Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Effect of *Mauritia flexuosa* L. leaf extract on *Staphylococcus aureus* and *Staphylococcus haemolyticus* biofilms adhered to stainless steel surface

Efeito do extrato de folhas de *Mauritia flexuosa* L. sobre biofilmes de *Staphylococcus aureus* e *Staphylococcus haemolyticus* aderidos a superfície de aço inoxidável

E. G. A. Mariano[a], A. G. F. Michel[a], F. Morais-Costa[a], B. S. O. Conceição[a], F. O. Silvério[a], S. R. Arrudas[b], Y. R. F. Nunes[b], M. S. Pinto[a], R. T. Careli[a] and E. R. Duarte[a*]

[a]Universidade Federal de Minas Gerais – UFMG, Instituto de Ciências Agrárias, Montes Claros, MG, Brasil
[b]Universidade Estadual de Montes Claros – UNIMONTES, Montes Claros, MG, Brasil

Abstract

Staphylococcus spp. represents the main mastitis agents in ruminants and contaminants of milk due to their expressive capacity to make biofilms. The aims in this study was evaluate evaluated the antimicrobial activity of *Mauritia flexuosa* L. extracts against *Staphylococcus* spp. adhered to a stainless steel surface. Two isolates from cows with clinical mastitis were evaluated; one was identified as *Staphylococcus aureus*, and the other *Staphylococcus haemolyticus*. Additionally the ATCC 25923 strain, *S. aureus* from human was evaluated. The chemical profile obtained from gas chromatography revealed the presence of carbohydrates, organic acids, and flavonoids. The minimum bactericidal concentrations of the ethanolic extract (EE) and aqueous extract (AE) were 4.4 and 5.82 mg/mL, respectively. After EE treatment at 4.4 mg/mL for 2.5 min, total removal of mature biofilms grown on stainless steel coupons was observed (reduction by 3.85-4.81 log units). This extract from *M. flexuosa* shows potential as an effective sanitizer and may represent a natural alternative against *Staphylococcus* spp.

Keywords: bovine mastitis, Arecaceae, bacterial adhesion, natural bactericide, alternative sanitizer.

Resumo

Bactérias do gênero *Staphylococcus* spp. são os principais agentes da mastite em ruminantes e contaminantes do leite devido à expressiva capacidade de formação de biofilmes. Neste estudo o objetivo foi avaliar a atividade antimicrobiana de extratos de *Mauritia flexuosa* L. (Buritizeiro) contra *Staphylococcus* spp. aderidos à superfície de aço inoxidável. Foram avaliados dois isolados de vacas com mastite clínica; o um isolado foi identificado como *Staphylococcus aureus* e o outro como *Staphylococcus haemolyticus*. Adicionalmente foi também avaliada a e a cepa *S. aureus* ATCC 25923 de origem humana. O perfil químico obtido por cromatografia gasosa revelou a presença de carboidratos, ácidos orgânicos e flavonóides. As concentrações bactericidas mínimas do extrato etanólico (EE) e do extrato aquoso (AE) foram 4,4 e 5,82 mg / mL, respectivamente. Após o tratamento com EE a 4,4 mg / mL por 2,5 min, foi observada remoção total de biofilmes maduros cultivados em cupons de aço inoxidável (redução de 3,85-4,81 unidades log). O EE de folhas de *M. flexuosa* apresenta potencial como um desinfetante eficaz e pode representar uma alternativa natural contra *Staphylococcus* spp.

Palavras-chave: mastite bovina, Arecaceae, adesão bacteriana, bactericida natural, desinfetante alternativo.

1. Introduction

Staphylococcus aureus is the most important mastitis pathogen and may form biofilms on surfaces used in the collection, production, and storage of milk (Saidi et al., 2013; Sampimon et al., 2011). The incorrect and indiscriminate use of antimicrobial agents in animals has selected multi-resistant bacteria (Moritz and Moritz, 2016), as evidenced in *Staphylococcus* spp. strains isolated from cows (Liu et al., 2017; Wang et al., 2013; Kalayu et al., 2020). Livestock-associated *S. aureus* showing this multi-resistance in people with occupational contact with these cattle has represented an important public health risks (Verkade and Kluytmans 2014).

Additionally, stainless steel surfaces used in food storage and processing equipment represent important sources of microbial contamination (Cabeça et al., 2006). In particular, contamination with biofilm-forming bacteria poses a risk to the consumer´s health, as well as causing damage to the industry by deterioration of foods containing milk (Chmielewski and Frank, 2003).

*e-mail: duartevet@hotmail.com
Received: April 17, 2022 – Accepted: November 10, 2022

The ineffectiveness of disinfectants and antibiotics has been frequently related to bacterial biofilms (Mafu et al., 2011), because the biofilms cells are up to 1,000 times more resistant to these antimicrobial agents (Nader et al., 2014). Due to the difficulties in reducing bacteria in biofilms, new control strategies have been evaluated (Donlan, 2002). Studies have shown the potential of some plant metabolites, such as flavonoids, to reduce biofilm formation (Bazargani and Rohloff, 2016; Borges et al., 2014) by breaking the outer membrane and inhibiting bacterial enzymatic activity (Nascimento et al., 2000).

Plants are reservoir of potentially useful biomolecules with unique properties which make them attractive candidates for the development of novel nature antimicrobial agents (Evans, 2022). *Mauritia flexuosa* L. (Arecaceae), known as "buriti", is a palm that can reach up to 40 m in height. Native to the Amazon region (Nascimento, 2010), it grows in areas flooded with moist savannas from the central west of Brazil and in the north and northeast regions (Ferreira, 2005). In a previous study, the stem extract of this plant showed an inhibitory effect on the growth of methicillan-resistant strains of *S. aureus* (Siqueira et al., 2014). However, the effects of leaf extracts on the inhibition of bacterial growth and the reduction of mature bacterial biofilms are unknown. In this study, we investigated the bactericidal effects of leaf extracts from this plant on *Staphylococcus* spp. and its activity against mature biofilms on stainless steel surfaces.

