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Chemical characterization UPLC-ESI-QToF-MSE, antibacterial and antibiofilm potential of *Sarcomphalus joazeiro* (MART.) Hauenschild

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ABSTRACT

The present study aims to demonstrate the chemical composition of the ethanolic extract of *Sarcomphalus joazeiro* bark and to evaluate its antibacterial and antibiofilm activity. For that, the ethanolic extract of the stem bark was prepared, and chemical characterization was carried out in UPLC-ESI-QToF-MSE. Minimum inhibitory concentration and antibiotic modification test were performed by the microdilution method. Formation and anti-formation assays were evaluated by the crystal violet method. In the chemical characterization, the presence of 24 peaks was observed. Antibacterial activity showed minimum inhibitory concentration ≥2048 μg/mL. In the evaluations of the potentiating activity, synergism was the most evident. In the formation analysis, it was observed that most strains showed moderate biofilm formation. It was concluded that saponin derivatives are the major secondary metabolites in the analyzed extract and that it presents relevant synergism with the antibiotics studied and a significant ability to inhibit bacterial biofilm formation.

1. Introduction

Biofilms are organizations of microbial cells in a self-produced matrix, implicated in a variety of human infections (Kadam; Chattopadhyay; Kaushik, 2022). In general, bacteria that organize themselves in this way are more resistant to both antibiotics and the host's immune system (Zhao et al., 2022).

Bacteria organized in the form of biofilms can adapt to food and oxygen shortages, stop growing and become resistant to the action of antibiotics, in addition to having a greater capacity for colonization and greater ability to resist the immune system. In addition to these factors responsible for resistance, the polysaccharide matrix that acts as a physical barrier, the variety of metabolic activity present in the system, the sense quorum and efflux pumps are mentioned (Huang et al., 2022; Bolivar-Vargas; Torres -Caycedo; Sanchez-Neira, 2021).

Biofilms are present in pathologies such as endocarditis, osteomyelitis, periodontitis and are also responsible for the chronicity, persistence and recurrence of several infections, thus increasing their morbidity and mortality, thus becoming a serious public health problem (Dumaru; Baral; Shrestha, 2019; Pozo, 2017). These organizations are extremely resistant to elimination via the immune system, antibiotics and antiseptics, demonstrating the need to invest in the search for new control techniques (Leite et al., 2018; Oliveira; Brugnera; Piccoli, 2013).

Research using plant products has proved to be a valid alternative for

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the production of products capable of combating multidrug-resistant organisms, in addition to important activity of inhibition of the formation and even of reduction of the bacterial biofilm by plant extracts. In this scenario, plant extracts have shown significant results because they present in their composition secondary metabolites that make them substances of great therapeutic potential (Andrade et al., 2019a; Nader et al., 2018).

Sarcomphalus joazeiro (Mart.) Hauenshild (basionym: *Ziziphus joazeiro* Mart.) is a species belonging to the Rhamnaceae family that is part of the caatinga biome and is endemic in northeastern Brazil. The plant is used in food, industrial production and mainly in traditional medicine in the treatment of fever, bacterial infections, respiratory and gastrointestinal tract infections (Santos et al., 2021).

The use of natural products in the fight against bacterial infections, including the process of inhibition of biofilm formation, has been the subject of several researches, due to the great potential that these substances have, such research has shown that plant products are capable of exerting an important activity. antibacterial and anti-biofilm. Based on the above, the present research aimed to characterize the chemical composition of the ethanolic extract of *Sarcomphalus joazeiro* bark, in addition to evaluating its antibacterial activity, production and anti-biofilm activity.

2. Materials and methods

2.1. Collection of plant material

The material was collected at Sitio Ipueiras, with geographic coordinates, 07°28′54.4″S/39°01′47.2″W, rural area of the city of Brejo Santo, Ceará, located in the surroundings of Chapada do Araripe. Exsiccates were produced from the collected material and later deposited in the Herbarium Caririense Dárdano de Andrade Lima of the Regional University of Cariri – URCA under the number 13,346. The plant material was screened to evaluate the general condition and absence of contamination and then washed with distilled water and placed in the laboratory for the preparation of the extract.

2.2. Obtaining the ethanolic extract of Sarcomphalus joazeiro

The ethanolic extract of *Sarcomphalus joazeiro* (EECCSJ) was prepared by maceration with cold extraction (Matos, 2002). The stem barks were dried at room temperature (30–32 °C), and then ground in a mechanical mill, dried in absolute ethanol solution, placed in a container protected from the action of light and air, and after 72 h they were filtered. and concentrated in a rotary evaporator (model Q-344 B – Quimis, Brazil). The extract was frozen and taken to the lyophilizer (−60 °C) until all the water was removed. The powdered extract was stored under refrigeration for testing.

2.3. Identification of compounds by UPLC-ESI-QToF-MS^E

The compounds present in the extract were identified using an Acquity® UPLC system coupled to a Quadrupole/Time of Flight system (UPLC-ESI-QTOFMS) (*Waters Corporation*, Milford, USA), belonging to Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA. Chromatographies were performed on a Waters Acquity UPLC BEH column (150 × 2.1 mm, 1.7 μm), fixed temperature of 40 °C, mobile phases water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), gradient ranging from 2% to 95% B (15 min), flow rate of 0.4 mL/min and injection volume of 5 μL. The ESI- mode was acquired in the range of 110–1180 Da, source temperature fixed at 120 °C, desolvation temperature 350 °C, desolvation gas flow of 500 L/h, extraction cone of 0.5 V, capillary voltage of 2.6 kV. The ESI + mode was acquired in the range of 110–1180 Da, fixed source temperature of 120 °C, desolvation temperature of 350 °C, desolvation gas flow of 500 L/h and capillary voltage of 3.2 kV. Leucine enkephalin was used as lock mass. The acquisition mode was MSE and the instrument was controlled by Masslynx 4.1(*Waters Corporation*, Milford, USA) software.

2.4. Bacterial strains

Six bacterial strains were used, the strains *Streptococcus mutans* INCQS 00446 (ATCC 25175), *Enterococcus faecalis* INCQS 00018 (ATCC 14506), *Staphylococcus epidermidis* INCQS 00016 (ATCC 12228) were obtained from the Collection of Reference Microorganisms in Health Surveillance (CMRVS), Oswaldo Cruz Foundation – FIOCRUZ - INCQS, Rio de Janeiro, RJ. The strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 259223 were obtained from the Mycology Laboratory of the Federal University of Paraíba (João Pessoa, Paraíba, Brazil). To perform the tests, each sample was subcultured in BHI agar medium and incubated at 37 °C for 24 h, after which a small number of cells was removed and diluted in 0.85% NaCl, and adjusted in a spectrophotometer (600 nm), for a concentration of 5×10^5 CFU/mL (5×10^4 CFU/μL well).

2.5. Determination of the minimum inhibitory concentration (MIC)

The determination of the MIC of *S. joazeiro* extract was performed by the microdilution technique, using plates containing 96 wells and in triplicate. Each well contained 100 μL of concentrated BHI medium, microbial suspension (10%) and 100 μL of extract at a concentration of 2048 μg/mL were deposited in the first well and underwent serial dilution until the penultimate well. The last well was the growth control. The plates were incubated for 24 h at 35 °C and the bacterial MIC was revealed using resazurin (Javadpour et al., 1996). The concentrations chosen to carry out the research were based on previous studies.

