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Antimicrobial potential of Jurema preta and umburana, native species of the Caatinga biome, on *Staphylococcus* isolated from small ruminants with mastitis

Potencial antimicrobiano de jurema preta e umburana, espécies nativas do bioma caatinga, frente a isolados de *Staphylococcus* provenientes de casos de mastite em pequenos ruminantes

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Highlights:
The flavonoid myricetin is found in high concentrations in extracts of *C. leptophloeos*.
Native plant extracts of the Caatinga biome demonstrate antibacterial activity.
The toxicity test suggests the absence of toxic potential in the natural extract of *C. leptophloeos*.

Abstract

Mastitis is responsible for both damage to animal health and economic losses to the industry. To identify effective treatments for this disease, products extracted from a variety of plants with antimicrobial potential have gained attention. The present study aimed to assess the *in vitro* antibacterial potential of the ethanolic extract of two plant species from the Caatinga biome against bacteria isolated from small ruminants with subclinical mastitis. The leaves of *Mimosa tenuiflora* and *Commiphora leptophloeos* were dried and processed to obtain crude ethanolic extracts and their phenolic composition was evaluated. In total, 33 *Staphylococcus* spp. isolates from the bacterial collection of the Laboratory of Food Quality Control of IF SERTÃO-PE were used for evaluation of biofilm production. Furthermore, an antimicrobial susceptibility test was conducted using the minimal bactericidal concentration (MBC) method against the two ethanolic extracts. The toxic potential was measured through a toxicity test with *Artemia salina*. The quantification of the phenolic compounds revealed that the ethanolic extracts of *M. tenuiflora* and *C. leptophloeos* possessed higher amounts of myricetin (43.2 and 294.9 mg in 10 g, respectively) in relation to the other compounds. A 39.4% positivity rate was observed in the *nuc* gene

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investigation. The biofilm production analysis revealed that 96.9% of the isolates produced biofilm, evidencing the evolution the microorganisms regarding the development of resistance mechanisms. The MBC results showed an inhibition range between 195.30 and 3125.00 μg mL^{-1} and between 781.20 and 6250.00 μg mL^{-1} for the extracts of M. *tenuiflora* and *C. leptophloeos*, respectively. The *M. tenuiflora* extract showed the highest activity, suppressing 100% of the bacterial isolates (n=26), whereas the extract of *C. leptophloeos* showed an inhibition percentage of 69.23%. The crude ethanolic extract (EEB) of *M. tenuiflora* was found to be toxic, presenting a DL50 of 118.356 μg mL^{-1}. In contrast, the EEB of *C. leptophloeos* was found to be non-toxic (DL50 = 1527.430 μg mL^{-1}). In conclusion, both native Caatinga species presented antibacterial activity and myricetin was the major compound. These findings highlight the need for further studies regarding the identification of anti-mastitis products from natural extracts.
Key words: Antibacterial potential. Goat and sheep farming. *Commiphora leptophloeos*. Mastitis. *Mimosa tenuiflora*.