2. Materials and Methods

2.1. Preparation of aqueous and ethanolic extracts

Leaves of *M. flexuosa* were collected in the Vereda Água Doce, North of Minas Gerais, Brazil (15°13'30" S 44°55'04" W) in October 2016. The climate of this region is tropical wet with dry summer (As) according to the Köppen classification (Alvares et al., 2013), marked by a dry season from May to October and a rainy period in December, January, and February. After discarding damaged leaves, the selected specimens were washed in running water and dehydrated in a forced air circulation stove at 40±5°C for 72 h (TE 394/4, Tecnal Equipamentos Científicos Tecnal, Piracicaba, SP, Brazil). Dried leaves were ground in a Wiley mill (CE- 430/Macro, Cienlab, SP, Brazil) and stored in paper bags in the dark. Plant samples were deposited in the Montes Claros Herbarium of Universidade Estadual de Montes Claros, as voucher specimen 5.777.

Aqueous extracts (AEs) were produced by placing the ground dried leaves in a distilled water bath at 40°C for 60 min. Ethanolic extracts (EEs) were obtained from macerated dried leaves suspended in absolute ethanol in amber-colored glass containers in the dark for seven days. Extracts were filtered through a gauze funnel, evaporated at 40°C for 48 h under forced air circulation until completely dry, and stored at 4°C until use (Morais-Costa et al., 2016). In this study, both EEs and AEs were completely soluble in distilled water and did not require any other solvents for antimicrobial analysis.

2.2. Characterization of the extracts

For derivatization, aliquots (1.0 mg) of the plant extracts were weighed in an internally conical glass and then dissolved in 60 µL of pyridine and 100 µL of BSTFA (N,O-bis(trimethylsilyl)-trifluoroacetamide) containing 1% chlorotrimethylsilane. The reaction mixture was heated at 60°C for 30 min.

The derivatized extracts were analyzed with a gas chromatograph (Agilent Technologies, GC 7890A) equipped with an electron impact ionization detector (CG-EM) and a DB-5MS capillary column (Agilent Technologies, 30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). Helium (99.9999% purity) was used as carrier gas at the rate of 1 mL/min. Using a self-injector (CTC combiPaL), 1 µL of the solution was injected into the chromatograph at a 1:10 split ratio. The split/splitless injector was maintained at 290°C. The chromatographic column, initially maintained at an 80°C isotherm for 5 min, was heated at a rate of 4°C/min at 260°C for 10 min. After compound separation, the temperature was raised to 300°C, and maintained for 2 min (post-run). The interface temperature was maintained at 280°C, and the ionization was performed at 70 eV. The scanning range of m/z was from 30 to 600 Da, and all the procedures were done in triplicate.

2.3. Bacteria strains

The antibacterial effects of the plant extracts were evaluated against *Staphylococcus aureus* ATCC 25923 (human strain, resistant to penicillin, clindamycin, oxacillin and vancomycin) and a milk-isolated strain from cows with mastitis (SA 178), from northern Minas Gerais, Brazil (resistant to the above antibiotics in addition to tetracycline and erythromycin). In addition, an isolate of *Staphylococcus haemolyticus* (SH182) from a cow with mastitis was evaluated and was found to be sensitive to these six antimicrobials (Ribeiro et al., 2018). All bacteria were cultured in Brain Heart Infusion broth, and subsamples were stored at −80°C in glycerol (1:1). DNA from these bovine isolates was extracted and then amplified via polymerase chain reaction (PCR) using primers 27F (5′-AGAGTTTGATCCTGGC TCAG-3′) and 1492R (5′-GGTTACCTTGTTACGA CTT-3′). The 16S ribosomal RNA (rRNA) was sequenced in an automatic sequencer (Mega-BACE® 1000, GE Life Sciences, USA), and the results were analyzed using the SeqScanner Software® v1.0 (Applied Biosystems, USA) and compared online using the BLAST database (BLAST, 2022). The bacterial species were identified with a similarity level of ≥ 99% (Ribeiro et al., 2018).

2.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

After filtration of the extracts, we determined the MIC necessary to inhibit the growth of the microorganism (McFarland turbidity standard No. 0.5; approximately 1.3×10^8 colony-forming units (CFU)/mL) by macro-dilution in Mueller-Hinton broth, as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). Extract solutions were prepared at the double final concentration (NCCLS, 2003; Balouiri et al., 2016) and then

diluted to a final volume of 5 mL with an equal volume of medium. The final concentrations were 5.82, 2.91, 1.46, 0.73 and 0.34 mg/mL for the AE and 4.4, 2.2, 1.1, 0.55 and 0.275 mg/mL for the EE. From these bacterial inocula, 120 µL were added to 2.48 mL Mueller Hinto broth and 2.5 mL of extract solution. For the controls, we used growth control tubes containing broth without extract for each bacterium tested and tubes without bacteria containing broth alone or broth with extract additions. All tubes were incubated at 35°C for 24 h in a thermo-shaker incubator (Novatécnica, São Paulo, SP, Brazil) to ensure homogenization. After this period, bacterial growth was assessed by adding 125 µL 0.5% triphenyl tetrazolium chloride (TTC) solution. This reagent indicates cell multiplication by developing of a reddish color, thus enabling MIC determination (Klancnik et al., 2010).

For MBC determination, 100 µL aliquots from the dilution tubes of the MIC assay and the control without extracts were inoculated on Mueller-Hinton agar and incubated at 37°C for 24 h. In addition, the absence of bacterial growth on the agar plates was checked to determine the MBCs. All these procedures were carried out in triplicate.

