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Efficacy of *Caryocar brasiliense* Camb. and *Annona crassiflora* Mart. leaves in the reduction of biotransferred *Escherichia coli* from *Lactuca sativa* L. leaves to polypropylene

Eficácia de extratos de folhas de *Caryocar brasiliense* Camb. e *Annona crassiflora* Mart. na redução de *Escherichia coli* biotransferida de folhas de *Lactuca sativa* L. para polipropileno

Eficacia de hojas de *Caryocar brasiliense* Camb. y *Annona crassiflora* Mart. en la reducción de *Escherichia coli* biotransferida de hojas de *Lactuca sativa* L. a polipropileno

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Abstract
The antimicrobial activity of *Caryocar brasiliense* and *Annona crassiflora* leaves extracts was evaluated against *Escherichia coli* strains through a sensitivity test by the agar diffusion method and by the evaluation of minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) by the microdilution technique followed by plating. The sanitizing efficiency of these extracts in reducing *E. coli* cells biotransferred from *L. sativa* leaves to polypropylene surfaces was evaluated after an exposure time of 5 minutes. All strains were sensitive to antibacterials and the formation of an inhibition halo was observed for the extracts against all strains tested. The *C. brasiliense* extract showed better antimicrobial action against *E. coli* strains, with a MIC of 1.09 mg/mL, while the MIC of *A. crassiflora* extract was 5.58 mg/mL. No MBC was found for plant extracts. The strains were not able to form biofilms under the conditions studied, however, there was biotransfer and adhesion of *E. coli* to polypropylene. The highest counts of *E. coli* cells biotransferred and, consequently, adhere to the polypropylene coupons were observed when 5 log CFU/mL was inoculated, with a mean count of 4.53 ± 0.66 log CFU/cm^2. It was verified that the treatment with the extract solutions in the minimum inhibitory concentrations (MIC) totally reduced the number of *E. coli* cells adhered to the polypropylene coupons. The results obtained indicate that the use of extracts from both species as antibacterials is promising.
Keywords: Bacterial adhesion; Biofilms; Cross contamination; Sanitization.

Resumo
A atividade antimicrobiana de extratos de folhas de *Caryocar brasiliense* e *Annona crassiflora* foi avaliada frente cepas de *Escherichia coli* através de teste de sensibilidade pelo método de difusão em ágar e pela avaliação das concentrações inibitórias mínimas (CIM) e as concentrações bactericidas mínimas (CBM) pela técnica de

microdiluição seguida de plaqueamento. A eficiência sanitizante desses extratos na redução de células de *E. coli* biotransferidas de folhas de *Lactuca sativa* para superfícies de polipropileno, foi avaliada após o tempo de exposição de 5 minutos. Todas as cepas foram sensíveis aos antibacterianos e observou-se a formação de halo de inibição para os extratos em estudo frente a todas as cepas testadas. O extrato de *C. brasiliense* apresentou melhor ação antimicrobiana frente às cepas de *E. coli*, com CIM de 1,09 mg/mL, enquanto que a CIM do extrato de *A. crassiflora* foi de 5,58 mg/mL. Não foi encontrada CBM para os extratos vegetais. As estirpes não foram capazes de formar biofilmes nas condições estudadas, no entanto, houve biotransferência e adesão de *E. coli* ao polipropileno. As maiores contagens de células de *E. coli* biotransferidas e, consequentemente, aderidas aos cupons de polipropileno foram observadas quando se inoculou 5 log UFC/mL com contagem média de 4,53 ± 0,66 log UFC/cm^2. Verificou-se que o tratamento com as soluções dos extratos nas concentrações inibitórias mínimas (CIM), reduziu totalmente o número de células de *E. coli* aderidas nos cupons de polipropileno. Os resultados obtidos indicam que a utilização dos extratos de ambas as espécies como antibacterianos é promissora.
Palavras-chave: Adesão bacteriana; Biofilmes; Contaminação cruzada; Sanitização.

