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ORIGINAL ARTICLE

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Antibiofilm activity in vitro of *Rosmarinus officinalis* and *Syzygium cumini* glycolic extracts on *Staphylococcus* spp. of dentistry interest

Atividade antibiofilme in vitro dos extratos glicólicos de *Rosmarinus officinalis* e *Syzygium cumini* em *Staphylococcus* spp. de interesse odontológico

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ABSTRACT

Objectives: The aim of this study was to identify the slime production and evaluate the effects of *Rosmarinus officinalis* (rosemary) and *Syzygium cumini* (jambolan) glycolic extracts, and 0.12% chlorhexidine (CHX) in biofilms formed by strains of coagulase-positive *Staphylococcus* - CPS and coagulase negative *Staphylococcus* - CNS isolated from the oral cavity. **Material and Methods:** Slime production was evaluated by two methods: the color of colony presented in Congo red agar, and through the amount of slime adhered to polystyrene. Biofilms were grown in acrylic resin discs immersed in broth, inoculated with microbial suspension (106 cells/ml) and incubated at 37°C/48 h. After formation, the biofilms were exposed for 5 minutes to glycol extracts, CHX or saline solution. The viability of biofilms was determined by counting the colony-forming units per milliliter (CFU/ml) in agar, and analyzed statistically by Tukey test ($p < 0.05$). **Results:** The strains S. aureus, S. schleiferi and S. epidermidis obtained the highest values of slime adhered to polystyrene. R. officinalis promoted reductions ranging from 12.1% to 78.7% in biofilms formed by isolates of CPS, and 9.2% to 73.7% in the biofilms of CNS. S. cumini reduced 12% to 55.7% in biofilms of CPS, and 7.9% to 71.5% in biofilms of CNS. With exception of S. saprophyticus, glycol extracts produced significant reductions in biofilms. For five isolates studied, R. officinalis produced greater reductions than CHX. **Conclusion:** R. officinalis and S. cumini showed effective antibiofilm activity against isolates that

RESUMO

Objetivos: O objetivo deste estudo foi identificar a produção de slime e avaliar os efeitos dos extratos glicólicos de *Rosmarinus officinalis* (alecrim), *Syzygium cumini* (jambolão) e 0,12% de clorexidina (CLX) em biofilmes formados por cepas de *Staphylococcus* coagulase positivo (SCP) e *Staphylococcus* coagulase negativo (SCN) da cavidade oral. **Material e Métodos:** A produção de slime foi avaliada por dois métodos: a cor da colônia apresentada em ágar vermelho Congo e pela quantidade de slime aderido ao poliestireno. Os biofilmes foram crescidos em discos de resina acrílica imersos em caldo, inoculados com suspensão microbiana (106 células/ml) e incubados a 37°C/48h. Após a formação, os biofilmes foram expostos durante 5 minutos aos extractos glicólicos, CLX ou solução salina. A viabilidade dos biofilmes foi determinada pela contagem das unidades formadoras de colônias por mililitro (UFC/ml) em ágar e analisada estatisticamente pelo teste de Tukey ($p < 0,05$). **Resultados:** As cepas S. aureus, S. schleiferi e S. epidermidis obtiveram os maiores valores de aderência ao poliestireno. R. officinalis promoveu reduções variando de 12,1% a 78,7% em biofilmes formados por isolados de SCP e 9,2% a 73,7% nos biofilmes de SCN. S. cumini reduziu de 12% a 55,7% nos biofilmes de SCP, e 7,9% a 71,5% nos biofilmes de SCN. Com exceção de S. saprophyticus, os extratos glicólicos produziram reduções estatísticas nos biofilmes. Para cinco isolados estudados, R. officinalis produziu maiores reduções do que CLX. **Conclusão:** R. officinalis e S. cumini mostraram

showed slime production.

atividade antibiofilme efetiva contra isolados que apresentaram produção de slime.

KEYWORDS

Biofilm; *Rosmarinus officinalis*; Slime; *Staphylococcus*; *Syzygium cumini*.