Resumo

A mastite é responsável por danos à saúde animal e prejuízos financeiros ao produtor. Na busca por alternativas eficazes no controle da mastite, destacam-se os produtos naturais extraídos de diversas plantas que possuem ação antibacteriana. Neste contexto, o presente trabalho teve por objetivo avaliar o potencial antimicrobiano *in vitro* do extrato etanólico de duas espécies do bioma caatinga frente a bactérias isoladas de casos de mastite subclínica em pequenos ruminantes. As folhas de jurema preta e umburana de cambão foram secas e processadas para obtenção dos extratos etanólicos bruto para o estudo da composição fenólica. Foram utilizados 33 isolados de *Staphylococcus* spp. provenientes da bacterioteca do Laboratório de Controle de Qualidade de Alimentos do IF SERTÃO-PE. Com os isolados de *Staphylococcus* spp. realizou-se a análise de quantificação da produção de biofilme. Além disso, empregou-se o teste de sensibilidade antimicrobiana, utilizando-se a técnica da concentração bactericida mínima (CBM) frente aos dois extratos etanólicos. Para conhecer o potencial tóxico, utilizou-se o teste de toxicidade com *Artemia salina*. A análise de quantificação dos compostos fenólicos demonstrou que os extratos etanólicos de jurema preta e umburana de cambão apresentaram a miricetina em quantidades bastante elevadas, 43,2 e 294,9 mg em 10 g respectivamente, em relação aos demais compostos detectados. Na pesquisa do gene *nuc*, observou-se uma positividade de 39,4%. A análise da produção de biofilme revelou que 96,9% dos isolados apresentaram a capacidade de produção do biofilme, evidenciando a evolução destes micro-organismos quanto ao desenvolvimento de mecanismos de resistência. Os resultados referentes à concentração bactericida mínima indicaram uma faixa de inibição entre 195,30 a 3125,00 e 781,20 a 6250,00 μg mL^{-1} para os extratos de *jurema preta* e *umburana de cambão*, respectivamente. Observou-se maior atividade para o extrato de *jurema preta, que* inibiu 100% dos isolados bacterianos (n=26), enquanto o extrato de umburana de cambão apresentou um percentual de inibição de 69,23%. Evidenciou-se a atividade tóxica do extrato etanólico bruto (EEB) de jurema preta, que apresentou uma DL50 de 118,35 μg mL^{-1}. Por outro lado, o EEB de umburana de cambão apresentou-se como atóxico (DL50 = 1527,43 μg mL^{-1}). Considerando que as duas espécies nativas da caatinga apresentaram atividade antibacteriana e que a miricetina foi o composto majoritário, tem-se a possibilidade de desenvolvimento de novas linhas de investigação, visando a obtenção produtos antimastíticos à base de extrato natural.
Palavras-chave: Atividade antibacteriana. Caprinovinocultura. *Commiphora leptophloeos* (Mart.) J. B. Gillett. Mastite. *Mimosa tenuiflora* (Willd.) Poir.

Introduction

Goat and sheep farming are an important part of the regional economy of northeastern Brazil, and are among the key activities in familial agriculture (K. A. Ribeiro & Alencar, 2018). This region harbors more than 90% and 60% of the national herds of

goats and sheep, respectively (Instituto Brasileiro de Geografia e Estatística [IBGE], 2017).

Although the Northeast region contains the majority of these herds, various challenges, especially those arising due to unsanitary conditions, have hindered the expansion of animal farming activities throughout the region. For example, mastitis is a significant disease, usually caused by bacteria, characterized by the inflammation of mammary glands. In terms of etiology, in dairy goats, intramammary infections are usually caused by different species of the genus *Staphylococcus* (Contreras et al., 2007; Santos et al., 2019).

Mastitis causes damages to animal health and economic losses to the livestock industry (Peixoto, Mota, & Costa, 2010a). Mastitis is commonly controlled using a variety of medicinal products, such as antibiotics. However, their constant and indiscriminate use has raised serious concerns regarding clinical safety and public health due to residues in the milk owing to noncompliance with treatment guidelines. The bacteria present in the milk are not always fully eliminated even after pasteurization. In addition, bacterial resistance may be exacerbated (Cades et al., 2017; Oliveira et al., 2019).

Some issues, such as the non-commercialization of milk produced by treated animals, high costs due to antibiotic dependence, and bacterial resistance to these compounds, have prompted researchers to attempt identification of new alternatives to treat mastitis (Loguercio et al., 2006). Pereira et al. (2015) recognized that natural products are an economic therapeutic option in the control of diseases in developing countries.

Despite the genetic diversity of Brazilian plant species, less than 10% have been biologically characterized, and less than 5% have been subjected to detailed phytochemical studies. Even with research increasing in this area, plants are an underused, yet promising, source for the discovery of new biologically active substances (Luna et al., 2005).

Therefore, in the search for effective alternatives for mastitis treatment, the potential use of a variety of plants with antibacterial activities is worth investigating. The study of phytotherapeutic species is considered to be of global importance plant-derived compounds have low toxicity toward humans and animals and are less likely to induce bacterial resistance (Oliveira et al., 2019). Therefore, this study aimed to evaluate the *in vitro* antimicrobial potential of the ethanolic extract of two native Caatinga species against *Staphylococcus*.

Material and Methods

This study was conducted in the Laboratory of Chemistry, the Laboratory of Food Quality Control and the Laboratory of Liquid Chromatography of the IF SERTÃO-PE, and the Laboratory of Microbiology and Animal Immunology of the Federal University of São Francisco Valley (UNIVASF), respectively.