Resumen
La actividad antimicrobiana de los extractos de hojas de *Caryocar brasiliense* y *Annona crassiflora* se evaluó frente a cepas de *Escherichia coli* mediante una prueba de sensibilidad por el método de difusión en agar y mediante la evaluación de concentraciones mínimas inhibitorias (CMI) y concentraciones mínimas bactericidas (CMB) mediante la técnica de microdilución seguida de enchapado. La eficacia higienizante de estos extractos para reducir la biotransferencia de células de *E. coli* de hojas de *L. sativa* a superficies de polipropileno se evaluó después de un tiempo de exposición de 5 minutos. Todas las cepas fueron sensibles a los antibacterianos y se observó la formación de un halo de inhibición para los extractos en estudio frente a todas las cepas probadas. El extracto de *C. brasiliense* mostró una mejor acción antimicrobiana contra las cepas de *E. coli*, con una CMI de 1.09 mg/mL, mientras que la CMI del extracto de *A. crassiflora* fue de 5.58 mg/mL. No se encontró CMB para extractos de plantas. Las cepas no pudieron formar biopelículas en las condiciones estudiadas, sin embargo, hubo biotransferencia y adhesión de *E. coli* al polipropileno. Los recuentos más altos de células de *E. coli* biotransferidas y, en consecuencia, adheridas a los cupones de polipropileno se observaron cuando se inocularon 5 log UFC/mL con un recuento medio de 4,53 ± 0,66 log UFC/cm^2. Se verificó que el tratamiento con las soluciones de extracto en las concentraciones mínimas inhibitorias (CMI), redujo totalmente el número de células de *E. coli* adheridas a los cupones de polipropileno. Los resultados obtenidos indican que el uso de extractos de ambas especies como antibacterianos es prometedor.
Palabras clave: Adhesión bacteriana; Biopelículas; Contaminación cruzada; Higienización.

1. Introduction

The formation of biofilms on food contact surfaces represents one of the main problems in food processing because has a significant impact on operational efficiency, overall product quality, and safety in food production (Franco et al., 2021). Biofilms are communities of microorganisms adhered to a surface and each other, which are embedded in a protective extracellular matrix, produced by the microorganisms themselves (Kannan et al., 2017). When there is deficient cleaning of the surfaces of equipment and utensils, without total removal of organic and inorganic residues, the adhesion of microorganisms and the formation of biofilms are favored. Biofilms, in addition to reducing the efficiency and useful life of equipment, are more resistant to the disinfection process and can cause cross-contamination and transmission of foodborne pathogens (Galié et al., 2018; Koo et al., 2013; Kregiel, 2014; Sanchez-Vizuete et al., 2015).

Escherichia coli is one of the most important pathogenic bacteria capable of forming biofilms. It was demonstrated that *E. coli* strains can adhere and form biofilms on a variety of surfaces, including stainless steel, polystyrene, and polypropylene (Nahar et al., 2021; Vidács et al., 2018; Weerarathne et al., 2021). This bacterium is part of the intestinal microbiota of humans and warm-blooded animals, where it does not represent a health problem. However, some *E. coli* strains are pathogenic and the consumption of contaminated food is one of the main ways of dissemination of these strains (Galié et al., 2018).

Lactuca sativa L. (lettuce) is one of the most common food contaminated by *Escherichia coli* (Feltes et al., 2017). Fresh products such as *L. sativa* can be contaminated as a result of the use of contaminated water for irrigation, application of contaminated organic fertilizer, and due to inadequate conditions of handling, washing, processing, transport, and packaging (Ceuppens et al., 2014; Luna-Guevara et al., 2019).

