. Studies involving a reasonable ingestion of CJP are important to assess this hypothesis.

Conflicts of interest

The authors declare that they have no competing interests

Acknowledgments

We are thankful to Dr Alexandre Sylvio for providing the refractometer used in this study. RMDS is currently a recipient of a Masters of Science scholarship from CNPq, and MVDS is supported by grants from FAPEMIG.

REFERENCES

- [1] Marçal PHF, Dias-Souza MV, Villela EG, Luz LM, Pereira MK, Vale CHB, Rabelo FLA. Qualidade Físico-Química, Microbiológica e Microscópica de Polpas de Frutas Congeladas Comercializadas no Município de Governador Valadares, MG. *Nova Rev Cienc* 2014;2:1–10.
- [2] Deenanath ED, Rumbold K, Daramola M, Falcon R, Iyuke S. Evaluation of physicochemical properties of South African cashew apple juice as a biofuel feedstock. *Scientifica* 2015;2015:764196.
- [3] Assunção RB, Mercadante AZ. Carotenoids and ascorbic acid from cashew apple (*Anacardium occidentale* L.): variety and geographic effects. *Food Chem* 2003;81:495–502.
- [4] Ojewole JA. Potentiation of the antiinflammatory effect of *Anacardium occidentale* (Linn.) stem–bark aqueous extract by grapefruit juice. *Methods Find Exp Clin Pharmacol* 2004;26:183–8.
- [5] Melo AFM, Santos EJV, Souza LFC, Carvalho AAT, Pereira MSV, Higino JS. Atividade antimicrobiana *in vitro* do extrato de *Anacardium occidentale* L. sobre espécies de *Streptococcus*. *Rev Bras Farmacogn* 2006;16:202–5.
- [6] Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. *Nat Rev Microbiol* 2015;13:529–43.
- [7] Gnanadhas DP, Elango M, Janardhanraj S, Srinandan CS, Datey A, Strugnell RA, Gopalan J, Chakravorty D. Successful treatment of biofilm infections using shock waves combined with antibiotic therapy. *Sci Rep* 2015;10:17440.
- [8] Flemming HC, Wingender J. The biofilm matrix. *Nat Rev Microbiol* 2010;8:623–33.
- [9] Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Disc* 2015;14(2):111–29.
- [10] Food Chemicals Codex. Committee of Food Chemicals Codex, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. 4th ed. Washington, D.C.: National Academy Press; 1996. 882 pp.
- [11] Zabidah AA, Kong KW, Amin I. Antioxidant properties of tropical juices and their effects on *in vitro* hemoglobin and low density lipoprotein (LDL) oxidations. *Int Food Res J* 2011;18:549–56.
- [12] Monteiro A, Domingues VS, Dias-Souza MV, Lula I, Gonçalves DB, de Siqueira EP, Santos VL. Bioconversion of biodiesel refinery waste in the bioemulsifier by *Trichosporon mycotoxinivorans* CLA2. *Biotechnol Biofuels* 2012;5:29.
- [13] Pratt DE. Natural antioxidants of soybean and other oil-seeds. In: Simic MG, Karel M, editors. *Autoxidation in food and biological systems*. New York: Plenum Press; 1980. p. 283–92.
- [14] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard. 10th ed (M07–A10). Wayne, PA: CLSI; 2015.
- [15] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; Approved standard. 12th ed (M02–A12). Wayne, PA: CLSI; 2015.
- [16] Dias-Souza MV, Andrade S, Aguiar AP, Monteiro AS. Evaluation of Antimicrobial and Anti-biofilm activities of *Anacardium occidentale* stem bark extract. *J Nat Prod* 2013;26:198–205.
- [17] Dias-Souza MV, Caldoncelli JL, Monteiro AS. *Anacardium occidentale* stem bark extract can decrease the efficacy of antimicrobial drugs. *J Med Biol Sci* 2013;12:161–5.
- [18] Hafidh RR, Abdulamir AS, Vern LS, Abu-Bakar F, Abas F, Jahanshiri F, Sekawi Z. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *Open Microbiol J* 2011;5:96–106.
- [19] Talasila U, Vechalapu RR, Shaik KB. Clarification, preservation, and shelf life evaluation of cashew apple juice. *Food Sci Biotechnol* 2012;21:709–14.
- [20] Bueno SM, Lopes MRV, Graciano RAS, Fernandes ECB, Garcia-Cruz CH. Avaliação da qualidade de polpas de frutas congeladas. *Rev Inst Adolfo Lutz* 2002;62:121–6.
- [21] BRASIL, 2000. Instrução normativa do Ministério da Agricultura n. 1, de 7 de janeiro de 2000. Regulamento técnico geral para fixação dos padrões de identidade e qualidade para polpa de fruta. *Diário Oficial da União, Brasília, DF*, 10 jan. Seção I.
- [22] Dos Santos RM, Pimenta G, Dias-Souza MV. Carotenoids and flavonoids can impair the effectiveness of some antimicrobial drugs against clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. *Int Food Res J* 2015;5:1777–82.
- [23] Broinizi PRB, Andrade-Wartha ERS, Silva AMO, Novoa AJV, Torres RP, Azeredo HMC, Alves RE, Mancini-Filho J. Avaliação da atividade antioxidante dos compostos fenólicos naturalmente presentes em subprodutos do pseudofruto de caju (*Anacardium occidentale* L.). *Food Sci Technol* 2007;27:902–8.
- [24] Stefanović OD, Tešić JD, Čomić LR. *Melilotus albus* and *Dorycnium herbaceum* extracts as source of phenolic compounds and their antimicrobial, antibiofilm, and antioxidant potentials. *J Food Drug Anal* 2015;23:417–24.

- [25] Joshi A, Prasad SK, Joshi VK, Hemalatha S. Phytochemical standardization, antioxidant, and antibacterial evaluations of *Leea macrophylla*: a wild edible plant. *J Food Drug Anal* 2016;24:324–31.
- [26] Vivek MN, Manasa M, Pallavi S, Sachidananda-Swamy HC, Prashith-Kekuda TR. Antibacterial potential of cashew apple (*Anacardium occidentale* L.) juice against clinical isolates of *Staphylococcus aureus* and *Streptococcus mutans*. *Sci Tech Arts Res J* 2013;2:144–6.
- [27] Chabi KS, Sina H, Adoukonou-Sagbadja H, Ahoton LE, Roko GO, Saidou A, Adéoti K, Ahanchede A, Baba-Moussa L. Antimicrobial activity of *Anacardium occidentale* L. leaves and barks extracts on pathogenic bacteria. *Afr J Microbiol Res* 2014;8:2458–68.
- [28] Cabaleiro J. Flavoring meds for children and adults so it goes down easy! *Home Healthc Nurse* 2003;21:295–8.
- [29] Matsui D. Assessing the palatability of medications in children. *Paediatr Perinatal Drug Ther* 2007;8:55–60.
- [30] Dos Santos RM, Pimenta G, Figueiredo FJB, Dias-Souza MV. Interference of flavonoids and carotenoids on the antimicrobial activity of some drugs against clinical isolates of *Pseudomonas aeruginosa*. *Int Food Res J* 2016;23(3):1268–73.