Collection and identification of vegetation

The leaves of *Mimosa tenuiflora* (Willd.) Poir. (Jurema preta) and *Commiphora leptophloeos* (Mart.) J. B. Gillett (Umburana de cambão) were collected in the municipality of Petrolina-PE at the following coordinates: 09º20'14.9" S, 040º41'30.3" W and 09º20'37.6" S, 040º41'25.3" W, respectively. Exsiccates were prepared from the botanic material, presenting the following registration number: *5.276 for M. tenuiflora* and *5.277 for C. leptophloeos*.

Processing of the vegetation

The leaves were subjected to drying in a forced-air oven at a temperature of 40ºC for three days, and then crushed in a cutting mill until a powdered material was obtained. Afterward, the preparation of the crude ethanolic extracts (EEBs) began, in which

the powdered material was subjected to exhaustive maceration in 99.3% ethanol. Four extractions were performed at 72 h intervals.

Analysis of phenolic compounds - HPCL-DAD

The method employed for the determination of the phenolic compound profiles of the extracts was validated by Padilha et al. (2017), with adaptations from Dutra, Rodrigues, Oliveira, Pedreira and Lima (2018). The individual phenolic compounds were determined by HPLC-DAD (High Performance Liquid Chromatography) using an LC Agilent 1260 Infinity system (Agilent Technologies, Santa Clara - EUA), a liquid chromatograph attached to a quaternary solvent pump (model G1311C) and deaerator, a thermostatted column compartment (G1316A), an automatic sample changer (G1329B), and a photodiode array detector (DAD) (model G1315D).

The data were processed using OpenLAB CDS ChemStation Edition software (Agilent Technologies, Santa Clara - EUA). The column used was a Zorbax Eclipse Plus RP-C18 (100 × 4.6 mm, 3.5 μm), and the pre-column was a Zorbax C18 (12.6 × 4.6 mm, 5 μm) (Zorbax, EUA). The temperature of the oven was 35°C, and the injection volume of the sample solution was 20 μL, previously diluted in phase A (10 mg mL^{-1}) and filtered through a 0.45 μm membrane (Millipore, Barueri, SP, Brazil). The solvent flow was 0.8 mL per minute. The gradient used during the separation was from 0 to 5 minutes: 5% B; 5 to 14 minutes: 23% B; 14 to 30 minutes: 50% B; 0 to 33 minutes: 80% B, in which solvent A was a phosphoric acid solution (0.1 M, pH = 2.0) and solvent B was acidified methanol with 0.5% H_3PO_4. Detection of the compounds was performed at 220, 280, 320, 360, and 520 nm, and identification and quantification were performed through comparison to external standards.

Genotypic identification of isolates of Staphylococcus spp.

In total, 33 bacterial isolates, phenotypically recognized as *Staphylococcus* spp., were used prevenient from the bacterial collection of the Laboratory of Food Quality Control of IF SERTÃO-PE. The DNA of these isolates were extracted and purified according to the methodology proposed by Aldous, Pounder, Cloud and Woods (2005), with adaptations. The genotypic identification of the isolates of *Staphylococcus* spp. was performed using PCR amplification of the *nuc* gene (Kateete et al., 2010).

Biofilm quantification

Biofilm quantification was performed through the microplate adhesion technique (Merino et al., 2009) using *Staphylococcus aureus* ATCC 25923 as a positive control and sterile Tryptone Soya Broth (TSB) as a negative control.

A bacterial suspension was formed from each isolate, with turbidity equivalent to the 0.5 tube of the MacFarland standards. Afterward, inoculations were performed in 3 mL of the TSB broth enriched with 0.25% glucose and incubated at 37ºC for 24 h. Later, 195 μL of TSB glucose was added to 96-well microplates along with the previously incubated bacterial solution. Once again, the microplate was subjected to incubation at 37ºC for 24 h, then washed three times with 200 μL of sterile distilled water. Next, the wells were stained with 100 μL of gentian violet (0.25%) for 5 min and washed again three times with sterile distilled water. To complete the process, 200 μL of alcohol-acetone (80:20) was added. The microplates were subjected to absorbance analysis through a microplate scanner (model EXPERT PLUSUV) with a wavelength of 620 nm. The assays were held on triplicate samples, and, according to the optical density values (O.D.) obtained in the reading, the isolates were classified on the basis of biofilm production as negative, weak, moderate, or strong producers.