2.5. Bacterial biofilm formation and milk biotransference

Stainless steel coupons (AISI 304 #4) used in the food industry with dimensions of 2.0 cm × 2.0 cm × 0.1 cm were sanitized and sterilized, according to Rossoni and Gaylarde (2000). For the experimental system of biofilm formation, 1 mL of 5 log CFU/mL of each bacterial strain was inoculated in a sterile glass jar containing 99 mL of skimmed UHT milk and four stainless steel coupons. These materials were maintained at 28°C for 24 h under constant agitation on an orbital shaker at 60 rpm to simulate the agitation of the milk in the expansion tank. The coupons were then rinsed with distilled water and transferred to a new sample of sterilized skim milk without inoculation. This new system was maintained under agitation at 60 rpm for a further 24 h, totaling 48 h of bacterial adhesion.

To evaluate the biotransfer potential of the adhered cells from the coupons to the non-inoculated skim milk, aliquots of 1 mL were withdrawn and subjected to successive serial decimal dilutions, followed by plating on Mannitol Agar Salt at 35 ± 2 °C for 24 h (Careli et al., 2009). All procedures were carried out in four repetitions, and the results were expressed in units of CFU/mL.

For quantification of the adhered bacterial cells, the coupons were removed, rinsed for 1 min in 10 mL of 0.85% w/v NaCl sterile solution to remove planktonic cells, transferred to another 10 mL of 0.85% w/v NaCl solution, and placed in an ultrasonic water bath for 2 min to detach sessile cells. Decimal dilutions of each sample were performed by plating on mannitol salt agar at 35 ± 2 °C for 24 h. The procedures were carried out in five repetitions, and the results were expressed in units of CFU/cm^2.

2.6. Sanitizing activity of extracts on bacterial biofilms

The coupons coated with the mature biofilms were rinsed with 0.85% w/v sterile NaCl solution and then transferred to the sanitizing solution containing the EE and AE at the MICs determined from the previous tests. Sterilized distilled water was used as a control. The contact times of the coupons in the solutions containing the extracts and control were 2.5, 5.0, and 10 min. Adhered bacterial cells were then removed and quantified following the procedures described by Careli et al. (2009). The samples were planted on Mannitol Salt Agar at 35°C for 48 h. The procedures were carried out in five repetitions, and the results were expressed in units of CFU/cm^2).

2.7 Statititcal analysis

To evaluate the biotransfer potential of the adhered cells from the coupons and quantification of the adhered bacterial cells, the results were transformed in Log (x+10) in entirely casualized designs considering the four repetitions. For analyses of the sanitizing activity of extracts on biofilms, the data of five repetitions were transformed in Log (x+10) and compared in a 3×3 factorial scheme (3 treatments and 3 times). The SAEG 9.0 statistical package was used to compare mean activities using Tukey's test at a significance level of 5%.

3. Results and Discussion

3.1. Chemical characterization of evaluated extracts

After analysis of phytochemical profiles by gas chromatography, 10 and 15 major compounds were detected within the EE and AE of *M. flexuosa* leaves, respectively (Table 1, Figure 1). The main active compounds of the EE, which was efficient in the total removal of mature biofilms of *Staphylococcus* spp., were the carbohydrates d-fructose (18.59%) and glycopyranoside (16.24%), which were present at a 2.5-fold higher concentration compared with the EA. Glycerol, hexadecanoic acid (C6), octadecanoic acid, and 12-hydroxyoctadecanoic acid were detected only in the EE and could be contributing to its antibiofilm action, which should be elucidated in future studies. Previous studies demonstrated that C6 kills *S. aureus* (Takigawa et al., 2005; Clarke et al., 2007). In other research, this acid showed high antibacterial activity at pH 5.5, with only 0.5% survival after 2 h of treatment with C6 at 5 µg/mL, thus exhibiting clear dose dependence (Cartron et al., 2014).

In other studies that evaluated alternative compounds for the control of biofilms, phenolic acids (gallic acid and ferulic acid), isothiocyanates, and 2-phenyl ethyl isothiocyanate present in plants have also shown the potential to reduce the formation of mature biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Borges et al., 2014, Bazargani and Rohloff, 2016).

The main compounds detected in the EE of leaves of *M. flexuosa* were carbohydrates (Table 1), which could also be involved in the observed antibiofilm activity on stainless steel. Sánchez et al. (2016) evaluated the methanol extracts from cladodes of *Opuntia ficus-indica* Mill. (Nopal cactus, Cactaceae) and leaves of *Prosopis laevigata* M.C. Johnston (Fabacea) against *S. aureus* and observed MBCs of 1.0 ± 0.2 and 0.7 ± 0.01 mg/mL, respectively. The specific biofilm formation index (SBF) was evaluated before and after adding plant extracts (MBC × 0.75). A major reduction in

Table 1. Main compounds identified by gas chromatography in leaf extracts from *Mauritia flexuosa* L. and their area (%) in the chromatographic profile.

Etanolic extract				Aqueous extract			
PN	**RT**	**Compounds**	**Area**	**PN**	**RT**	**Compounds**	**Area**
1	15.129	Glycerol	1.11	1	14.923	Phosphate	0.56
2	31.416	D-fructose	18.59	2	22.128	Malic acid	1.02
3	32.888	Carbohydrate N.I.	30.26	3	24.330	Thraionic acid	0.70
4	35.616	Isopropanol of glycopyranose	7.73	4	31.287	Carbohydrate N.I.	11.56
5	37.124	Hexadecanoic acid	0.93	5	31.449	Isocitric acid	2.06
6	38.008	Inositol isomer	2.92	6	31.067	D-fructose	6.55
7	41.548	Octadecanoico acid	0.23	7	32.457	Carbohydrate N.I.	13.71
8	45.487	12-hydroxyoctadecanoic acid	0.52	8	32.787	Glucitol	14.24
9	49.377	Glycopyranoside	16.84	9	34.158	D-mannitol	0.05
10	54.810	Catechin	0.22	10	35.529	Talose	8.83
				11	37.920	Inositol	10.34
				12	49.222	Glycopyranoside	6.48
				13	52.541	Carbohydrate N.I.	2.23
				14	51.545	2,5-Dihydroxybenzoic acid	0.42
				15	54.569	Catechin	0.39

Note: RT - Retention time (min.); N.I. - Not identified; PN - Peak number.