Biofilms are more resistant to antimicrobial products compared to planktonic cells, making their removal from surfaces a major challenge (Flemming et al., 2016). In addition, bacteria can develop resistance to the action of disinfectants over time, interfering with the minimal bactericidal efficiency of these products (Carlie et al., 2020). In this context, an alternative for the food industries is natural products with antimicrobial properties, such as essential oils and plant extracts, which seem to be a promising alternative (Calo et al., 2015; Gonelimali et al., 2018). By using natural agents, the generation of harmful effects to health by toxic products can be avoided, and the demand for effective but non-toxic and ecological products can be attended (Galié et al., 2018; Vidács et al., 2018).

Based on this, the Brazilian Cerrado can be considered a granary of plant species for these types of studies, being the second-largest plant formation in Brazil (Del-claro & Torezan-silingardi, 2019). Among the native plant species of the Cerrado, "pequi" (*Caryocar brasiliense* Camb. - Caryocaraceae) and "panã" (*Annona crassiflora* Mart. - Annonaceae) stand out, with some studies showing the antimicrobial action of extracts from these species (Paula-Junior et al., 2006; Ribeiro et al., 2018).

In this context, the study aimed to evaluate the biofilm formation capacity of *Escherichia coli* strains biotransferred from *Lactuca sativa* leaves to polypropylene surfaces and to verify the antimicrobial activity and sanitizing efficiency of ethanol extracts from leaves of *Caryocar brasiliense* and *Annona crassiflora*.

2. Material and Methods

2.1 Microorganisms

The antibacterial effects of the extracts were evaluated with *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 8739 strains and a polyurethane sponge isolated strain used in a cafe located in Montes Claros, MG.

To identify the isolated strain, proteomic analysis was performed following the standard extraction protocol adapted from Freiwald e Sauer (2009). A pure bacterial culture loop was resuspended in 1.2 mL of a 75% (v/v) ethanol solution. The sample was centrifuged and the supernatant removed. 50 µL of acetonitrile, formic acid, and water (50:35:15 v/v) were added to the formed pellet, which was vortexed for 1 min for cell extraction. Another centrifugation was performed and 0.3 µL of supernatant was deposited in a three-well plate and dried at room temperature. 0.3 µL of a saturated solution of alpha-cyano-4-hydroxycyanhydric acid, acetonitrile, water, and TFA (50:47:5:2.5 v/v) was then added. Matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF MS) analysis was performed according to Dušková *et al.* (2012), using Microflex TM MALDI-TOF MS (BrukerDaltonics, Billerica, Massachusetts, USA). The identifications were expressed by BioTyper log (scores), indicating the similarity of the unknown strain by MALDI TOF MS with the profile available in databases.

2.2 Obtention of the extracts

Leaves of *C. brasiliense* and *A. crassiflora*, with deposit numbers 338 and 1492, respectively, in the herbarium of the State University of Montes Claros, were collected at the Instituto de Ciências Agrárias of UFMG in Montes Claros, Minas Gerais. This region is located approximately at 16º51' of latitude and 44º55' of longitude, and the climate is tropical and humid with dry summers according to Köppen classification (Alvares et al., 2014).

The leaves were dehydrated under a forced air circulation oven at 38 ºC for 72 h, crushed in an industrial blender, and stored in an opaque package. Ethanol extracts were obtained by adding 100 g of plant material to 1000 mL of ethanol PA. This mixture was stored in an amber bottle for ten days and stored in a dry, light-free place at room temperature. After this period, the extracts were filtered (funnel with gauze and cotton), dehydrated in an oven with forced air circulation at 40 °C for

approximately three days, and stored at 4 °C until the time of analysis (Morais-costa et al., 2016). To perform the analyses, the extracts were diluted in distilled water and filtered through a 0.2 µm millipore cellulose membrane. Aliquots of the obtained solutions were subjected to dry matter determination to calculate the concentrations to be tested (Cunnif, 1995).