Antimicrobial susceptibility test

In total, 25 isolates, eight *S. aureus* and 17 *Staphylococcus* spp., were used for the susceptibility test with the plant extracts. The Methicillin-sensitive *S. aureus* (MSSA) strain, lineage ATCC 25923 reference strain from the American Type Culture Collection, was also used in the assay, as recommended by Cos, Vlietinck, Berghe and Maes (2006).

The susceptibility tests were initiated with 0.25 g of each ethanolic extract diluted in 10 mL of the defined diluent (40% ethanol for the EEB of *M. tenuiflora* and 92.3% ethanol for the EEB of *C. leptophloeos*) to obtain a stock solution at a concentration of 25 mg mL^{-1}. Determination of the minimal bactericidal concentration (MBC), based on the M7-A7 document (Clinical and Laboratory Standard Institute [CLSI], 2006), consisted of the distribution of 200 µL of the Mueller-Hinton broth in microtitration plates. Afterward, 200 µL of the stock solution of the extract was added to the first well and, after homogenization, was transferred to the second well, and so on, until the following final concentrations were obtained: 12,500, 6,250, 3,125, 1,562.5, 781.2, 390.6, 195.3, and 97.6 µg mL^{-1}. An equal proceeding was performed for the control of the diluent, in which the stock solution was replaced by the solvent, taking into consideration the smaller MBC than the one obtained by the diluent.

In the preparation of the inoculum, colonies obtained in Mueller-Hinton (MH) agar were used to obtain a bacterial suspension with turbidity equivalent to the 0.5 tube of the MacFarland standards, later adjusted in a spectrophotometer at 625 nm. From this suspension, 10 µL was inoculated into the wells of the microplates containing the diluted ethanolic extract. The material was incubated at 37°C for 24 h under aerobic conditions. After, the microplate content was pricked out with a sterile re-chipper, cultivated in MH agar, and incubated for 48 h at 37ºC for the determination of the MBC as the lowest concentration of the ethanolic extract in the study able to cause bacterial death. All assays were conducted in triplicate. The data were treated through descriptive and inferential statistical analysis, with the distribution of values in absolute and relative numbers. The MBC mean values were analyzed by the Mann-Whitney test.

Toxicity test with Artemia salina

The toxicity test was performed according to the methodology of Meyer et al. (1982), with adaptations. A saline solution was prepared (with sea salt and sterile distilled water) at a concentration of 30 g L^{-1}. This solution was used to hatch the eggs of *A. salina* and in the preparation of the remaining dilutions. The eggs hatched in the saline solution over 24 h in an aquarium divided by partitions with 0.02 cm holes. On one side of the aquarium, 40 mg of cists of *A. salina* were added under careful observation to ensure that the cists did not trespass across the partition. This section of the aquarium was then covered with aluminum foil so that the organisms, once hatched, would be attracted by the artificial light that had been set up on the other side of the aquarium, forcing the passage of these organisms through the partition. After the eggs hatched, approximately 10 nauplii of *A. salina* were transferred with a micropipette to containers with the saline solution, and the samples were tested under different concentrations (1000 µg mL^{-1}, 100 µg mL^{-1}, 10 µg mL^{-1}, and 1 µg mL^{-1}). The test was followed by a negative control. After 24 h of contact with the tested substances, the living and dead nauplii were counted. The tested substances were crude ethanolic extracts (EEBs) of *Commiphora leptophloeos* and *Mimosa tenuiflora*. Each tested concentration was performed in triplicate. The dose that caused a lethality of 50% of the nauplii (DL50) was obtained through calculations using the PROBIT method of analysis, with a 95% reliability rate.

Results and Discussion

The extraction of the compounds of *M. tenuiflora* and *C. leptophloeos* presented yields of 14.34% and 6.3%, respectively. Specific factors, including the extraction methods, solvents, temperature, and characteristics of the vegetation, are fundamental to the quality and yield of the compounds extracted from a plant. These factors considerably affect the content of the secondary metabolites extracted, thereby affecting the yield and final composition of the extracts (Karabegović et al., 2014).