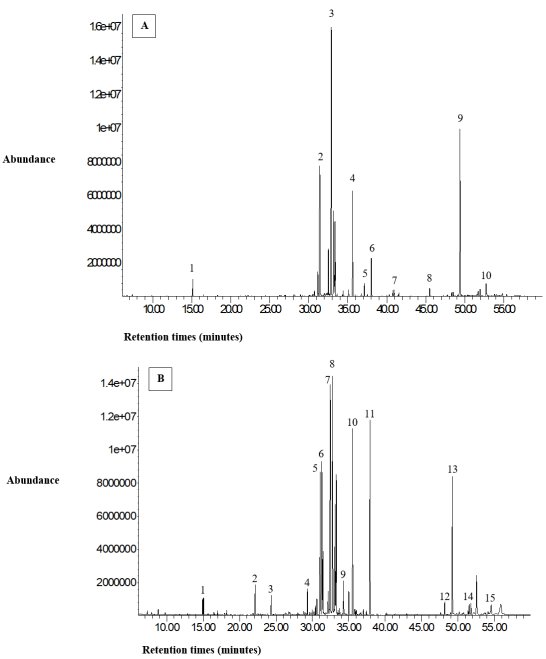

Figure 1. Gas chromatographic profile of extracts from Mauritia flexuosa L. leaves. (A) Ethanolic extract and (B) Aqueous extract. The peaks numbers correspond to the phenolic compounds and carbohydrates listed in Table 1.

SBF was observed in response to the *O. ficus-indica* extract, which also contained carbohydrates.

The antibacterial effect of *Berberis lycium* Royle root bark aqueous extract was evaluated and showed zones of inhibition at 10±1.5 mm *to S. aureu and* 13.3±0.8 mm to *Streptococcus pyogenes. the extrac was* effective against this bacterial pathogens and also had antioxidant potential (Mughal et al., 2024).

In another study, the inhibition of biofilm formation was explained by the presence of flavonoids such as quercetin, kaempferol, and naringenin, which could reduce biofilm synthesis by suppressing the activity of the autoinducer responsible for cell-to-cell communication (Vikram et al., 2010). Palm leaves represent an alternative, sustainable use of the native tropical forest, using extractive management to obtain economical, social, and environmental benefits, while respecting this ecosystem and its sustainability (Henderson et al., 2000).

In the present study was detected catechin in boths extracts. This substance is included a diverse group involved in plant interaction, These findings hightlight the potential use of the extract as a natural defensive agent (Rabaioli and Silva, 2016)

3.2. Minimum inhibitory concentration and minimum bactericidal concentration

The three bacterial strains showed a similar sensitivity profile to both extracts evaluated. The determined concentrations were 5.82 mg/mL (MIC and MBC) for the AE and 4.4 mg/mL (MIC and MBC) for the EE. These results corroborate those of Siqueira et al. (2014), who evaluated the inhibitory activity of hexane, dichloromethane, ethyl acetate and the AE from *M. flexuosa* against resistant or non-resistant strains of *S. aureus* to methicillin. Only the purified fraction of the dichloromethane extract from stems inhibited these bacterial strains, with a MIC of 31.3 µg/mL.

Other studies that evaluated plants from Cerrado reported higher MICs for *Staphylococcus* spp. than those reported in this study but did not detect MBCs. The EE of *Annona crassiflora* leaves showed an MIC of 25 mg/mL against multi-resistant *S. aureus* from humans, with alkaloids and flavonoids as the main active compounds in this extract (Silva et al., 2014). Amaral et al. (2014) reported an MIC of 11.25 mg/mL for the EE of the *Caryocar brasiliense* leaves against *S. aureus*.

3.3. Milk biotransfer and adhesion potential of the evaluated strains

Among the bacterial strains evaluated, ATCC 25923 presented a higher biotransfer potential in milk ($P < 0.05$). However, higher adhesion ability to stainless steel was detected for the bovine isolates in comparison with the ATCC 25923 strain ($P < 0.05$, Table 2). This observed milk biotransference is preoccupant since the concentrations of transferred bacterial cells are considered high, which compromises the safety of milk.

Concentrations of *Staphylococcus* spp. above 1.0×10^5 CFU/mL may promote enterotoxin production, constituting a risk to public health. Among coagulase-positive species, *S. aureus* is most frequently associated with food poisoning outbreaks due to its ability to produce different types of enterotoxins (Omoe et al., 2005). These results of biotransfer potential corroborated the study of Boari et al. (2009), which detected a concentration of *S. aureus* planktonic cells of 6.9 log CFU/mL in milk after two days of inoculation with 5 log CFU/mL.

Table 2. Potential of *Staphylococcus* spp. strains to milk transference and adhesion in stainless steel coupons (AISI 304 #4).

***Staphylococcus* spp. strains**	**Milk transference (Log UFC / mL)**	**Adhesion in stainless stell (Log UFC / cm²)**
S.A. ATCC 25923	7.07 ± 1.59 a	4.70 ± 0.47 b
SA. 178	5.98 ± 0.19 b	6.22 ± 0.49 a
SH. 182	5.75 ± 0.31 b	5.11 ± 0.98 a

Averages followed by the same letter in the column did not differ from each other by the Scott Knott test at 5% probability.