2.6. Antibiotic action modification Test

Sub-inhibitory concentrations (MIC/8) of the extract were used. A volume of 100 μL of a solution containing 10% BHI, inoculum and the natural product was distributed in each well in the alphabetical direction of the plate. Soon after, 100 μL of each antimicrobial, individually, was mixed to the first well, proceeding to microdilution in series, in a proportion of 1:1 until the penultimate well. Antimicrobial concentrations gradually varied from 512 to 0.5 μg/mL. The plates were incubated for 24 h at 37 °C (Coutinho et al., 2010). The revelation was performed using resazurin.

2.7. Biofilm formation and anti-biofilm activity

Biofilm formation by the isolates was evaluated in microtiter plates, using the Crystal Violet method described by Stepanovic et al. (2007), with some modifications. (Andrade et al., 2019). 160 μL of culture medium, 20 μL of distilled water and 20 μL of bacterial inoculum adjusted to 1.5×10^8 CFU/mL were added to the microtiter plates. In the sterility control, the bacterial inoculum was replaced by distilled water. After incubation for 24 h at 37 °C, the two substrates were washed three times with saline (0.9%) to remove planktonic cells, and then incubated at 55 °C for biofilm fixation. Subsequently, 200 μL of crystal violet was added for 15 min. After this period, the plates were washed with distilled water, followed by elution with 100% ethanol to obtain density and optical readings at a wavelength of 570 nm. From the readings (OD570), the average of the absorbance values of each sample (ODa) was determined in comparison with the absorbance of the sterility control (ODc). Samples were classified as strongly ($4\times$ ODc $<$ ODa), moderately ($2\times$ ODc $<$ ODa $\leq 4\times$ ODc) and weakly (ODc $<$ ODa $\leq 2\times$ ODc) biofilm forming. The isolates that showed absorbance values equal to or lower than the control were classified as non-biofilm producers.

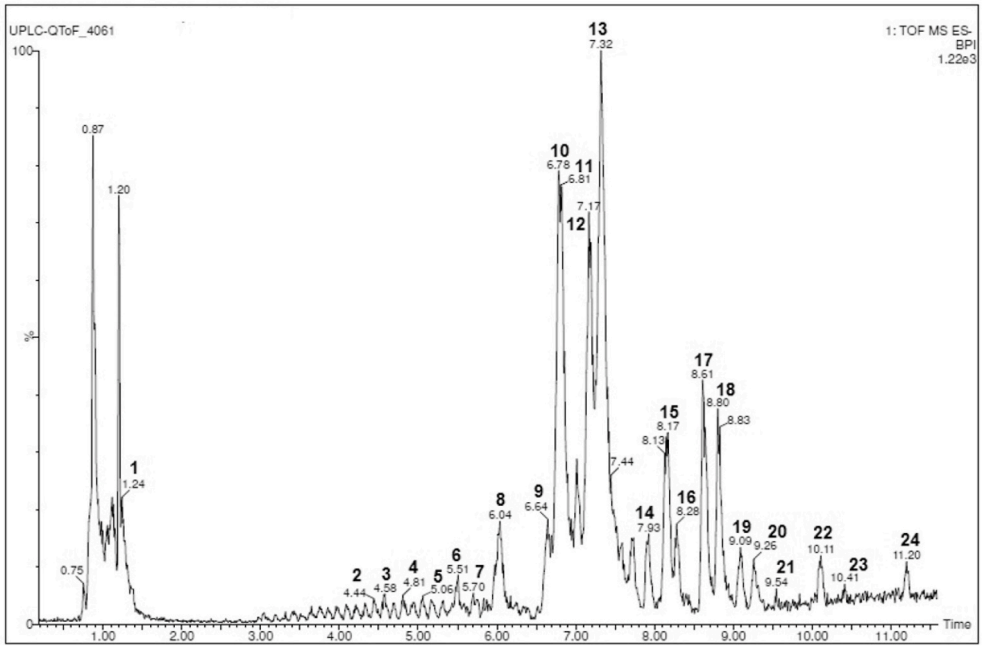

Fig. 1. EECCSJ compound peaks by ultra-performance liquid chromatography coupled to quadrupole/time of flight system.

2.8. Biofilm formation inhibition assay

For the evaluation of the biofilm formation inhibition, 20 μL of concentrations of 1 mg/mL and 0.1 mg/mL of the product were added to the microtiter plate, together with 20 μL of bacterial inoculum (1.5×10^8 CFU/mL), and 160 μL of brain-heart infusion broth growth medium. To control bacterial growth, the product was replaced by distilled water and to control the sterility of the experiment, the inoculum and the product were replaced by distilled water. The plate was incubated for 24 h at 37 °C, after which the planktonic cells were removed by washing the plate three times with 0.9% saline. To fix the biofilm, the plate was incubated at 55 °C for 1 h and then the biofilm was stained with 0.4% crystal violet for 15 min. Subsequently, the plate was washed three times with saline and eluted in ethanol (100%) to obtain the optical density at 570 nm. The percentage of anti-biofilm activity was evaluated comparing with the bacterial biofilm growth control. The concentrations chosen to carry out the research were based on previous studies, with an increase in the concentrations of the anti-formation assays due to the absence of direct inhibition of the substances.

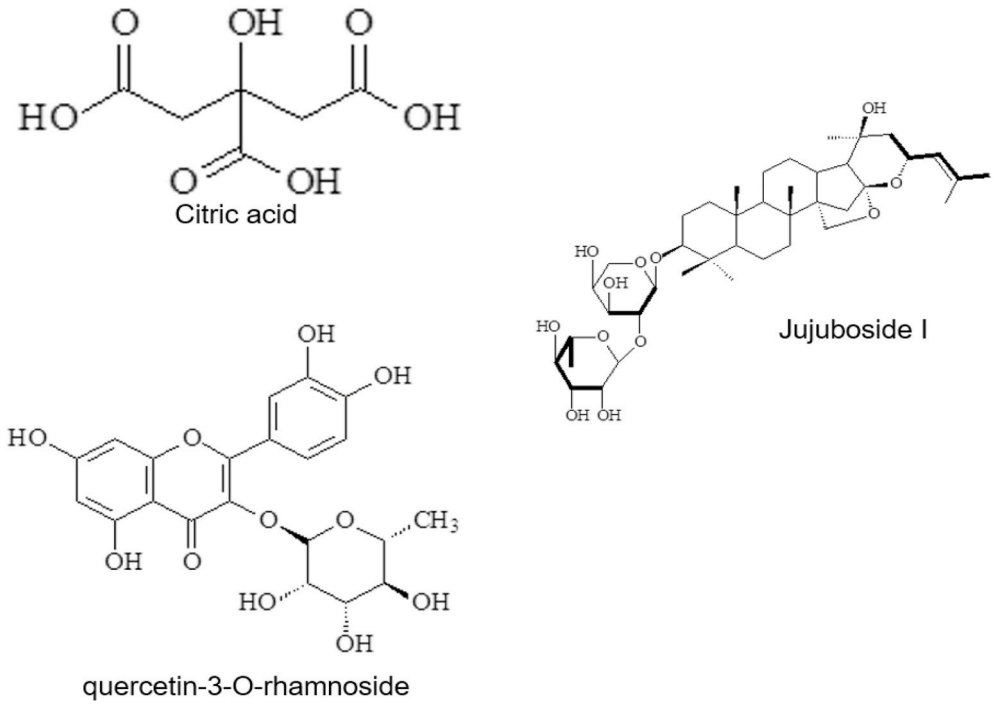

Fig. 2. Chemical structure of the metabolites identified in the extract.

