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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



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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.

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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]. Antiproliferative, 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

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