In this study, the highest adhesion potential of *Staphylococcus* spp. from cows with mastitis occurred possibly because of a better adaptation to bacterial growth using milk constituents since the ATCC 25923 strain is of human origin. Similar values were reported by Parizzi et al. (2004), who evaluated the adhesion of *S. aureus* ATCC 6538 to stainless steel coupons AISI 304, polycarbonate, and polypropylene.

According to Ronner and Wong (1993) > 5 log UFC/cm^{-2} may characterize mature biofilm formation in stainless steel. In this study, the two bacteria strains from cows were able to form biofilms on stainless steel surfaces, with values > 5 log of CFU/cm^2. This is a concerning result since the formation of these biofilms may hinder the procedures for cleaning equipment made of stainless steel. Furthermore, this bacterial concentration may also favor the production of milk-borne thermotolerant toxins, causing food poisoning in humans (Ciupescu et al., 2018).

3.4. Sanitizing activity of the extracts on the biofilms of Staphylococcus spp.

Considering the sanitizing effects of EEs and AEs for biofilm removal, we observed a significant interaction between treatment time and the extracts used (Table 3, $P < 0.01$). For the AE treatment, 7.5 min of contact promoted a greater reduction of adhered cells for both bacterial strains evaluated ($P < 0.05$) than other evaluated periods. Notably, a total reduction of the bacteria adhered to the

Table 3. Cell counts of *Staphylococcus* spp. adhered to a stainless steel surface and efficacy of reduction (%) after 2.5, 5 and 7.5 min contact with a control solution or aqueous (5.8 mg/mL) or ethanolic (4.4 mg/mL) extracts from *Mauritia flexuosa* L. leaves.

Strains/contact time (min.)	**Control (H_2O)**	**Aqueous extract**		**Etanolic extract**	
	Log UFC/cm^{-2}	**Log UFC/cm^{-2}**	**Efficacy (%)***	**Log UFC/cm^{-2}**	**Efficacy (%)***
Staphylococcus aureus ATCC 25923					
2.5 min	4.81 Aa	4.49 Aa	0.93	0.0	100.0
5.0 min	4.79 Aa	4.16 Ba	0.87	0.0	100.0
7.5 min	3.87 Ba	3.58 Cb	0.92	0.0	100.0
Staphylococcus aureus S.A. 178					
2.5 min	4.79 Aa	4.49 Aa	0.94	0.0	100.0
5.0 min	4.77 Aa	4.19 Ba	0.88	0.0	100.0
7.5 min	3.94 Ba	3.82 Cab	0.97	0.0	100.0
Staphylococcus haemoliticus S.H 182					
2.5 min	4.27 Ab	4.27Ab	1.00	0.0	100,0
5.0 min	4.23 Ab	4.15 Aa	0.98	0.0	100.0
7.5 min	3.85 Ba	3.72 Bab	0.97	0.0	100.0
CV (%)	1.17%	1.89%		0.00%	

Means followed by the same capital letter in the column and lowercase in the row do not differ by the Tukey test, at 5% probability. CV = Coefficient of variation %. *Efficacy = 1 - (log UFC / cm² of Extract / log UFC/cm² Control) x 100.

coupons was observed following the EE treatment, and no differences between the bacterial strains were detected ($P > 0.05$). Only 2.5 min of contact with this extract at 4.4 mg/mL was sufficient for the total removal of adhered bacterial cells from the stainless steel (Table 2).

According to the American Public Health Association (APHA, 2015), efficient sanitizing agents should eliminate all bacteria after 5 min of contact. Thus, the EE from leaves of *M. flexuosa* at 4.4 mg/mL could be an efficient sanitizing agent for the reduction of *Staphylococcus* spp. in mature biofilms on stainless steel.

In the scientific literature, there are no reports of efficient plant extracts with demonstrable sanitizing effects against mature biofilms. However, the anti-biofilm activity of essential oils from *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano), and *Origanum vulgare* (carvacrol) were also found to be effective. Concentrations between 0.05% and 0.1% were required to reduce 7 log CFU of *Salmonella typhimurim* biofilm to an undetectable level on polystyrene and stainless steel surfaces during 1 h of exposure to these extracts (Soni et al., 2013).

The ethanolic extract of *Asphodelus fistulosus* showed significant antimicrobial and antiprotozoal activities in studies carried out by Alam et al. (2018). The authors observed that metabolites, anthraquinones, flavonoids, terpenes and phenolics could be responsible for the such activity of this plant.

Considered as a potential source of substances of *Red propolis Alagoas* with antimicrobial Silva et al. (2019) reported the importance to identify and isolate the active compounds responsible for such activities it is necessary to carry out studies on the bioactive potential of this natural product,.

Studies that evaluated aqueous and ethanolic extracts of *Asphodelus fistulosus*, showed an inhibitory effect on the growth of *S. aureus* The ethanol extract is the most effective. Where it is also necessary to elucidate the components of the crude extract of *A. fistulosus* or the mechanism of how these constituents and these types of bacteria (Al-Qudah, 2022). The extrats of *M. flexuaosa* could be an alternative adjunct to other methods in the controle of bacterial biofilms of *Staphylococcus* spp. or other bacterias and it may be evaluated in futures studies.

In conclusion, the natural extracts from *Mauritia flexuosa* leaves are bactericides to *S. aureus and S. haemolyticus* isolates from cows with clinical mastitis. In addition, the ethanolic extract is more efficient than the aqueous extract, reducing mature biofilms on stainless steel after 2.5 min, indicating its potential as a natural sanitizer.

Acknowledgements

This study was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior fund. 0001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and Pró-reitoria de Pesquisa da Universidade Federal de Minas Gerais.