Table 1
Identification of EECCZJ compounds by ultra-performance liquid chromatography coupled to a quadrupole/time-of-flight system.

Peak no.	Rt min	[M-H]⁻Observed	[M-H]⁻Calculated	Product Ions (MS/MS)	Empirical Formula	Ppm (error)	Putative Name	References
1	1.24	191.0163	191.0192	155.9530, 111.0095	$C_6H_8O_7$	15.2	Citric acid	Silva et al. (2015)
2	4.44	503.1573	503.1612	341.1059, 293.0838, 179.0538	$C_{18}H_{31}O_{16}$	7.8	NI	-
3	4.58	503.1597	503.1612	341.1083, 179.0605	$C_{18}H_{31}O_{16}$	3.0	NI	-
4	4.81	503.1660	503.1612	341.1087, 179.0521	$C_{18}H_{31}O_{16}$	9.5	NI	-
5	5.06	503.1620	503.1612	341.1016, 179.0510	$C_{18}H_{31}O_{16}$	1.6	NI	-
6	5.51	1077.5726	1077.5728	977.4636, 945.5278	$C_{52}H_{85}O_{23}$	0,2	Saponin derivative	Pu et al. (2017)
7	5.70	447.2257	447.2230	301.8223, 447.2272	$C_{21}H_{20}O_{11}$	6,0	quercetin-3-*O*-rhamnoside	Pu et al. (2017)
8	6.04	1123.5718	1123.5748	1105.5701, 935.4473	$C_{50}H_{91}O_{27}$	2.7	Saponin derivative	Pu et al. (2017)
9	6.64	1153.5878	1153.5853	1107.5796, 1105.5793	$C_{51}H_{93}O_{28}$	2.2	Saponin derivative	Pu et al. (2017)
10	6.78	1077.5577	1077.5482	1059.5676, 945.5198	$C_{52}H_{85}O_{23}$	0.2	Saponin deriative	Pu et al. (2017)
11	6.82	1077.5604	1077.5482	1075.5516, 945.5286	$C_{52}H_{85}O_{23}$	11.3	Saponin deriative	Pu et al. (2017)
12	7.17	1091.5786	1091.5791	959.5322, 945.5393	$C_{57}H_{87}O_{20}$	0.5	Saponin derivative	Pu et al. (2017)
13	7.32	1091.5721	1091.5697	959.5394	$C_{46}H_{91}O_{28}$	1.0	Saponin derivative	Pu et al. (2017)
14	7.93	897.5013	897.5000	502.3310, 501.3203	$C_{50}H_{73}O_{14}$	1.4	NI	–
15	8.17	1041.5500	1041.5540	503.1776, 311.1776	$C_{42}H_{89}O_{28}$	3.8	Saponin derivative	Pu et al. (2017)
16	8.28	1073.5743	1073.5744	1119.5930, 911.3109	$C_{41}H_{66}O_{12}$	0.1	Jujuboside I	Pu et al. (2017)
17	8.61	977.4520	977.4535	311.1743	$C_{53}H_{69}O_{17}$	1.5	NI	–
18	8.80	1121.5026	1121.5016	1119.6132, 977.4628	$C_{52}H_{81}O_{26}$	0.9	Saponin derivative	Pu et al. (2017)
19	9.09	991.4826	991.4809	665.2281, 503.1765	$C_{40}H_{79}O_{27}$	1.7	NI	–
20	9.26	1135.5151	1135.5173	1121.4932, 991.4810, 665.3224	$C_{53}H_{83}O_{26}$	1.9	Saponin derivative	Pu et al. (2017)
21	9.54	503.1657	503.1612	341.1030, 179.0535	$C_{18}H_{31}O_{16}$	8.9	NI	–
22	10.11	485.3291	485.3267	439.2801, 341.1075	$C_{30}H_{45}O_5$	4.9	Quillaja sapogenin	Pu et al. (2017)
23	10.41	485.3266	485.3267	439.2929, 325.1811	$C_{30}H_{45}O_5$	0.2	Quillaja sapogenin	Pu et al. (2017)
24	11.20	607.3710	607.3694	503.1711, 325.1867	$C_{30}H_{55}O_{12}$	2.6	NI	–

*NI- Not Identified.

2.9. Statistical analysis

For statistical analysis, the software Graphpad Prism v. 5.0 (San Diego, California, USA). Data were analyzed using the arithmetic mean of triplicates for each concentration tested and further analyzed using two-way ANOVA ($P < 0.05$; *$P < 0.1$; ****$P < 0.0001$), comparing the values for each extract concentration, point by point, using the *post-hoc* Bonferroni test. A general comparison of the behaviour of each substance with the tested strains was inferred through the analysis. Each variable was compared to biofilm growth control and chlorhexidine gluconate.

3. Results and discussion

3.1. Chemical characterization by UPLC-ESI-QToF-MSE

The identification of compounds was performed using UPLC-ESI-QToF-MSE and the chromatogram obtained can be visualized in Fig. 1, where the presence of 24 peaks can be observed, of which 15 were identified, among them jujuboside I (Fig. 2) and quillaja sapogenin. All compounds are listed in Table 1. Among the phenolic compounds, the presence of quercetin and citric acid (Fig. 2) was observed as representative of the class of organic acids. Jujuboside was also found by Lyrio (2016) in the extract obtained from *Sarcomphalus joazeiro* bark. Souza-Neto (2016) identified in chemical analysis the presence of saponins, quercetin and isoquercetin in the hydroethanolic extract of leaves. Quercetin and saponin derivatives were also identified by

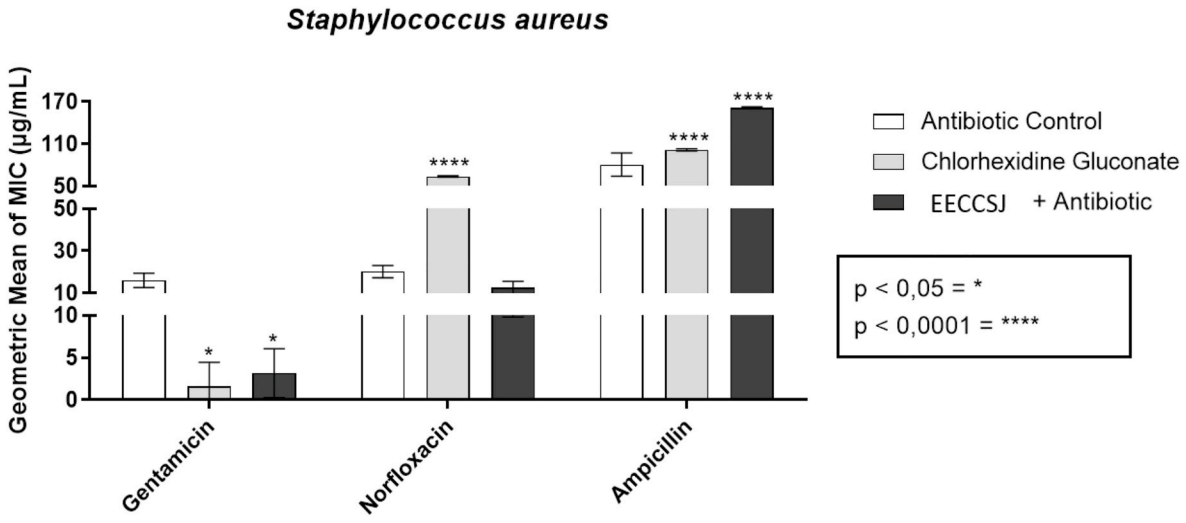

Fig. 3. Modifying effect of the antibiotic activity of an ethanolic extract of *Sarcomphalus joazeiro* on multiresistant strains of *Staphylococcus aureus*, in association with the antibiotics Gentamicin, Norfloxacin and Ampicillin.