2.3 Antibacterial susceptibility

Antimicrobial susceptibility testing was performed according to NCCLS (2003) and conducted in triplicate. For *E. coli* strains the following antimicrobial discs were added to the surface of the medium: chloramphenicol 30 µg, gentamicin 10 µg, ciprofloxacin 5 µg, sulfazotrim 25 µg, and norfloxacin 10 µg. In addition, extracts discs were tested together with the antimicrobial discs. For this, filter paper discs with 5 mm of diameter were autoclaved and impregnated with 20 µL of the standardized extracts at concentrations of 27.36 mg/mL for the *C. brasiliense* extract and 27.92 mg/mL for the *A. crassiflora* extract. The plates were incubated at 35 ºC for 24 hours, and the diameters of the inhibition zones were measured in millimeters (mm).

2.4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Antibacterial activity was determined in triplicate using the microdilution method (CLSI, 2011). *E. coli* strains were cultivated in plates containing Mueller Hinton Agar (HIMEDIA®, Mumbai, India) and incubated at 37 °C for 24 h. A loopful of bacteria was inoculated onto 5.0 mL of sterile NaCl 0.85% (m/v). Turbidity equivalent to a 0.5 McFarland standard was used as a reference to adjust for approximately 10^6 colony-forming units (CFU)/mL.

For the Minimum inhibitory concentration (MIC) determination, in 96-well microdilution plate, 80 µL of Mueller Hinton Broth (HIMEDIA®, Mumbai, India) were mixed to 20 µL of extracts (27.36 to 0.22 mg/mL for *C. brasiliense* and 27.92 to 0.22 mg/mL for *A. crassiflora*), and 100 µL of bacterial suspensions (10^6 CFU/mL). For the controls, 100 µL of bacterial suspensions were added to 80 µL of Mueller-Hinton broth. The plates were incubated at 37 °C for 18 h. After this period, bacterial growth was evaluated using 100 µL of 1.0% triphenyl tetrazolium chloride (TTC) solution (m/v), which indicates cellular multiplication by the development of reddish color (Klančnik et al., 2010). For the minimum bactericidal concentration (MBC) determination, 100 microliters aliquots from the wells where there was no visible bacterial growth in the MIC test, were transferred to plates containing MacConkey Agar (HIMEDIA®, Mumbai, India), and incubated at 35 °C for 24 h to observe microbial growth.

2.5 Biotransfer of *E. coli* to polypropylene coupons

Samples of crisp *L. sativa*, variety Vitória de Santo Antão, commercially obtained in Montes Claros-MG and stored at 7 ºC ± 2 ºC, were used. Initially, a microbiological exclusion test was performed on the *L. sativa* e samples to verify the absence of contamination by *E. coli*, using the most probable number method (MPN) (Kornacki & Johnson, 2001). Before the biotransfer study, *L. sativa* leaves were sanitized according to the procedures described by Lima et al., (2013). Then, under aseptic conditions, *L. sativa* leaves were cut into coupons measuring 2 x 2 cm. Polypropylene coupons (2.0 cm x 2.0 cm x 0.2 cm) were sanitized at 25 ºC ± 2 ºC with potable water and neutral detergent. Subsequently, they were rinsed with distilled water and sanitized with 70% ethyl alcohol (v/v). The coupons were oven-dried at 60 °C for 2 h and sterilized at 121 °C for 15 min (Rossoni & Gaylarde, 2000). To assess the biotransfer potential, *L. sativa* e coupons contaminated with inoculums of Log 2 CFU/mL or Log 5 CFU/mL of each strain were placed in contact with polypropylene coupons for 24 h at 7 ºC ± 2 ºC. Subsequently, the number of transferred cells was quantified in MacConkey Agar, and the results were expressed in CFU/cm², according to Careli et al., (2009).