References

ALAM, M. M., NAZREEN, S. and AL-FAHAD, A. J., 2018. *In vitro* antioxidant, antimicrobial and antiprotozoal activities of ethanolic extract and its various fractions from asphodelus fistulosus seeds. *Asian Journal of Biological and Life Sciences*, 7(3), 81-86. http://dx.doi.org/10.5530/ajbls.2018.7.9.

AL-QUDAH, M.M.A., 2022. Antibacterial effect of *Asphodelus fistulosus* aqueous and ethanolic crude extracts on gram positive and gram negative bacteria. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, pp. e260029. http://dx.doi.org/10.1590/1519-6984.260029. PMid:35857941.

ALVARES, C.A., STAPE, J.L., SENTELHAS, P.C., DE MORAES GONÇALVES, J.L. and SPAROVEK, G., 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift (Berlin)*, vol. 22, no. 6, pp. 711-728. http://dx.doi.org/10.1127/0941-2948/2013/0507.

AMARAL, L.F.B., MORIEL, P., FOGLIO, M.A. and MAZZOLA, P.G., 2014. *Caryocar brasiliense* supercritical CO_2 extract possesses antimicrobial and antioxidant properties useful for personal care products. *BMC Complementary and Alternative Medicine*, vol. 14, pp. 73. http://dx.doi.org/10.1186/1472-6882-14-73. PMid:24565304.

AMERICAN PUBLIC HEALTH ASSOCIATION – APHA, 2015. *Compendium of methods for the microbiological examination of foods*. Washington: American Public Health Association.

BALOUIRI, M., SADIKI, M. and IBNSOUDA, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity. A review. *Journal of Pharmaceutical Analysis*, vol. 6, no. 2, pp. 71-79. http://dx.doi.org/10.1016/j.jpha.2015.11.005. PMid:29403965.

BASIC LOCAL ALIGNMENT SEARCH TOOL – BLAST, 2022 [viewed 16 April 2022]. *Basic Local Alignment Search Tool* [online]. Available from: https://blast.ncbi.nlm.nih.gov/Blast.cgi

BAZARGANI, M.M. and ROHLOFF, J., 2016. Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. *Food Control*, vol. 61, pp. 156-164. http://dx.doi.org/10.1016/j.foodcont.2015.09.036.

BOARI, C.A., ALVES, M.P., TEBALDI, V.M.R., SAVIAN, T.V. and PICCOLI, R.H., 2009. Formação de biofilme em aço inoxidável por Aeromonas hydrophila e Staphylococcus aureus usando leite e diferentes condições de cultivo. *Food Science and Technology (Campinas)*, vol. 29, no. 4, pp. 886-895. http://dx.doi.org/10.1590/S0101-20612009000400029.

BORGES, A., SAAVEDRA, M.J. and SIMÕES, M., 2014. Antibiofilm activity of plant molecules against *Pseudomonas aeruginosa* and *Staphylococcus aureus. Planta Medica*, vol. 80, no. 16, pp. 16. http://dx.doi.org/10.1055/s-0034-1394777.

CABEÇA, T.K., PIZZOLITTO, A.C. and PIZZOLITTO, E.L., 2006. Assessment of action of disinfectants against *Listeria monocytogenes* biofilms. *Brazilian Journal of Microbiology*, vol. 17, pp. 121-125.

CARELI, R.T., ANDRADE, N.J., SOARES, N.F., RIBEIRO JÚNIOR, J.I., ROSADO, M.S. and BERNARDES, P.C., 2009. The adherence of *Pseudomonas fluorescens* to marble, granite, synthetic polymers, and stainless steel. *Food Science and Technology (Campinas)*, vol. 29, no. 1, pp. 171-176. http://dx.doi.org/10.1590/S0101-20612009000100026.

CARTRON, M.L., ENGLAND, S.R., CHIRIAC, A.L., JOSTEN, M., TURNER, R., RAUTER, Y., HURD, A., SAHL, H.G., JONES, S. and FOSTER, S.J., 2014. Bactericidal activity of the human skin fatty acid cis-6-hexadecanoic acid on *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy*, vol. 58, no. 7, pp. 3599-3609. http://dx.doi.org/10.1128/AAC.01043-13. PMid:24709265.

CHMIELEWSKI, R.A.N. and FRANK, J.F., 2003. Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety*, vol. 2, no. 1, pp. 22-32. http://dx.doi.org/10.1111/j.1541-4337.2003.tb00012.x. PMid:33451238.

CIUPESCU, L.M., AUVRAY, F., NICORESCU, I.M., MEHEUT, T., CIUPESCU, V., LARDEUX, A.L., TANASUICA, R. and HENNEKINNE, J.A., 2018. Characterization of *Staphylococcus aureus* strains and evidence for the involvement of non-classical enterotoxin genes in food poisoning outbreaks. *FEMS Microbiology Letters*, vol. 365, no. 13, pp. 13. http://dx.doi.org/10.1093/femsle/fny139. PMid:29878105.

CLARKE, S.R., MOHAMED, R., BIAN, L., ROUTH, A.F., KOKAI-KUN, J.F., MOND, J.J., TARKOWSKI, A. and FOSTER, S.J., 2007. The *Staphylococcus aureus* surface protein IsdA mediates resistance to innate defenses of human skin. *Cell Host & Microbe*, vol. 1, no. 3, pp. 199-212. http://dx.doi.org/10.1016/j.chom.2007.04.005. PMid:18005699.

DONLAN, R.M., 2002. Biofilms, microbial life on surfaces. *Emerging Infectious Diseases*, vol. 8, no. 9, pp. 881-890. http://dx.doi.org/10.3201/eid0809.020063. PMid:12194761.

EVANS, W.C., 2022. *Trease and Evans' pharmacognosy*. Singapore: Souders Company.