In the chromatograms obtained from the ethanolic extract of *M. tenuiflora*, the main peaks corresponded to the following substances: gallic acid (280 nm); epigallocatechin gallate (220 nm); trans-resveratrol (320 nm), and Myricetin (360 nm) (Figure 1).

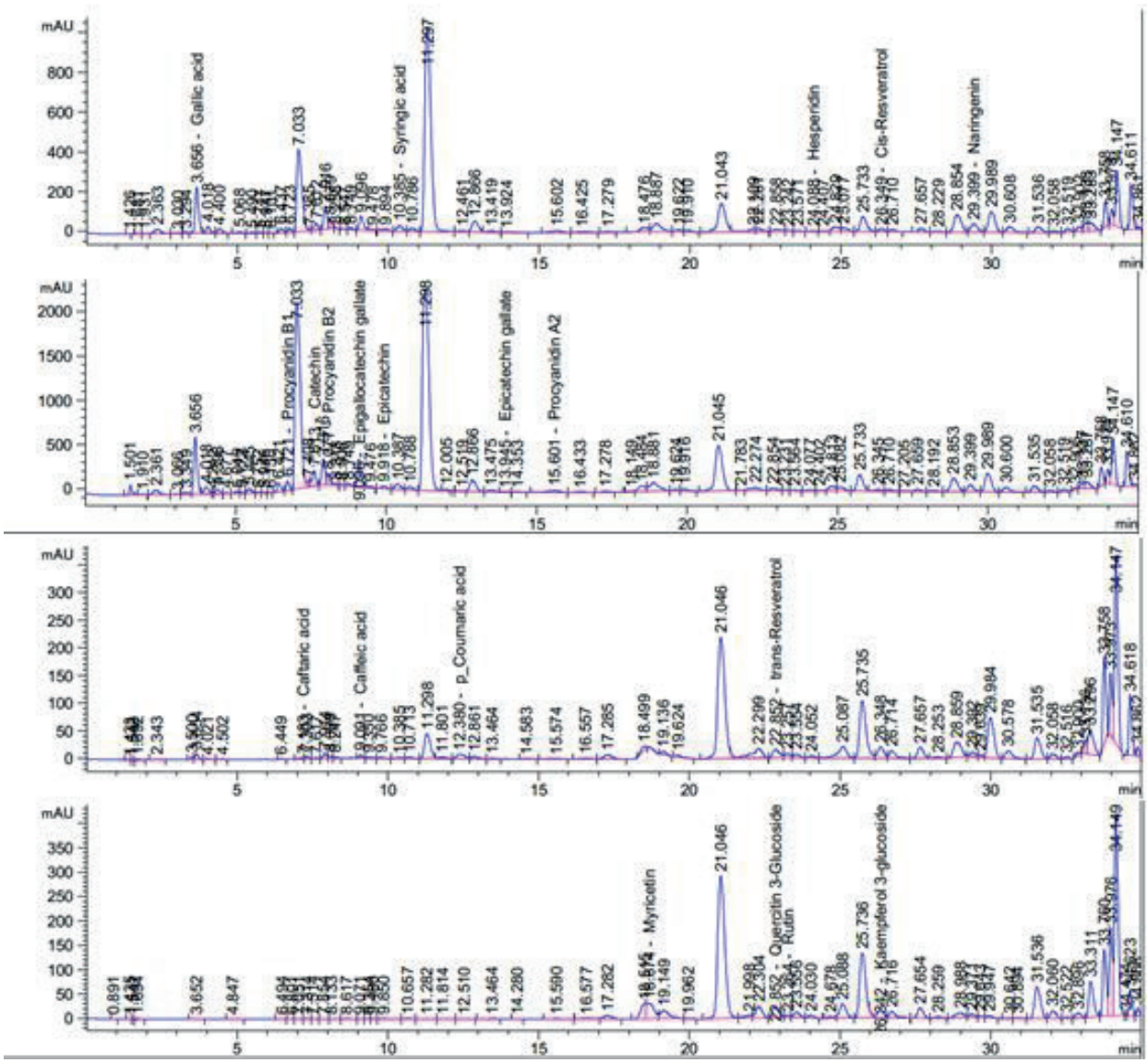

Figure 1. Chromatograms of the ethanolic extract of *Mimosa tenuiflora* with the detection of wavelengths of 280, 220, 320, and 360 nm.