2.6 Sanitizing efficiency of extracts

After 24 hours of biotransfer and bacterial adhesion, the coupons with cells adhered to polypropylene were subjected to the following treatments: immersion in solutions of *C. brasiliense* and *A. crassiflora* extracts at concentrations of 1.09 mg/mL and 5 .58 mg/mL, respectively, and sterile distilled water control solution. For comparison, a 200 mg/L (v/v) sodium hypochlorite solution was used, as it is the most used commercial product for reducing the microbiota of food processing surfaces. With the aid of sterilized tweezers, the coupons were immersed separately in 0.85 % NaCl (m/v) to remove planktonic cells (weakly adhered). Then, they were immersed in the tested solutions, and the sanitizing action was evaluated after 5 min of contact under static conditions at a temperature of 25 ± 2 °C. The coupons from each treatment were transferred to 10 mL of 0.85% saline solution (m/v) and sonicated for 2 min using an ultrasound bath (QUIMIS®, Q335D) at 40 kHz to remove adhered cells surviving in the coupon surfaces (Malheiros et al., 2010). Successive serial decimal dilutions were performed, with inoculation on plates containing MacConkey Agar and incubation at 37 °C for 24 h. The results were expressed in CFU/cm^2, according to Careli et al., (2009).

2.7 Characterization of extracts

The chromatographic analysis of plant extracts of *C. brasiliense* and *A. crassiflora* was performed by HPLC (High-Performance Liquid Chromatography) in Merck-Hitachi equipment (Germany) comprising an L-6200A pump, AS-2000A automatic injector, UV-VIS L-4250 detector, and D-2500 integrator. An ODS column (250 x 4.0 mm dia.5 mm, Merck, Germany) was used, flow 1.0 mL/min, temperature 40 °C, eluting with a linear gradient of water (A) and acetonitrile (B): 0 min 90% A, 10% B; 60 min 10% A, 90% B, followed by 5 min of isocratic elution. Detection was carried out in UV at 261.5 nm. HPLC grade solvents (Merck, Germany) were used, and air removal was performed by sonication. For the analysis, the samples were dissolved in HPLC grade methanol at concentrations of 10 mg/mL and 5 mg/mL, and the solutions were centrifuged at 10.000 rpm for 10 min before injection. Aliquots of these solutions (5 µL) were automatically injected.

2.8 Statistical analysis

All analyzes were performed in three repetitions, and results were expressed as the mean ± standard deviation. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's test using Sisvar software. A value of $p<0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Characterization of bacterial isolate

The isolated strain under study was identified by proteomic analysis as *Escherichia coli* with 99.9% similarity with the profile available in the database.

3.2 Antibacterial activity of Cerrado plants extracts

Escherichia coli ATCC 25922, *E. coli* ATCC 8739 strains, and the isolate were sensitive to all antibacterials tested (Table 1). Regarding the antimicrobial properties of the ethanol extracts from the leaves of *C. brasiliense* and A. crassiflora, the results showed that the extracts efficiently suppress the growth of *E. coli* strains. The formation of inhibition halos was detected for the two plant species under study against all strains tested (Table 2). There were no significant differences ($p > 0.05$) for the action of *C. brasiliense* extract between the strains under study. However, for the *A. crassiflora* extract, there was a lower antibacterial effect ($p < 0.05$) against *E. coli* ATCC 25922 (Table 2).

Table 1. Inhibition halos (mm) of *Escherichia coli* in antibiogram test.

Antibacterials	*E. coli* ATCC 25922	*E. coli* ATCC 8739	Isolated *E. coli*
Chloramphenicol 30 µg	27.0 ± 1.0	30.0 ± 0.0	25.0 ± 0.0
Gentamicin 10 µg	20.0 ± 0.0	28.5 ± 1.5	22.0 ± 0.0
Ciprofloxacin 5 µg	36.5 ± 0.5	40.0 ± 0.0	32.5 ± 2.5
Sulfazotrim 25 µg	24.5 ± 0.5	36.0 ± 1.0	24.0 ± 1.0
Norfloxacin 10 µg	36.5 ± 0.5	36.5 ± 0.5	32.5 ± 2.5

Values described are mean ± standard deviation. Source: Authors (2021).