PALAVRAS-CHAVE

Biofilme; *Rosmarinus officinalis*; Slime; *Staphylococcus*; *Syzygium cumini*.

INTRODUCTION

Some *Staphylococcus* species produce mucus, called slime, composed of exopolysaccharide and teichoic acids, considered an important virulence factor that facilitates the adhesion and biofilm formation [1]. Slime allows bacterial cells from clumping together in multilayer biofilm, making them less accessible to the host immune system and to antimicrobial agents [2]. These microbial communities present important therapeutic barriers against many antibiotics and the search for new agents which could inhibit and prevent their formation would be of great use. The search, in medicine and dentistry, for natural products with antibiofilm activity has been increasing in recent years.

Rosmarinus officinalis, popularly known as rosemary, is a medicinal plant originated from the Mediterranean region of Europe and grown in almost all countries with temperate and tropical climates. In culinary, this plant is used as tea or spice. These species have been used in the prevention and/or cure of diseases, such as lack of appetite, asthma, tonsillitis, nasal obstruction and constipation [3].

Syzygium cumini (Syn. Syzygium jambolana, Eugenia jambolana or Eugenia cumini; Family Myrtaceae) commonly known as "jambolão" in Brazil, is a medicinal plant native to India, and it grows naturally in regions with tropical and subtropical climate, and can be commonly found in most Brazilian states. In traditional folk medicine S. cumini has a recognized role as one of the world's most commonly used plants for the treatment of diabetes mellitus, inflammation, ulcers and diarrhea and preclinical studies have also shown it to possess antineoplastic, chemopreventive and radioprotective properties [4].

The aim of the study was to identify the slime production and evaluate the effects of *Rosmarinus officinalis* (rosemary) and Syzygim cumini (jambolan) glycolic extracts compared with 0.12% chlorhexidine (CHX) in biofilms formed by strains of coagulase-positive *Staphylococcus* CPS (S. aureus, S. schleiferi, S. warneri and S. xylosus) and coagulase negative *Staphylococcus* - CNS (S. epidermidis, S. haemolyticus, S. capitis and S. saprophyticus), species of dentistry interest collected from the oral cavity.

METHODS

Microorganisms

Eight *Staphylococcus* isolates from the oral cavity of healthy individuals maintained in our laboratory stock collection were included in the study [5]. The isolates of coagulase-positive used were: S. aureus, S. schleiferi, S. warneri and S. xylosus, and coagulase-negative were: S. epidermidis, S. haemolyticus, S. capitis and S. saprophyticus.

Slime production

Detection of slime by Congo Red Agar (CRA) method

Two different tests were used for the slime production to leave no doubt that the strains used in this study produce this virulence factor.

The method developed by Freeman et al. [6] was used in this study. For detection of slime, the strains were equidistant spot inoculated (~6 mm) in brain heart infusion (BHI) agar supplemented with sucrose (5%) and Congo Red stain (0.08%). Plates were incubated at 37ºC for 24 h. The results were classified as follows: (++) isolates that produced black colonies with dry crystalline consistency were regarded as strong slime production; (+) colonies with color almost black were regarded as moderate slime production; and (-) those showing pink colonies were negative for slime production. All experiments were repeated at least twice. Detection of slime adhered to polystyrene by Microplate (MP) method

Quantitative determination was carried out by the micromethod proposed by Pfaller et al. [7] using tissue culture sterilized plates of 96 flat-bottomed wells (Costar Corning, New York, NY, USA). Each well was filled with 0.2 ml of 105 cells/ml of a bacterial suspension in Tryptic Soy Broth (TSB) supplemented with 0.25% glucose. After 48 h incubation in aerobiosis at 35ºC, the contents were aspirated and the plates were washed twice with phosphate-buffered saline (pH 7.2). The wells were stained with 0.25% safranin for 30 s. The plates were read in an enzyme-linked immunosorbent assay reader (ELX 808, Bio Tek Instruments, USA) to 490 nm. Sterile TSB supplemented with 0.25% glucose was used as negative control. All experiments were repeated at least twice; the values of optical density (OD) were then averaged. A three-grade scale was used to evaluate the slime producing ability: (++) strong positive OD >1.500, (+) positive OD 0.500-1.500 and (-) negative OD <0.500.