FERREIRA, M.G.R., 2005 [viewed 25 January 2018]. *Buriti . Mauritia flexuosa L.* [online]. Rondônia: Centro de Pesquisa Agroflorestal de Rondônia Porto Velho. Available from: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/24785/1/folder-buriti.pdf

HENDERSON, A., FISCHER, B., SCARIOT, A., PACHECO, M.A.W. and PARDINI, R., 2000. Flowering phenology of a palm community in a central Amazon forest. *Brittonia*, vol. 52, no. 2, pp. 149-159. http://dx.doi.org/10.2307/2666506.

KALAYU, A.A., WOLDETSADIK, D.A., WOLDEAMANUEL, Y., WANG, S.H., GEBREYES, W.A. and TEFERI, T., 2020. Burden and antimicrobial resistance of S. aureus in dairy farms in Mekelle, Northern Ethiopia. *BMC Veterinary Research*, vol. 16, no. 1, pp. 20. http://dx.doi.org/10.1186/s12917-020-2235-8. PMid:31969151.

KLANCNIK, A., PISKERNIK, S., JERSEK, B. and MOZINA, S.S., 2010. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods*, vol. 81, no. 2, pp. 121-126. http://dx.doi.org/10.1016/j.mimet.2010.02.004. PMid:20171250.

LIU, H., LI, S., MENG, L., DONG, L., ZHAO, S., LAN, X., WANG, J. and ZHENG, N., 2017. Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China. *Journal of Dairy Science*, vol. 100, no. 11, pp. 8796-8803. http://dx.doi.org/10.3168/jds.2017-13370. PMid:28865851.

MAFU, A.A., PLUMETY, C., DESCHÊNES, L. and GOULET, J., 2011. Adhesion of pathogenic bacteria to food contact surfaces, Influence of pH of culture. *International Journal of Microbiology*, vol. 2011, pp. 972494. http://dx.doi.org/10.1155/2011/972494. PMid:20981289.

MORAIS-COSTA, F., BASTOS, G.A., SOARES, A.C.M., COSTA, E.G.L., VASCONCELOS, V.O., OLIVEIRA, N.J.F., BRAGA, F.C., DUARTE, E.R. and LIMA, W.S., 2016. *In vitro* and *in vivo* action of *Piptadenia viridiflora*. Kunth. Benth against *Haemonchus contortus* in sheep. *Veterinary Parasitology*, vol. 223, pp. 43-49. http://dx.doi.org/10.1016/j.vetpar.2016.04.002. PMid:27198776.

MORITZ, F. and MORITZ, C.M.F., 2016. Resistência aos antimicrobianos em *Staphylococcus* spp associados à mastite bovina. *Revista de Ciência Veterinária e Saúde Pública*, vol. 3, no. 2, pp. 132-136. http://dx.doi.org/10.4025/revcivet.v3i2.34435.

MUGHAL, T. A., ALI, S., HASSAN, A., KAZMI, S. A. R, SALEEM, M. Z., SHAKIR, H. A., NAZER, S., FAROOQ, M. A., AWAN, M. Z., KHAN, M. A., ANDLEE, S., MUMTAZ, S., MUMTAZ, S., TAHIR, H. M. and GULZAR, N., 2024. Phytochemical screening, antimicrobial activity, in vitro and in vivo antioxidant activity of Berberis lycium Royle root bark extract. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 84, e249742. https://doi.org/10.1590/1519-6984.249742.

NADER, T.T., COPPEDE, J.S., AMARAL, L.A. and PEREIRA, A.M.S., 2014. Atividade antibiofilme de diterpeno isolado de *Croton antisyphiliticus* frente *Staphylococcus aureus. Ars Veterinária*, vol. 30, no. 1, pp. 32-37. http://dx.doi.org/10.15361/2175-0106.2014v30n1p32-37.

NASCIMENTO, A.R.T., 2010. Riqueza e etnobotânica de palmeiras no território Indígena Krahô. *Floresta*, vol. 40, no. 1, pp. 209-220.

NASCIMENTO, G.G.F., LOCATELLI, J., FREITAS, P.C. and SILVA, G.L., 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, vol. 31, no. 4, pp. 247-256. http://dx.doi.org/10.1590/S1517-83822000000400003.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS – NCCLS, 2003 [viewed 10 may 2018]. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard* [online]. 6th ed. Wayne, Pennsylvania. NCCLS document M7-A6 NCCLS. Available from: https://www.anvisa.gov.br/servicosaude/manuais/clsi/clsi_opasm7_a6.pdf

OMOE, K., HU, D.L., TAKAHASHI-OMOE, H., NAKANE, A. and SHINAGAWA, K., 2005. Comprehensive analysis of classical and newly described staphylococcal super antigenic toxin genes in *Staphylococcus aureus* isolates. *FEMS Microbiology Letters*, vol. 246, no. 2, pp. 191-198. http://dx.doi.org/10.1016/j.femsle.2005.04.007. PMid:15899405.

PARIZZI, S.Q.F., ANDRADE, N.J., SILVA, C.A.S., SOARES, N.F.F. and SILVA, E.A.M., 2004. Bacterial adherence to different inert surfaces evaluated by epifluorescence microscopy and plate count method. *Brazilian Archives of Biology and Technology*, vol. 47, no. 1, pp. 77-83. http://dx.doi.org/10.1590/S1516-89132004000100011.

RABAIOLI, V. and SILVA, C.P., 2016. Prospecting of different species of plants with biopesticides action in the agriculture of Mato Grosso do Sul. *Ensaios e Ciência: ciências Biológicas. Agrárias e da Saúde*, vol. 20, no. 3, pp. 188-195.

RIBEIRO, I.C.O., MARIANO, E.G.A.M., CARELI, R.T., MORAIS-COSTA, F., SANT'ANNA, F.M., PINTO, M.S., DE SOUZA, M.R. and DUARTE, E.R., 2018. Plants of the Cerrado with antimicrobial effects against *Staphylococcus* spp and *Escherichia coli* from cattle. *BMC Veterinary Research*, vol. 14, no. 1, pp. 32. http://dx.doi.org/10.1186/s12917-018-1351-1. PMid:29382347.