Table 2. Inhibition halos (mm) of *Escherichia coli* after addition of discs containing plant extracts of *C. brasiliense* and *A. crassiflora* at concentrations of 27.36 mg/mL and 27.92 mg/mL, respectively.

Extract	*E. coli* ATCC 25922	*E. coli* ATCC 8739	Isolated *E. coli*
Caryocar brasiliense	10.0 ± 0.0[a]	8.5 ± 0.5[a]	8.0 ± 1.0[a]
Annona crassiflora	7.0 ± 0.0[b]	9.5 ± 0.5[a]	10.0 ± 0.0[a]

Values described are mean ± standard deviation. Means in the same line not sharing the same letter are significantly different ($p < 0.05$) by Tukey test. Source: Authors (2021).

The inhibitory effect of extracts from *C. brasiliense* and *A. crassiflora* leaves was verified in other studies. For the *C. brasiliense* extract at concentrations of 1.5 and 2 mg/mL, lower than in this work, mean halos of 7.0 and 8.0 mm were obtained respectively for the *E. coli* ATCC 25922 strain (Paula-Junior et al., 2006). For extracts of *C. brasiliense* and *A. crassiflora* leaves at a concentration of 84 mg/mL, higher inhibition halos than in this study were found, with values of 11.3 mm and 10.1 mm, respectively (Ribeiro et al., 2018).

On the other hand, in a study with *C. brasiliense* peel extract on *E. coli* ATCC 25753 at concentrations of 200 to 500 mg/mL, no inhibition halo was obtained. The minor effect or absence of antibacterial activity of the extracts may be due to the concentration of the extract, leaves quality that can be altered by soil conditions, seasonality, type of harvest and actives compounds content, or even the lower sensitivity of the studied microorganisms (Pinho et al., 2012).

The extract of *C. brasiliense* showed the best antimicrobial action potential against the *E. coli* strains, with a MIC value of 1.09 mg/mL for the three strains under study. The MIC of the *A. crassiflora* extract was 5.58 mg/mL. Although both plant species have bacteriostatic action, no bactericidal action was detected for any of the extracts at the concentrations evaluated in this study.

The bacteriostatic action of extracts from these plant species was reported in other studies against *E. coli* ATCC 25922. For the ethanol extract of *C. brasiliense*, values of MIC of 25 mg/mL and 0.27 mg/mL were found, and for the extract of *A. crassiflora* of 6.24 mg/mL (Pinho et al., 2012; Ribeiro et al., 2018). Despite the absence of bactericidal action of the extracts evaluated in this study, Ribeiro et al., (2018) reported MBC values of 30 mg/mL and 6.24 mg/mL for ethanolic extracts of *C. brasiliense* and *A. crassiflora*.

3.3 Biotransfer of *E. coli* from *L. sativa* leaves to polypropylene

Regarding the study of biotransfer, it was observed that the concentration of the initial inoculum contributed to a significant difference ($p < 0.05$) in the number of cells transferred from the *L. sativa* coupons to the polypropylene surface. When the inoculum was 2 log CFU/mL, there was no biotransfer of *E. coli* to polypropylene, regardless of the strain evaluated.

When a count of 5 log CFU/mL was inoculated into the *L. sativa* coupons, there was biotransfer and, consequently, adhesion of *E. coli* cells to the polypropylene coupons, with a mean count of 4.53 ± 0.66 log CFU/cm^2. According to Andrade, Bridgeman e Zottola (1998), to consider a biofilm, a minimum number of 7 log CFU/cm^2 of surface is necessary. Thus, it was found that the strains were not able to form biofilms under the conditions studied.

Handling or storing contaminated *L. sativa* in containers and surfaces, such as polypropylene, followed by any insufficient decontamination process can contribute to the occurrence of cross-contamination and transmission of pathogens. Although the formation of biofilms by *E. coli* strains has not been verified, the adhesion process that occurred can not be underestimated, due to the fact that adhered bacteria are more resistant to sanitizers than those in suspension.