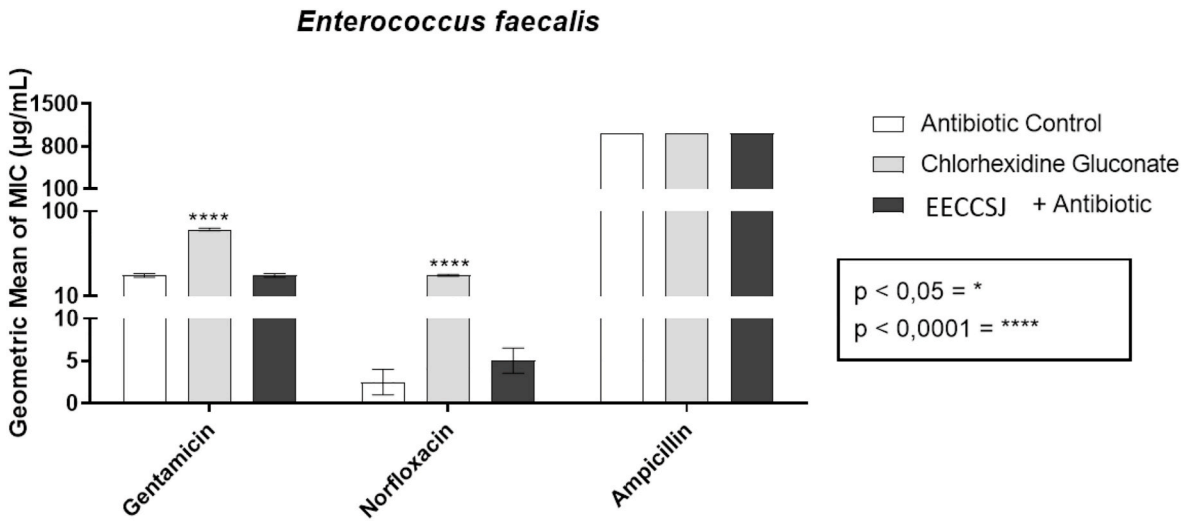

Fig. 4. Modifying effect of the antibiotic activity of an ethanolic extract of *Sarcomphalus joazeiro* on multiresistant strains of *Enterococcus faecalis*, in association with the antibiotics Gentamicin, Norfloxacin and Ampicillin.

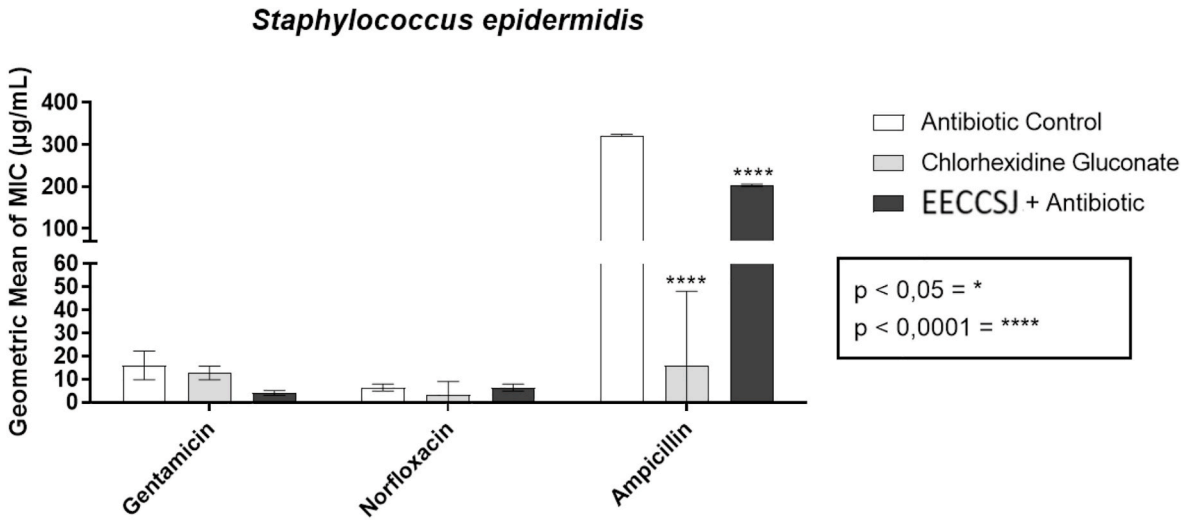

Fig. 5. Modifying effect of the antibiotic activity of an ethanolic extract of *Sarcomphalus joazeiro* on multiresistant strains of *Staphylococcus epidermidis*, in association with the antibiotics Gentamicin, Norfloxacin and Ampicillin.

Andrade et al. (2019a). Brito et al. (2015) identified the presence of saponins, quercetin and isoquercetin in the hydroalcoholic extract of *Sarcomphalus joazeiro*. Saponins were also identified in the phytochemical analysis of *Sarcomphalus joazeiro* stem bark carried out by Melo (2010). Almeida et al. (2017) mention in their research that the saponin derivatives identified as jujubosides are compounds observed in the bark of *Sarcomphalus joazeiro*, classified as an important part of the compounds that give it surfactant or detergent properties.

3.2. Assessment of antibacterial activity and potentiating antibiotic action

The evaluation of the antibacterial activity of the extract showed a value of minimum inhibitory concentration ≥2048 µg/mL, a value considered irrelevant. Silva et al. (2014) report in their results that one of the factors that contribute to the antimicrobial activity of *Sarcomphalus joazeiro* is the presence of saponins that have an inhibitory potential against bacteria. This action varies according to the concentration used. This information was corroborated by Lyrio (2016), who said that the antimicrobial power of a molecule depends on the concentrations, as well as the way it presents in the studied solution. Sidana, Singh and Sharma (2016) also reported the antimicrobial activity of saponins and correlated it with the concentration of the extracts used in the research.

The evaluations of potentiating activity of the antibiotic activity against a strain of *Staphylococcus aureus* (Fig. 3), demonstrated synergistic activity of the extract in association with gentamicin and norfloxacin with MIC points of 3.2 µg/mL for the association with gentamicin and of 12,7 µg/mL with norfloxacin. With ampicillin, the extract showed antagonistic activity with MIC of 161.3 µg/mL, as seen in Fig. 2. The antibiotic controls showed, respectively, MIC of 16 µg/mL, 20.1 µg/mL and 80.6 µg/mL The synergism with gentamicin was also a behaviour observed in the studies by Brito et al. (2015) in research carried out with the hydroalcoholic extract of *Sarcomphalus joazeiro* leaves.