Production of biofilms and antibacterial assays

The biofilm formation was developed as methodology proposed by Pereira et al. [8] with modifications. Standard suspensions of each isolate, with optical density relative to 106 cells/ml were prepared. For this purpose, the isolates were seeded onto BHI agar and incubated at 37°C for 24 h. After incubation, the growth was suspended in sterile physiological solution [0.9% sodium chloride (NaCl)] and the number of cells in suspension was counted in a spectrophotometer (B582, Micronal, São Paulo, SP, Brazil). The parameters of optical density and wavelength used were, respectively, 0.374 and 490 nm.

Three hundred and twenty acrylic resin (AC) discs (Clássico, São Paulo, SP, Brazil), with 11 mm diameter, sterilized in a 20 kGy gamma radiation chamber (cobalt 60) for 6 h (Embrarad, São Paulo, SP, Brazil) were used for growing the biofilms (40 for each microorganism). After sterilization, the AC discs were placed in empty wells - 24 wells (Costar Corning, New York, NY, EUA) with 2 ml of sterile BHI broth supplemented with 5% sucrose, and inoculated with 0.1 ml of bacterial suspension. The AC discs were then incubated at 37°C for 48 h. The media were not refreshed during the period of incubation.

For the biofilm susceptibility testing were used glycol extracts of *Rosmarinus officinalis* (rosemary) and *Syzygium cumini* (jambolan). The extracts were prepared from the leaves of plants and solvent (propylene glycol) at a concentration of 200 mg/ml (Yod Ervas, Campinas, SP, Brazil). The antibacterial effects of extracts were compared with the effect of 0.12% chlorhexidine (CHX) solution (Byoformula, São Paulo, SP, Brazil). The 0.9% NaCl was used as a negative control. The discs with biofilms established after 48 h were exposed for 5 min in contact with 2 ml of each solutions.

After the experimental periods, each biofilm was washed with 2 ml of 0.9% NaCl to remove the substances tested. The discs were placed in tubes containing 10 ml of 0.9% NaCl and sonicated (Sonoplus HD 2200, Bandelin Eletronic, Berlin, Berlin, Germany) for 30 s at 50 W to disperse the biofilms. Biofilms suspensions

were serially diluted in 0.9% NaCl to give dilutions of 10-1 to 10-5 times the original concentration. One hundred microliter aliquots of each dilution were seeded in duplicate on BHI agar. After 48 h of incubation, the number of colony-forming units per milliliter (CFU/ml) was determined. The results were submitted to analysis of variance (ANOVA) and the Tukey test ($p < 0.05$). The percentage of reduction presented was calculated having the 0.9% NaCl results as reference.

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to illustrate the biofilms formed and the antibacterial activity of the different solutions on biofilms. S. aureus and S. epidermidis isolates were used for formation of the biofilms. The discs were fixed for 2.5% glutaraldehyde for 1 h and dehydrated with several ethanol washes (10%, 25%, 50%, 75% and 90% for 20 min and 100% for 1 h). The samples were then incubated at 37°C for 24 h to dry the discs. The discs were transferred to aluminum stubs and covered with gold for 120 s at 40 mA (Denton Vacuum Desk II, Denton Vacuum, USA). After metallization, the biofilms were examined and photographed by SEM (JSM-5310, JEOL, Japan), operating at 15 kV in increments of 1000 and 5000 times.

RESULTS

The summarized results of CRA, MP tests, and the distribution of slime production in staphylococci isolates are shown in Table I. In CRA test S. aureus, S. schleiferi and S. epidermidis isolates produced black colonies, indicating strong production of slime. These same isolates were those who obtained the highest values of OD in MP test, however, S. aureus was the only isolate that has OD >1.500.