The results obtained in this work corroborate those described by Rocha et al. (2014), who evaluated the adhesion of *E. coli* ATCC 8739 for 12 h on a polypropylene surface used for food cutting. These authors did not observe the formation of biofilm by the evaluated strain, only the adhesion process with mean counts of 5 log CFU/cm^2.

3.4 Sanitizing efficiency of plant extracts

The sanitizing effectiveness of the extracts was evaluated by quantifying viable cells after sanitization. It was found that the treatments with the solutions of plant extracts of *C. brasiliense* and *A. crassiflora* were 100% effective in reducing *E. coli* cells adhered to polypropylene coupons for all strains under study (Table 3).

Table 3. Number of cells (log CFU/cm^2) of *E. coli* strains, on polypropylene surfaces, after treatments with hypochlorite solution and sanitizing solutions containing plant extracts for an exposure time of 5 min at 23 ± 2 °C

Treatments	Estirpes		
	E. coli ATCC 25922	*E. coli* ATCC 8739	Isolated *E. coli*
Control	3.77^A	4.82^A	4.99^A
Hypochlorite 200 ppm	0.00^B	0.00^B	0.00^B
C. brasiliense (1.09 mg/mL)	0.00^B	0.00^B	0.00^B
A. crassiflora (5.58 mg/mL)	0.00^B	0.00^B	0.00^B
CV (%)	5.63	5.16	8.42

CV: coefficient of variation. Means in the same column not sharing the same letter are significantly different ($p < 0.05$) by Tukey test. Source: Authors (2021).

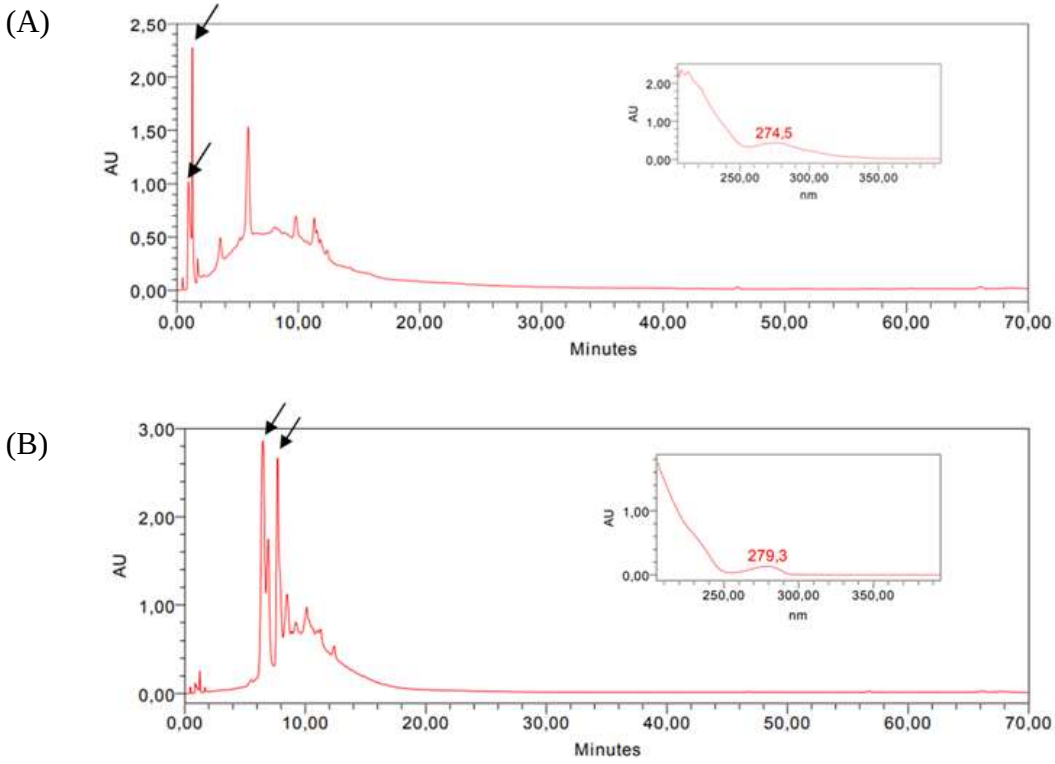
It was also observed that there were no significant differences ($p > 0.05$) between the treatment of polypropylene coupons with plant extracts compared to the conventional treatment with 200 mg/L (v/v) sodium hypochlorite solution (Table 3). Thus, the time of 5 minutes of contact with the sanitizing solutions at the concentrations evaluated was sufficient for the total removal of adhered cells from the *E. coli* strains on the polypropylene coupons. The use of *C. brasiliense* and *A. crassiflora* extracts in the sanitization of surfaces used in food processing has not yet been reported. However, with this work, it was possible to observe a mean reduction of 4.53 ± 0.66 logarithmic cycles in *E. coli* sessile cell counts in polypropylene coupons.