When inhibiting the growth of *Enterococcus faecalis*, the extract showed indifferent behaviour when in association with ampicillin (MIC of 1024 µg/mL) and gentamicin (MIC of 32 µg/mL) demonstrating the same MIC value of controls and antagonism when combined with norfloxacin (MIC 5 µg/mL) when compared to the control that presented MIC 2.5 µg/mL (Fig. 4).

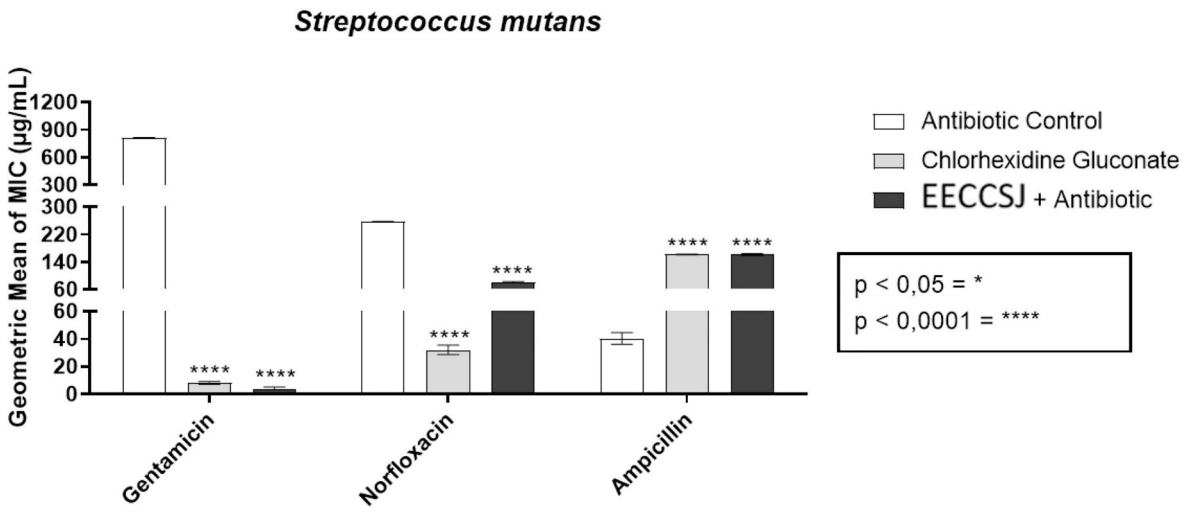

Fig. 6. Modifying effect of the antibiotic activity of an ethanolic extract of *Sarcomphalus joazeiro* on multiresistant strains of *Streptococcus mutans*, in association with the antibiotics Gentamicin, Norfloxacin and Ampicillin.

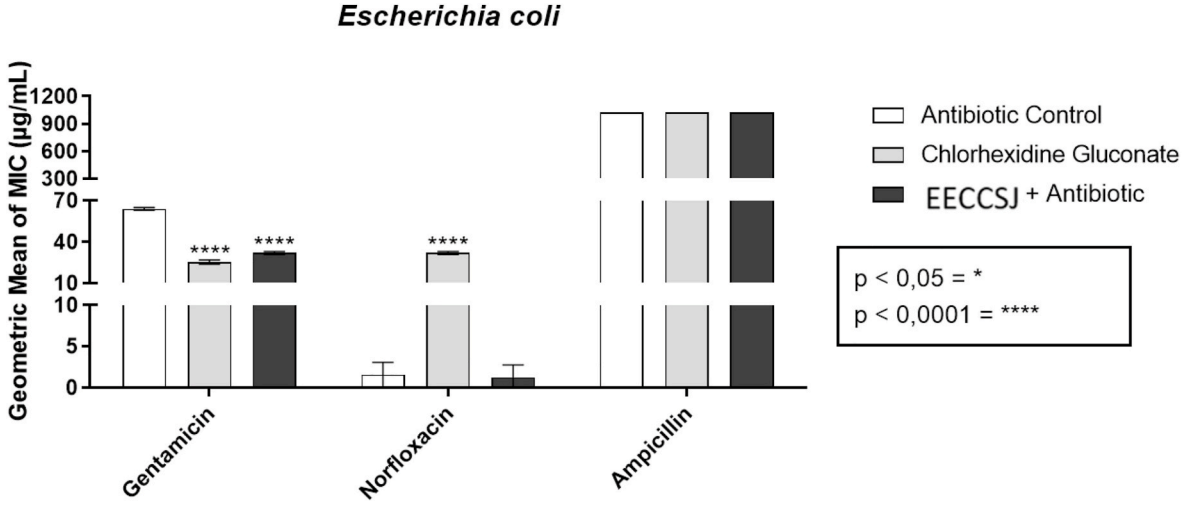

Fig. 7. Modifying effect of the antibiotic activity of an ethanolic extract of *Sarcomphalus joazeiro* on multiresistant strains of *Escherichia coli*, in association with the antibiotics Gentamicin, Norfloxacin and Ampicillin.

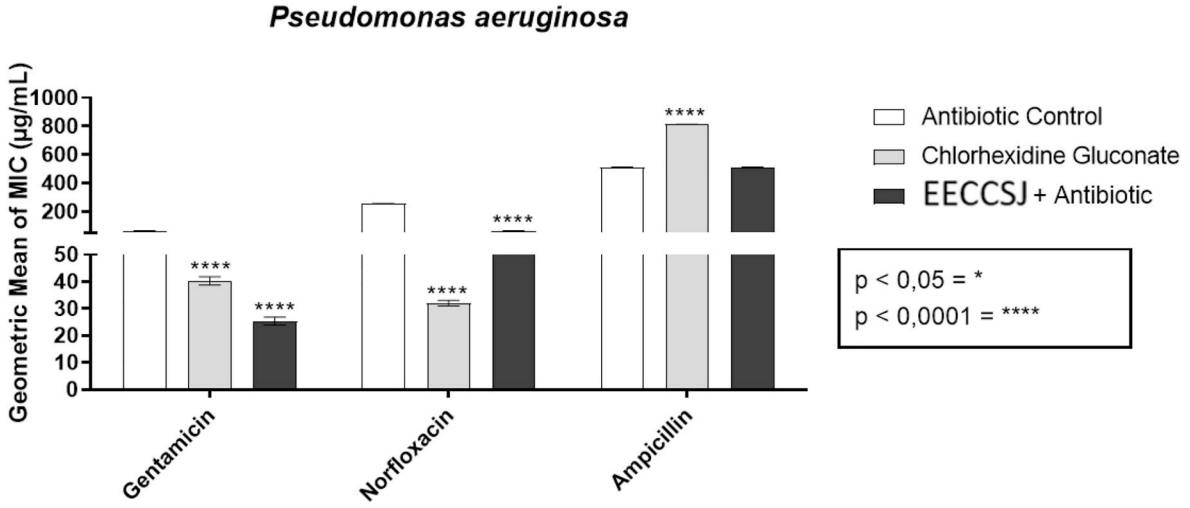

Fig. 8. Modifying effect of the antibiotic activity of an ethanolic extract of *Sarcomphalus joazeiro* on multiresistant strains of *Pseudomonas aeruginosa*, in association with the antibiotics Gentamicin, Norfloxacin and Ampicillin.