Mean and standard deviation values of the CFU/ml (log_{10}) obtained in the four experimental conditions tested for each biofilms formed with isolates of coagulase-positive staphylococci group are shown in Figure 1. With the exception of S. scheleiferi, for the remaining isolates of coagulase-positive staphylococci, the R. officinalis extract promoted a greater reduction than S. cumini extract and CHX in the biofilms. In the isolates of coagulase-negative staphylococci, both extracts promoted greater reductions than CHX in biofilms formed with the species of S. epidermidis and S. saprophyticus (Figure 2).

Table I - Distribution of slime production in Staphylococcus isolates with congo red agar and microplate methods

Isolates	CRA		MP	
	Color Colonies	Slime Production	Optical density	Slime Production
S. aureus	Black	++	1.651	++
S. schleiferi	Black	++	0.640	+
S. warneri	Almost black	+	0.510	+
S. xylosus	Almost black	+	0.515	+
S. epidermidis	Black	++	0.720	+
S. haemolyticus	Almost black	+	0.540	+
S. capitis	Almost black	+	0.616	+
S. saprophyticus	Almost black	+	0.580	+

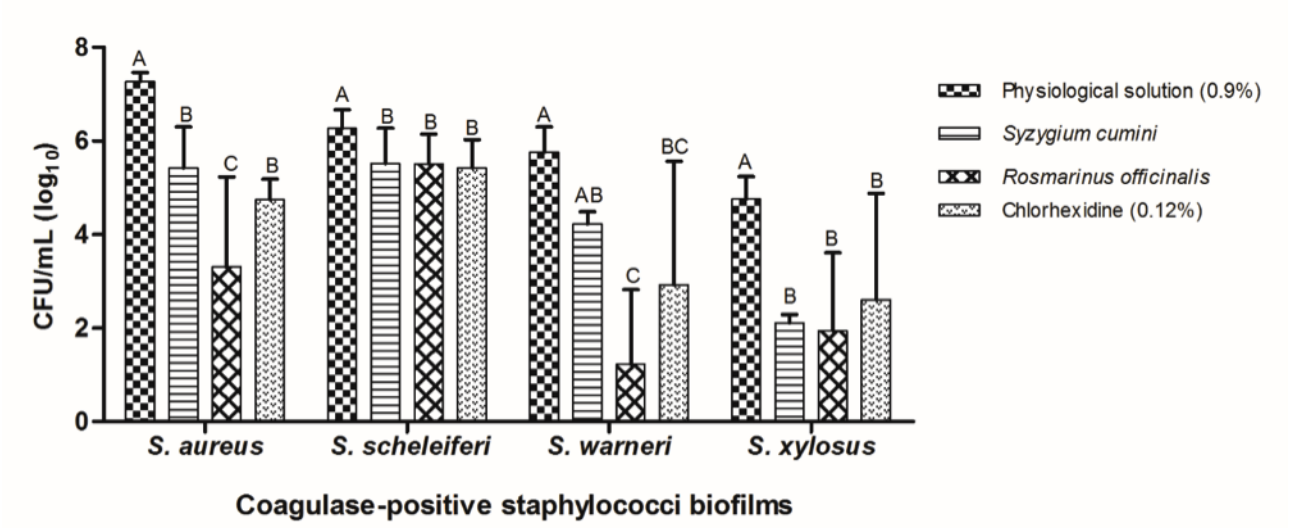

Figure 1 - Mean values and standard deviation of CFU/mL of CPS biofilms exposed to the following treatments: physiological solution (0.9% NaCl); Syzygium cumini glycol extract; Rosmarinus officinalis glycol extract; and chlorhexidine (0.12%). To analyze significance, a Tukey test was used. Values followed by different capital letters differed significantly among the experimental conditions (P<0.05).