RONNER, A.B. and WONG, A.C.L., 1993. Biofilm development and sanitizer inactivation of *Listeria monocytogenes* and *Salmonella Thyphimurium* on stainless steel and buna-n rubber. *Journal of Food Protection*, vol. 56, no. 9, pp. 750-758. http://dx.doi.org/10.4315/0362-028X-56.9.750. PMid:31113053.

ROSSONI, E.M. and GAYLARDE, C.C., 2000. Comparison of sodium hypochlorite and per acetic acid as sanitizing agents for stainless steel food processing surfaces using epifluorescence microscopy. *International Journal of Food Microbiology*, vol. 61, no. 1, pp. 81-85. http://dx.doi.org/10.1016/S0168-1605(00)00369-X. PMid:11028962.

SAIDI, R., KHELEF, D. and KAIDI, R., 2013. Bovine mastitis, prevalence of bacterial pathogens and evaluation of early screening test. *African Journal of Microbiological Research*, vol. 7, pp. 777-782.

SAMPIMON, O.C., LAM, T.J.G.M., MEVIUS, D.J., SCHUKKEN, Y.H. and ZADOKS, R.N., 2011. Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples.

Veterinary Microbiology, vol. 150, no. 1-2, pp. 173-179. http://dx.doi.org/10.1016/j.vetmic.2011.01.017. PMid:21333468.

SÁNCHEZ, E., RIVAS MORALES, C., CASTILLO, S., LEOS-RIVAS, C., GARCÍA-BECERRA, L. and ORTIZ MARTÍNEZ, D.M., 2016. Antibacterial and antibiofilm activity of methanolic plant extracts against nosocomial microorganisms. *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, pp. 1572697. http://dx.doi.org/10.1155/2016/1572697. PMid:27429633.

SILVA, F.R.G., MATIAS, T.M.S., SOUZA, L.I.O., MATOS-ROCHA, T.J., FONSECA, S.A., MOUSINHO, K.C. and SANTOS, A.F., 2019. Phytochemical screening and in vitro antibacterial, antifungal, antioxidant and antitumor activities of the red propolis Alagoas. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 79, no. 3, pp. 452-459. http://dx.doi.org/10.1590/1519-6984.182959. PMid:30379200.

SILVA, J.J., CERDEIRA, C.D., CHAVASCO, J.M., CINTRA, A.B.P., SILVA, C.B.P., MENDONÇA, A.N., ISHIKAWA, T., BORIOLLO, M.F.G. and CHAVASCO, J.K., 2014. *In vitro* screening antibacterial activity of *Bidens pilosa* Linné and *Annona crassiflora* Mart against oxacillin resistant *Staphylococcus aureus* (ORSA) from the aerial environment at the dental clinic. *Revista do Instituto de Medicina Tropical de São Paulo*, vol. 56, no. 4, pp. 333-340. http://dx.doi.org/10.1590/S0036-46652014000400011. PMid:25076435.

SIQUEIRA, E.P., ANDRADE, A.A., FAGUNDES, E.M.S., RAMOS, J.P., KOHLHOFF, M., NUNES, Y.R.F., VELOSO, M.D.M., CAMPOS, F.F., JOHANN, S., ALVES, T.M.A., ZANI, C.L. and COTA, B.B., 2014. *In vitro* antibacterial action on methicillin-susceptible. MSSA. and methicillin-resistant. MRSA. *Staphylococcus aureus* and antitumor potential of *Mauritia flexuosa* L F. *Journal of Medicinal Plants Research*, vol. 8, pp. 1408-1417.

SONI, K.A., OLADUNJOYE, A., NANNAPANENI, R., SCHILLING, M.W., SILVA, J.L., MIKEL, B. and BAILEY, R.H., 2013. Inhibition and inactivation of *Salmonella typhimurium* biofilms from polystyrene and stainless steel surfaces by essential oils and phenolic constituent carvacrol. *Journal of Food Protection*, vol. 76, no. 2, pp. 205-212. http://dx.doi.org/10.4315/0362-028X.JFP-12-196. PMid:23433366.

TAKIGAWA, H., NAKAGAWA, H., KUZUKAWA, M., MORI, H. and IMOKAWA, G., 2005. Deficient production of hexadecenoic acid in the skin is associated in part with the vulnerability of atopic dermatitis patients to colonization by *Staphylococcus aureus. Dermatology (Basel, Switzerland)*, vol. 211, no. 3, pp. 240-248. http://dx.doi.org/10.1159/000087018. PMid:16205069.

VERKADE, E. and KLUYTMANS, J., 2014. Livestock-associated *Staphylococcus aureus* CC398. animal reservoirs and human infections. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, vol. 21, pp. 523-530. http://dx.doi.org/10.1016/j.meegid.2013.02.013. PMid:23473831.

VIKRAM, A., JAYAPRAKASHA, G.K., JESUDHASAN, P.R., PILLAI, S.D. and PATIL, B.S., 2010. Suppression of bacterial cell-cell signalling, biofilin formation and type III secretion system by citrus flavonoids. *Journal of Applied Microbiology*, vol. 109, no. 2, pp. 515-527. http://dx.doi.org/10.1111/j.1365-2672.2010.04677.x. PMid:20163489.

WANG, S., WU, C., SHEN, J., WU, Y. and WANG, Y., 2013. Hypermutable *Staphylococcus aureus* strains present at high frequency in subclinical bovine mastitis isolates are associated with the development of antibiotic resistance. *Veterinary Microbiology*, vol. 165, no. 3-4, pp. 410-415. http://dx.doi.org/10.1016/j.vetmic.2013.04.009. PMid:23642648.