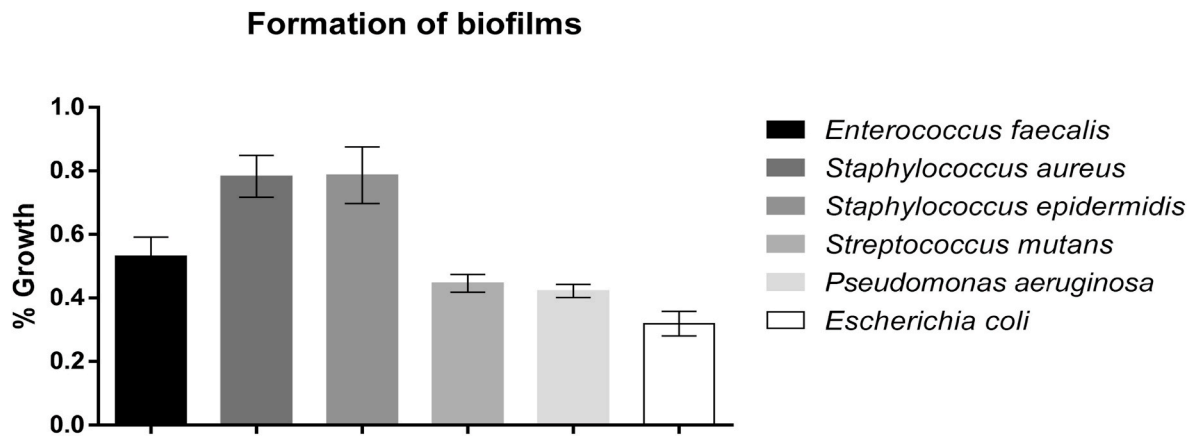

Fig. 9. Capacity of bacterial biofilm formation by the strains evaluated.

Figs. 5 and 6 show the combination of the extract with antibacterial drugs against strains of *Staphylococcus epidermidis* and *Streptococcus mutans*, respectively. It is possible to observe that in the tests with S. *epidermidis*, the ethanolic extract of *Sarcomphalus joazeiro* (EECCSJ) showed synergistic activity when associated with gentamicin with MIC 4 μg/mL and ampicillin with MIC 203.2 μg/mL. Drug controls had a MIC of 16 μg/mL and 322.5 μg/mL. In association with norfloxacin, the extract was indifferent, presenting MIC similar to the control (6.3 μg/mL). On the other hand, against *S. mutans*, synergistic activity of the extract is seen with gentamicin (MIC: 4 μg/mL) and with norfloxacin (MIC: 80.6). In this analysis, controls with MIC of 812.7 μg/mL and 256 μg/mL were observed, while the association with ampicillin showed antagonistic activity, where it was possible to observe a MIC of 40.3 μg/mL for the control and 161.2 μg/mL for the drug solution plus the extract.

In the evaluation of potentiating activity using *Escherichia coli* and *Pseudomonas aeruginosa* strains (Figs. 7 and 8), it is observed that the EECCSJ showed similar results in both cases. In *E. coli*, there was indifferent action with ampicillin (extract and control with MIC of 1024 μg/mL) and synergism in gentamicin, where extract with MIC: 32 μg/mL and control with MIC 64 μg/mL was verified. With norfloxacin, synergy was also proven with a MIC of 1.6 μg/mL for the control and 1.2 μg/mL for the extract. In front of *P. aeruginosa*, the indifferent activity with ampicillin was identified by obtaining a MIC of 512 μg/mL for the extract and the control. In gentamicin, it was possible to perceive a MIC of 64 μg/mL for the control and a MIC of 25.3 μg/mL for the extract. In norfloxacin, the MIC of the control was 256 μg/mL and that of the extract was 64 μg/mL, demonstrating synergism in both situations.

The analyzed extract showed, in most tests, activity potentiating the action of the analyzed antibiotics. One of the factors that may be associated with the observed phenomenon is its chemical constitution, more precisely due to the presence of secondary metabolites (Rodrigues, 2018). Brito et al. (2015) observed that the hydroalcoholic extract of *Sarcomphalus joazeiro* had a synergistic effect when combined with gentamicin. This research relates the antimicrobial potential of *Sarcomphalus joazeiro* with the presence of saponins and its ability to break the integrity of the cell membrane of microorganisms.

The weak inhibition of *E. faecalis* is similar to that obtained by Silva et al. (2011). Melo (2012), when carrying out a study of antibacterial activity with extracts from the fruit, leaf and stem bark of *Sarcomphalus joazeiro*, identified a lower inhibition of *S. aureus* and a good inhibition of *P. aeruginosa*. Results are different from those observed by Lima (2008) who concluded that the aqueous extract of *Sarcomphalus joazeiro* does not inhibit bacterial growth.

In all tests, there was an association with chlorhexidine gluconate. It was observed that the referred product showed an indifferent behaviour when combined with ampicillin both against *E. faecalis* and against *E. coli* with a MIC equal to the control (1024 μg/mL) in both cases. In tests involving *S. aureus*, chlorhexidine showed synergism with gentamicin, when presenting a MIC of 1.7 μg/mL and antagonism with norfloxacin, where it exhibited a MIC of 64 μg/mL, as well as with ampicillin, where there was a MIC 101.6 μg/mL for chlorhexidine.

Table 2
Potential for biofilm formation by bacterial strain.

Clinical isolates	Biofilm formation
Streptococcus mutans INCQS 00446 (ATCC 25175)	Moderate
Enterococcus faecalis INCQS 00018 (ATCC 14506)	Moderate
Staphylococcus epidermidis INCQS 00016 (ATCC 12228)	Strong
Staphylococcus aureus ATCC 25923	Strong
Pseudomonas aeruginosa ATCC 9027	Moderate
Escherichia coli ATCC 259223	Weak

With *E. faecalis*, an antagonistic behaviour of chlorhexidine was observed with gentamicin and norfloxacin, where an inhibitory concentration of chlorhexidine of 80 and 32 μg/mL was observed. In *S. epidermidis*, synergism was observed with gentamicin (MIC of 12.7), norfloxacin (MIC of 3.2 μg/mL) and ampicillin (MIC of: 16 μg/mL).

When performing tests with *S. mutans*, an antagonism with ampicillin was noticed, where the MIC of the drug was 161.2 μg/mL. With gentamicin and norfloxacin, chlorhexidine was shown to be synergistic with MIC of 8 and 32 μg/mL, respectively. In *E. coli* analyses, synergism of chlorhexidine was evidenced with a MIC of 25.3 μg/mL with gentamicin and 32 μg/mL with norfloxacin. Synergism was also present in the tests with *P. aeruginosa* where chlorhexidine expressed a MIC of 40.3 μg/mL with gentamicin and 32 μg/mL with norfloxacin.

In a test carried out by Monteiro et al. (2018), it was observed that chlorhexidine-based products in varying concentrations showed superior antibacterial activity than the other products used in the research against different strains of *S. aureus*. Alves et al. (2021) evaluated the effectiveness of mouthwashes against strains of *Streptococcus mutans* and identified that chlorhexidine-based compounds were the ones that showed the greatest effectiveness. Freitas and Andrade (2021) define chlorhexidine as a compound with important antibacterial properties at different concentrations.