In the ethanolic extract of *C. leptophloeos*, the main peaks corresponded to the following substances: hesperidin (280 nm); epicatechin gallate (220 nm); trans-resveratrol (320 nm), and myricetin and quercetin3-glycoside (both 360 nm) (Figure 2).

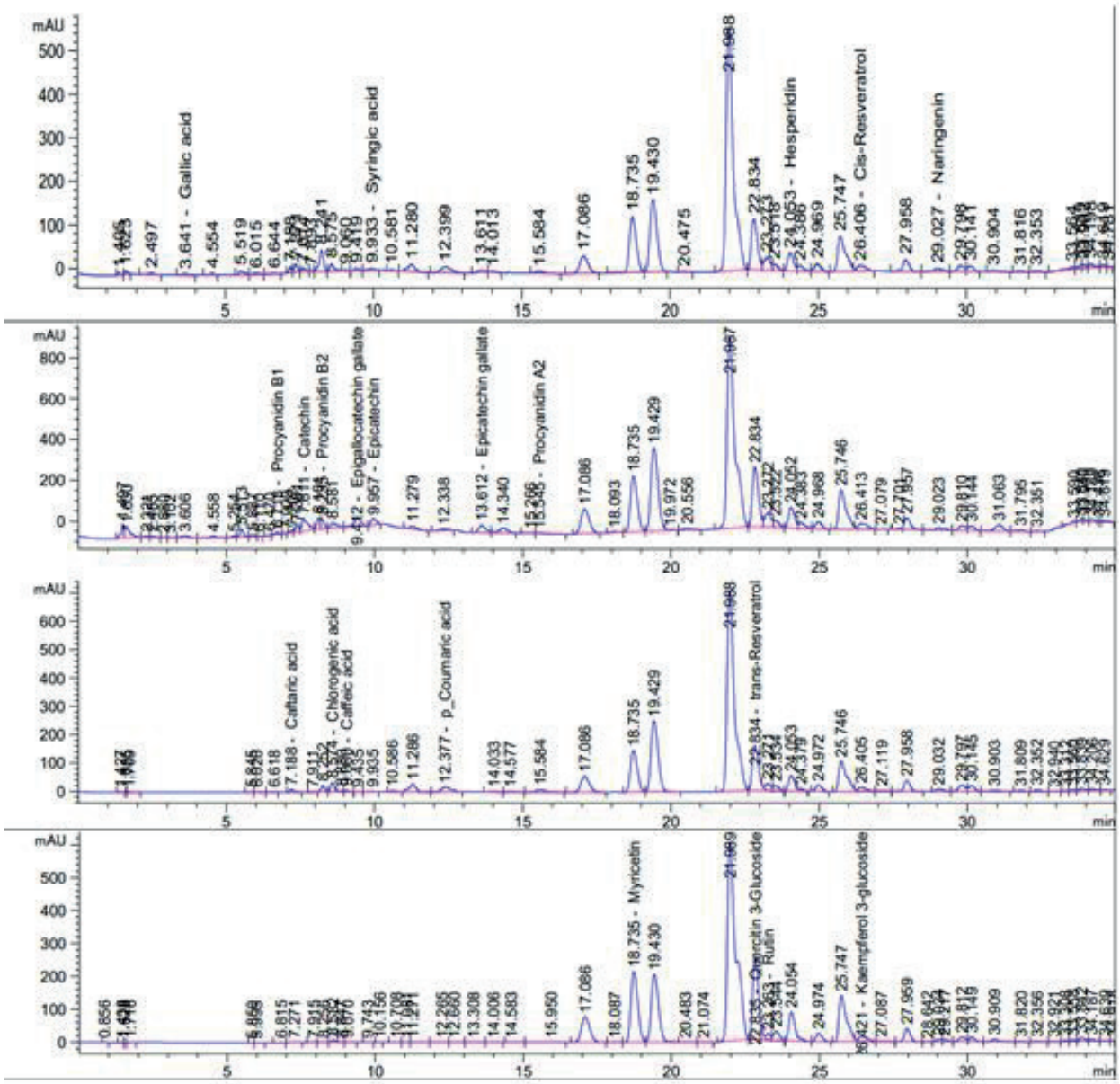

Figure 2. Chromatograms of the ethanolic extract of *Commiphora leptophloeos* with the detection of wavelengths of 280, 220, 320, and 360 nm.