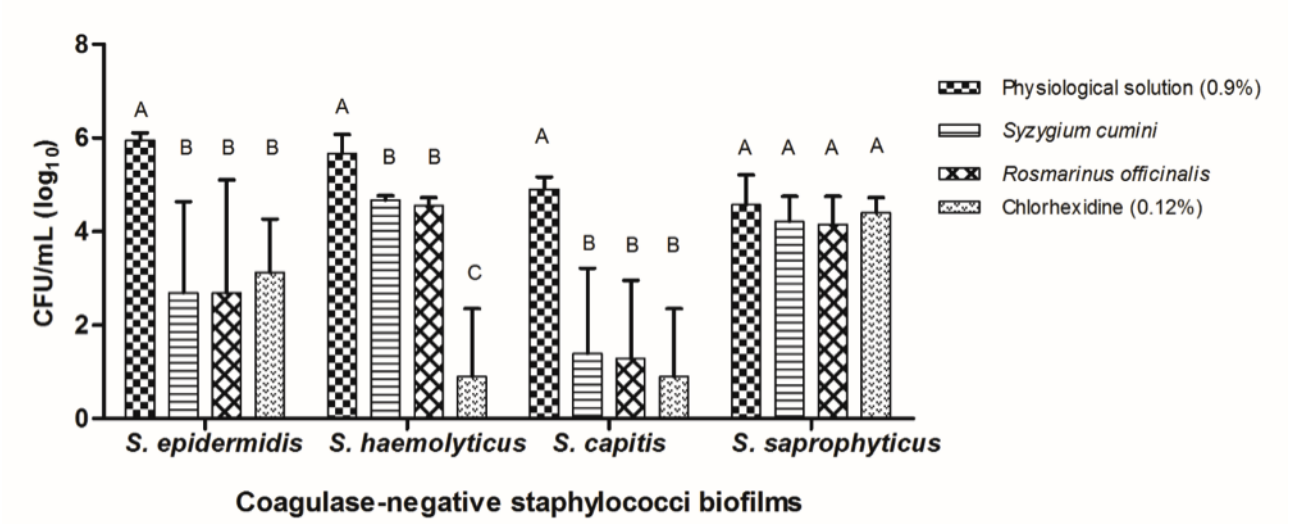

Figure 2 - Mean values and standard deviation of CFU/mL of CNS biofilms exposed to the following treatments: physiological solution (0.9% NaCl); Syzygium cumini glycol extract; Rosmarinus officinalis glycol extract; and chlorhexidine (0.12%). To analyze significance, a Tukey test was used. Values followed by different capital letters differed significantly among the experimental conditions (P<0.05).

In Table II are shown the mean (log_{10}) and percentages reduction for biofilms formed by each isolated treated with extracts or CHX, in relation to the untreated controls biofilms (that was treated only with physiological solution 0.9% NaCl). The highest percentage reduction with S. cumini extract was observed in S. capitis biofilms (71.5%). Already with R. officinalis extract, the largest percentage reduction was found in S. warneri biofilms (78.7%). CHX also promoted a large reduction in S. capitis biofilms (81.7%).

Table II - Percentages reduction for biofilms formed by each isolated exposed for 5 min

Staphylococcus spp.	Glycol extracts		Chlorhexidine (0.12%)
	R. officinalis	S. cumini	
S. aureus	54.3%	25.5%	34.7%
S. schleiferi	12.1%	12%	13.4%
S. warneri	78.7%	26.6%	49.1%
S. xylosus	59.2%	55.7%	45.2%
S. epidermidis	54.9%	54.8%	47.4%
S. haemolyticus	19.6%	17.5%	84.1%
S. capitis	73.7%	71.5%	81.7%
S. saprophyticus	9.2%	7.9%	3.9%

Percentages of reduction for R. officinalis or S. cumini glycol extracts, chlorhexidine (0.12%), in relation to the untreated controls biofilms (that was exposed only to physiological solution 0.9% NaCl).

Scanning electron microscopy (SEM) was used to evaluate the biofilms, as well as the effects of extracts and CHX on these microbial communities, and micrographs are shown in Figure 3 (S. aureus biofilms) and Figure 4 (S. epidermidis biofilms). In SEM analysis, the untreated controls biofilms formed by S. aureus and S. epidermidis species exhibited aggregated cocci covered with abundant extracellular matrix. When submitted to extracts or CHX, the biofilms formed were damaged exhibiting a few cells on the substratum and a decrease of the extracellular matrix.