3.3. Evaluation of bacterial biofilm formation and anti-formation

In Fig. 9 it is possible to observe the biofilm formation capacity of the bacterial strains used in the research and in Table 2 the degree of biofilm formation of each species is presented. *Streptococcus mutans*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* showed moderate biofilm formation, while *Staphylococcus epidermidis* and *Staphylococcus aureus* showed strong growth. The *E. coli* strain was excluded from the anti-formation assay because it showed poor growth. It can be seen that

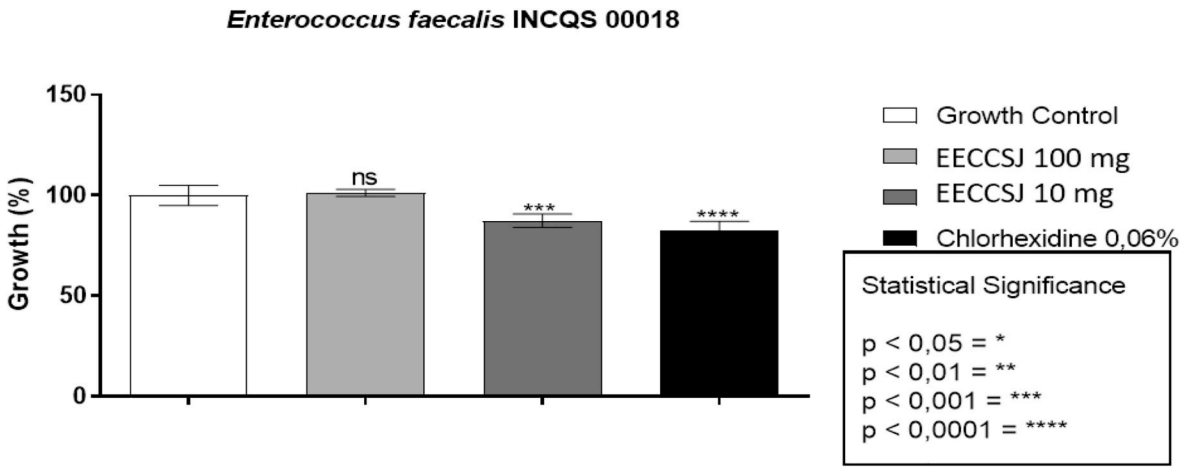

Fig. 10. Eradication of *Enterococus faecalis* biofilm by ethanolic extract of *Sarcomphalus joazeiro*.

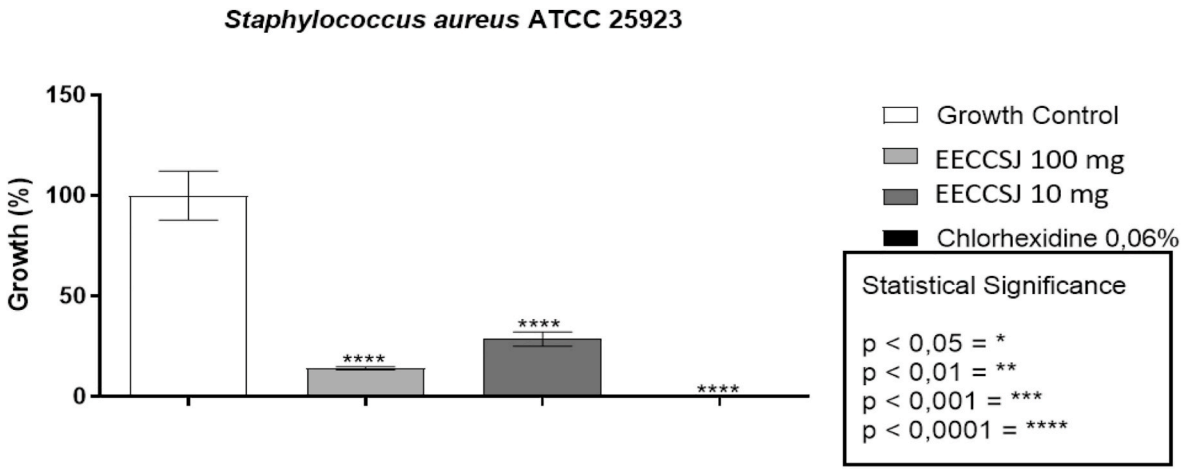

Fig. 11. Eradication of *Staphylococcus aureus* biofilm by ethanolic extract of *Sarcomphalus joazeiro*.

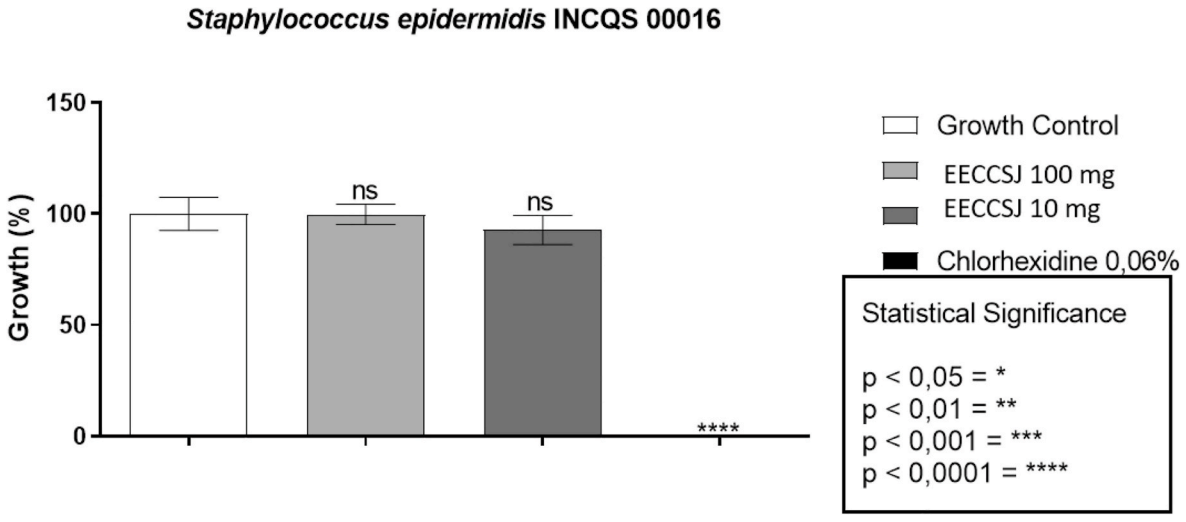

Fig. 12. Eradication of *Staphylococcus epidermidis* biofilm by ethanolic extract of *Sarcomphalus joazeiro*.

the bacterial strains GRAM positive (GRAM +) have a formation capacity ranging from moderate to strong, while the GRAM negative strains (GRAM -) have formation ranging from moderate to weak. Pavão et al. (2021), also identified in their studies the prevalence of a strong biofilm formation among GRAM + bacteria, while Pimentel et al. (2020), evaluated GRAM bacteria - from a hospital environment and identified a biofilm formation between moderate and strong, this fact was attributed to the possibility of growth of these bacteria even in