The quantification of the phenolic compounds demonstrated that the ethanolic extracts of *M. tenuiflora* and *C. leptophloeos* possessed higher quantities of myricetin, 43.2 and 294.9 mg in 10 g, respectively, in relation to the other detected compounds (Table 1). In a study on the antibacterial activity of 38 different flavonoids against *S. aureus,* some, including myricetin, datiscetin, quercetin, luteolin, and kaempferol, showed inhibitory activity against methicillin-resistant *S. aureus* (MRSA) (Xu & Lee, 2001).

In the ethanolic extract of *M. tenuiflora*, the flavonol group was the most abundant (56.5 mg in 10 g), followed by the phenolic acids (39.06 mg in 10 g). In the ethanolic extract of *C. leptophloeos,* too, the flavonol group was the most abundant (357.4 mg in 10 g). Myricetin being the major compound in both extracts.

Table 1
Phenolic profile of the ethanolic extracts of *Mimosa tenuiflora* and *Commiphora leptophloeos*

Phenolic compounds	*Mimosa tenuiflora* (mg per 10 g)	*Commiphora leptophloeos* (mg per 10 g)
Flavanols		
Catechin	6.9	2.6
Epicatechin	0.71	0.73
Epicatechin gallate	0.9	4
Epigallocatechin gallate	18	2.3
Procyanidin A2	3	0.64
Procyanidin B1	3	0.32
Procyanidin B2	6.7	2.6
∑ *Flavanols*	**38.6**	**13.19**
Flavonols		
Quercetin 3-glucoside	5.1	52.1
Rutin	1	3.4
Kaempferol 3-O-Glycoside	7.2	7
Myricetin	43.2	294.9
∑ Flavonols	**56.5**	**357.4**
Flavanones		
Hesperidin	1.6	18.8
Naringenin	7.9	1.3
∑ *Flavanones*	**9.5**	**20.1**
Phenolic acids		
Gallic acid	36.2	0.34
Syringic acid	1.6	0.28
p-Coumaric Acid	0.51	1.2
Caffeic Acid	0.24	0.22
Trans-caftaric acid	0.51	1.8
Chlorogenic Acid	ND	3.4
∑ *Phenolic acids*	**39.06**	**7.24**
Stilbenes		
trans-resveratrol	0.86	8.7
cis-resveratrol	2.6	2.1
∑ *stilbenes*	**3.46**	**10.8**

ND = Not detected.

The *nuc* gene analysis revealed a positivity rate of 39.4% (13/33). Therefore, these 13 isolates were identified as *S. aureus*. In a study performed in Brazil, *S. aureus* was one of the main agents involved in cases of mastitis in goats (Peixoto, França, Souza, Veschi, & Costa, 2010b).

The biofilm analysis revealed that the majority of the isolates (96.9%) had the potential for biofilm production, with 19 weak, eight moderate, and five strong producers; only one isolate did not produce biofilm (Table 2). This result represents the evolution of the microorganisms with regards to the development of resistance mechanisms. According to Dibbern, Botaro, Viziack, Silva and Santos (2015), the majority of mastitis cases are caused by bacteria, especially *S. aureus,* a microorganism capable of producing biofilm. Scherr, Heim, Morrison and Kielian (2014) define biofilm as a group of cells attached to a biotic or abiotic surface imbedded in a protective extracellular matrix, thus granting resistance to antimicrobial drugs used during the treatment of illness, as well as to disinfectants and the immune system of the host, allowing the infection to acquire a chronic nature (Arciola, Campoccia, Speziale, Montanaro, & Costerton, 2012; Guimarães et al., 2012; Tremblay, Caron, Blondeau, Messier, & Jacques, 2014).

Table 2
Number of isolates of *Staphylococcus* spp. and their classifications according to biofilm production

Classification	Nº of Isolates	Percentage (%)
Weak Production	19	57.6
Moderate Production	8	24.2
Strong Production	5	15.2
No Production	1	3.0
Total	33	100.00

The plants used in popular medicine are now being more thoroughly studied as potential sources of substances with activity against microorganisms that are pathogenic to humans (Mendes et al., 2011), agricultural plants, and livestock (Corrêa & Salgado, 2011). This is because the secondary metabolites found in plants have primary roles in defense against herbivores and microorganisms, protection against ultraviolet rays, attracting pollinators and seed dispersion animals, and the production of allelopathic substances (Simões, Schenkel, Gosmann, Melo, Mentz, & Petrovick, 2010).