3.5 Reversed-phase HPLC characterization of plant extracts

The antimicrobial effects observed for the extracts can be associated with their chemical composition. According to the observed UV spectra, the presence of flavonoids was detected in the region between 274.5 and 279.3 nm for the *C.*

brasiliense and *A. crassiflora* ethanol extracts (Figure 1). Flavonoids show intense UV absorption, exhibiting two bands: band I (320-385 nm) and band II (250-285 nm) (Wang et al., 2019). The antimicrobial effect presented by the extracts of *C. brasiliense* and *A. crassiflora* may be related to the flavonoids present in their composition. In fact, previous studies have already shown that flavonoids have antibacterial properties against pathogenic microorganisms (Adamczak, 2020; Górniak et al., 2018).

Figure 1. HPLC chromatographic profile, characteristics of the UV spectrum of flavonoids (peaks) and their respective retention times (RT). (A) - *Caryocar brasiliense* ethanolic extract (RT = 1.284 and UV = 274.5) and (B) - *Annona crassiflora* ethanolic extract (first RT = 6.484 and UV = 279.3)



Source: Authors (2021).

Although the analyzes realized in this study do not show the concentration or chemical structure of the metabolites present, other studies have already identified the presence of tannins and other phenolic compounds in the leaves of the studied species. For *A. crassiflora* species, the presence of the flavonoids epicatechin and quercetin, and tannins were detected (Machado et al., 2015; Oliveira et al., 2018; Ribeiro et al., 2018). In the species *C. brasiliense*, phytochemical tests were performed that identified the presence of flavonoids (quercetin, myricetin 3-O-hexoside, isoquercitrin) and tannins (Lopes et al., 2011; Morais et al., 2020; Ribeiro et al., 2018).

One of the main compounds associated with the antimicrobial activity of plant extracts has been the tannins, with studies reporting that the removal of tannins suppressed the antimicrobial activity of extracts (Djipa et al., 2000; Ribeiro et al., 2018). Ribeiro *et al.* (2018) reported that there was no antimicrobial activity of the extracts after the removal of tannins, indicating that this metabolite, acting alone or in association with other plant compounds, would be the main component with antibacterial action for the extracts of *C. brasiliense*, *A. crassiflora* and *Schinopsis brasiliensis*.

4. Conclusion

E. coli strains were sensitive to *C. brasiliense* and *A. crassiflora* ethanol extracts, showing halos of inhibition and MIC. The strains were not able to form biofilms under the conditions studied. However, the capacity for biotransfer followed by adhesion of *E. coli* strains on the polypropylene coupons surface was observed. The sanitizing solutions formulated with plant extracts were efficient in removing the cells present on the surfaces of the coupons. The results obtained from this study indicate that the use of plant extracts of *C. brasiliense* and *A. crassiflora* as antibacterials is promising. However, it is important to realize future studies on the mechanisms of action and toxicity of these extracts for safe and effective application in the sanitization of surfaces used in food processing.

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