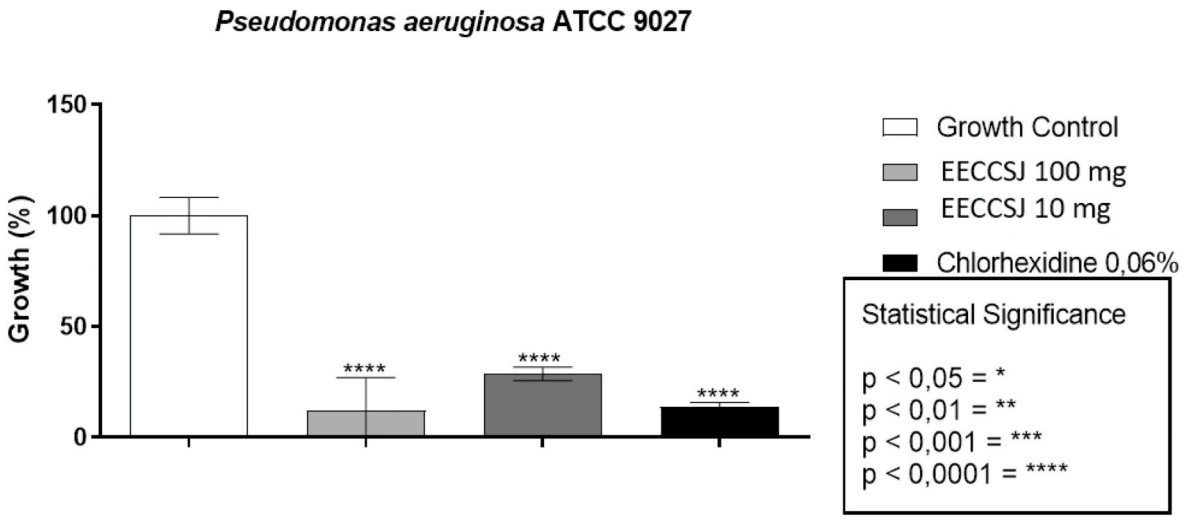

Fig. 13. Eradication of *Pseudomonas aeruginosa* biofilm by ethanolic extract of *Sarcomphalus joazeiro*.

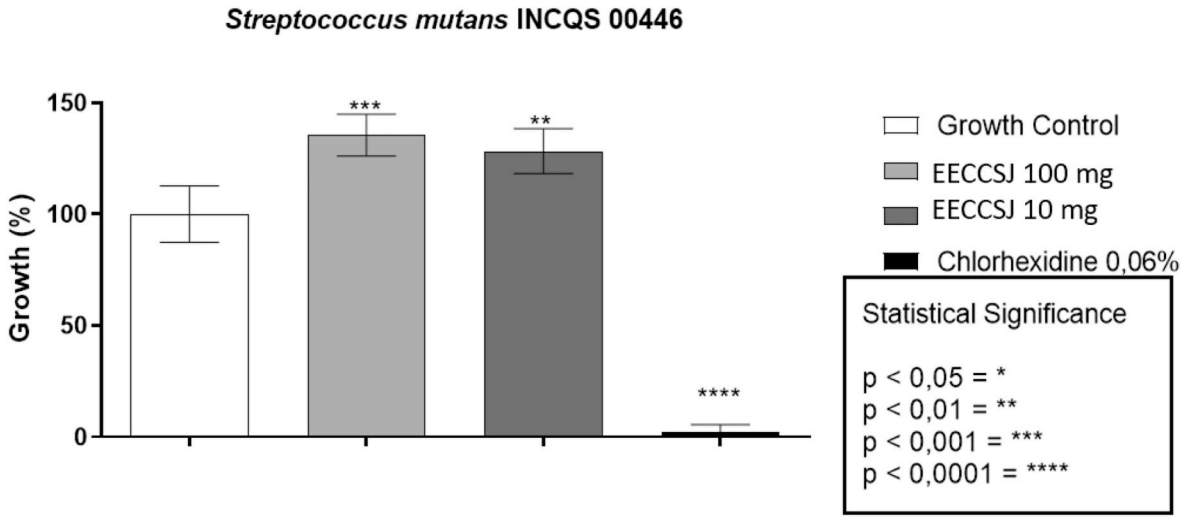

Fig. 14. Eradication of *Streptococcus mutans* biofilm by ethanolic extract of *Sarcomphalus joazeiro*.

environments with minimal supply of nutrients.

In the analysis of the potential for anti-biofilm activity of the ethanolic extract of *Sarcomphalus joazeiro*, a variation in activity was observed between the two concentrations of the extract. In the eradication of *Enterococcus faecalis* biofilm, represented in Fig. 10, EECCSJ at a concentration of 0.1 mg inhibited growth by 87.35%, approaching the inhibition of chlorhexidine gluconate, which was 82.67%.

Against *Staphylococcus aureus* (Fig. 11), the extract at concentrations of 1 mg and 0.1 mg showed inhibition of 14.03% and 28.62%, but with a lower significance than that of chlorhexidine gluconate.

In *Staphylococcus epidermidis* (Fig. 12) inhibition was observed with low significance when compared to chlorhexidine gluconate in the two concentrations that presented values of 99.77% and 92.77%.

The results of *P. aeruginosa* biomass reduction, observed in Fig. 13, demonstrate that the concentrations presented relevant results of 12.35% (1 mg) and 28.7% (0.1 mg). Inhibition obtained results similar to those of chlorhexidine gluconate (13.96%).

With the *S. mutans* strain (Fig. 14), it was observed that there was no inhibition of biofilm formation, since treatment with EECCSJ at concentrations of 1 mg and 0.1 mg presented results of 135.53% and 128.4%. Such results do not demonstrate significance, especially when compared with chlorhexidine gluconate, which showed a reduction of 2.03%.

In a study carried out by Andrade et al. (2019b), inhibition of biofilm masses was observed by the aqueous extract of *Sarcomphalus Joazeiro* stem bark, but it does not have considerable relevance when compared to the action of chlorhexidine gluconate. Against strains of *S. mutans*, Portela (2011) demonstrated that the extract of *Sarcomphalus joazeiro* has an effective effect in eliminating plaque or dental biofilm. Viana et al. (2021) presented results that classify *Sarcomphalus joazeiro* as a compound capable of significantly reducing the formation of biofilm by *S. aureus*.

The analyzes showed an anti-formation potential oscillating between good and low against the biofilm formed by bacteria GRAM+ and GRAM-with the exception of S. mutans, as previously reported. Similar inhibitory activity was identified in two studies carried out by Mizdal et al. (2018; 2018) in tests performed with bacteria belonging to the two groups.

The use of natural products in the control of bacterial biofilm has proven action in the scientific literature and shows great potential for the creation of new drugs. Such compounds act by inhibiting bacterial growth, adherence to surfaces and the reduction of substances such as acids and polysaccharides (Nascimento Júnior, 2020; Stefanello; Souza; Castro, 2017).

4. Final considerations

Based on the results presented, it can be concluded that the ethanolic extract of *Sarcomphalus joazeiro* has indirect antibacterial activity, with significant potentiating action and relevant synergistic activity. The present research is one of the few that presents UPLC-ESI-QToF-MSE characterization of *Sarcomphalus joazeiro*, demonstrating the need for investments in the area. This characterization showed that, among the secondary metabolites that make up the extract, saponin derivatives are the major compounds. Anti-biofilm formation analyzes demonstrated the presence of a significant ability to inhibit bacterial biofilm formation.

Author Contributions

Author Contributions: methodology, Coutinho hdm, Pinheiro jca, Testing, Araujo njs, Silva arp, Costa ms, Barbosa Filho jm, Silva cap, Souza eo, Statistical analysis, Freitas ts, writing, Araujo njs, review and editing, Pinheiro jca, Translation Pereira Junior fn. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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