With regards to the sensibility tests of the bacterial strains for the ethanolic extracts, the MBC analysis revealed an inhibition range from 195.30 to 3125.00 µg mL^{-1} and from 781.20 to 6250.00 µg mL^{-1} for *M. tenuiflora* and *C. leptophloeos*, respectively (Table 3).

Table 3
The susceptibility of isolates of *Staphylococcus* spp. to the ethanolic extracts of *Mimosa tenuiflora* and *Commiphora leptophloeos*

Species	Observed activity (%)	Minimal Bactericidal Concentration	
		Range	Mean (μg mL^{-1})
Mimosa tenuiflora	100 (26/26)	195.3 - 3,125	1,682.68 [a]
Commiphora leptophloeos	69.23 (18/26)	781.2 - 6,250	2,734.37 [a]

Means followed by the same letter are not statistically significantly different (p>0.05).

The extract of *M. tenuiflora showed high antimicrobial activity, inhibiting 100% of the bacterial isolates* (n=26), including *S. aureus* ATCC 25923. According to Pereira et al. (2015), tannic solutions isolated from *M. tenuiflora* showed *in vitro* antibacterial activity against lineages of *S. aureus* and warrant more detailed studies relating to their potential therapeutic use.

Studies relating to the antimicrobial activity of *C. leptophloeos* are scarce. The ethanolic extract of this plant presented an inhibition percentage of 69.23%. According to Ribeiro et al. (2014), regarding the therapeutic potential and use of medicinal plants in the Caatinga biome, *C. leptophloeos* presents itself as highly suitable for the treatment of several diseases, including inflammation.

In this study, it was verified that there was no significant difference between the MBC means, thus demonstrating the therapeutic potential of both studied species. This finding highlights the need for further studies relating to the antimicrobial activity of both plants and their attributes for the development of new investigation lines.

The toxicity evaluation of the ethanolic extracts in *A. salina* followed the classification established by Meyer et al. (1982), in which samples that showed DL50 > 1000 μg mL^{-1} are considered non-toxic, and samples that showed DL50 < 1000 μg mL^{-1} are considered toxic against microcrustaceans (Table 4).

Table 4
Toxicity evaluation in *Artemia salina* (DL50) of the ethanolic extracts of *Mimosa tenuiflora* and *Commiphora leptophloeos* and their respective limits of the confidence interval at 95%

Samples	DL50 (μg mL^{-1})	Confidence interval 95%	
		Inferior limit	Upper limit
EEB *Mimosa tenuiflora*	1527.430	60.110	38812.798
EEB *Commiphora leptophloeos*	118.356	20.042	698.952

The toxic activity of the ethanolic extract of *M. tenuiflora* was demonstrated by a DL50 of 118.35 μg mL^{-1}, which lower than the reference value. Bezerra and Falcão-Silva (2019) performed an assessment of the plants reported as toxic to ruminants in the semiarid northeast of Brazil, and the interviewees mentioned cases of intoxication of goats and pregnant sheep caused by the ingestion of the leaves of *M. tenuiflora*. They also reported that these intoxications played a role in congenital malformations during embryonic development, leading to serious issues for future reproduction in small ruminants.

In contrast, the extract of *C. leptophloeos* was non-toxic (DL50 = 1527.430 µg mL^{-1}), revealing its potential for use in the production of plant-based treatments. However, the bioassay with the nauplii of *A. salina* is only considered a screening test, and further tests are recommended for the verification of the toxic potential of potential therapeutics.

Tests with *A. salina* suggest its high potential for the study of biological activities, with the importance of this bioassay being established in the targeting of future phytochemical studies searching for bioactive substances (Amarante, Müller, Póvoa, & Dolabela, 2011).

Conclusions

Both tested species presented biological activity against *Staphylococcus* spp. and myricetin was the main compound in the extracts. Further studies should investigate other properties of these extracts, such as toxicity, with the aim of controlling pathogens without causing unwanted effects to the host. In addition, studies for the production of plant-based therapeutics to establish a therapeutic modalities with improved efficacy should be undertaken.

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