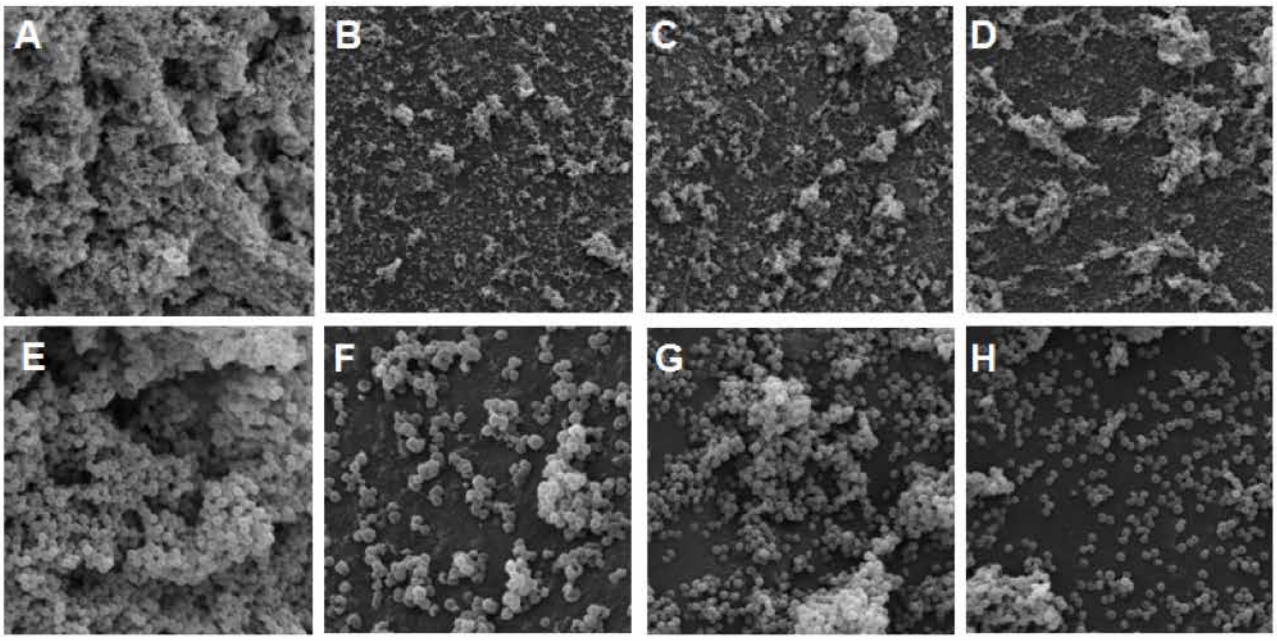

Figure 3- Scanning electron microscope (SEM) micrographs of Staphylococcus aureus biofilms: Images A and E refers to control biofilms exposed for 5 min to physiological solution 0.9% NaCl. Images B and F show biofilms exposed for 5 min to Rosmarinus officinalis glycol extract. Images C and G show biofilms exposed for 5 min to Syzygium cumini glycol extract. Images D and H show biofilms exposed for 5 min to chlorhexidine (0.12%). Magnification: A, B, C and D= 1000X; and, E, F, G and H =5000X.

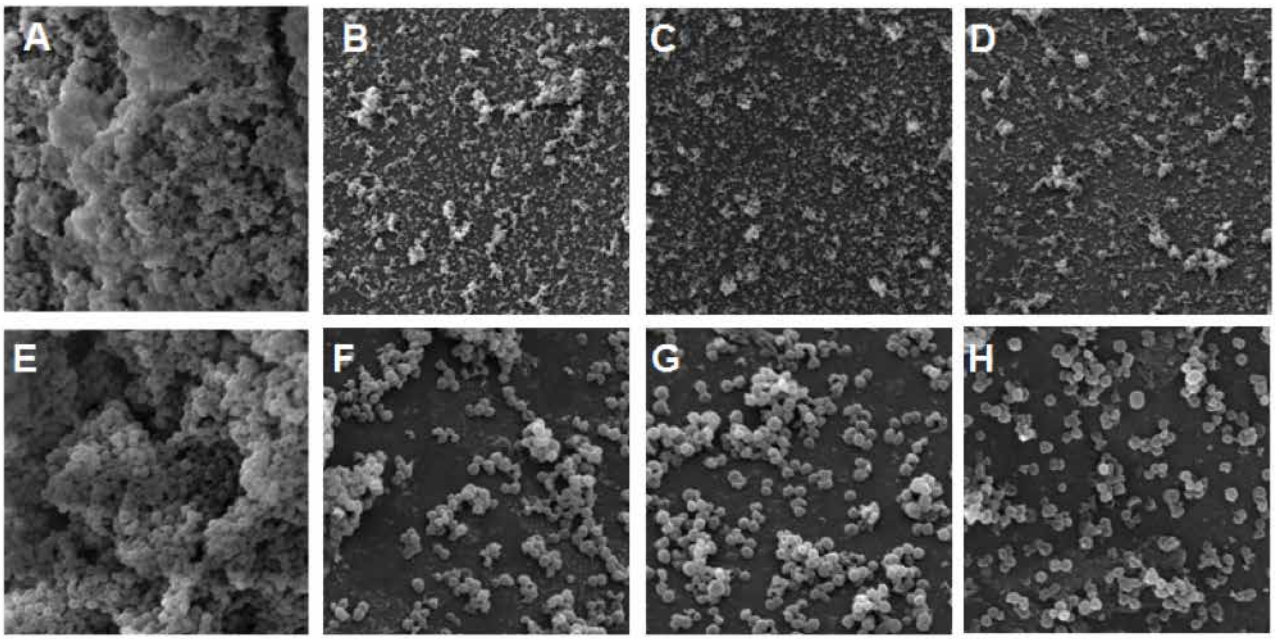

Figure 4 - Scanning electron microscope (SEM) micrographs of Staphylococcus epidermidis biofilms: Images A and E refers to control biofilms exposed for 5 min to physiological solution 0.9% NaCl. Images B and F show biofilms exposed for 5 min to Rosmarinus officinalis glycol extract. Images C and G show biofilms exposed for 5 min to Syzygium cumini glycol extract. Images D and H show biofilms exposed for 5 min to chlorhexidine (0.12%). Magnification: A, B, C and D= 1000X; and, E, F, G and H =5000X.

DISCUSSION

In this study S. aureus was the only species studied that showed strong production of slime in the two tests used. S. epidermidis and S. schleiferi, also demonstrated strong production of slime in the color of colonies in CRA, and were positive for slime production in the microplates test. Previous studies also gave an insight into the mechanism of slime production and adherence of slime-forming CNS to polystyrene plates and polypropylene tubes [9].

The search for new products with high pharmacological activity, less toxic and costs more affordable for the population have increased in the last years. Plant-derived compounds inhibit peptidoglycan synthesis and modulate quorum sensing [10] , damage microbial membrane structures [11], modify bacterial membrane surface hydrophobicity [12], all of which could influence biofilm formation.

Antibacterial activity of glycolic extracts of R. officinalis and S. cumini in biofilms formed by different species of staphylococci has also been tested in this study. With exception of S. saprophyticus, the glycolic extracts of R. officinalis and S. cumini produced reductions antibiofilm statistically significant ($p < 0.05$).

When we compare the extracts used, R. officinalis presented the best antibiofilm effect. R. officinalis extract utilized in this study promoted reductions ranging from 12.1% to 78.7% in biofilms formed by isolates of coagulase-positive staphylococci, and 9.2% to 73.7% in biofilms of coagulase-negative staphylococci. The antimicrobial activity of R. officinalis can be attributed to secondary metabolites, such as diterpenes. This metabolite has lipophilic character, and it acts in biological membranes, in the lipid matrix decreasing hydrophobic interactions, causing expansion of the membrane, increased fluidity, structural disorder and the inhibition of enzymes embedded in this site [13]. These effects may probably be responsible for the antibacterial activity promoted in biofilms by this extract in

this study. The study conducted by Quave et al. [14] with 168 extracts, botanical representing 104 botanical species, R. officinalis was one of the 10 extracts that exhibited antibiofilm activity against methicillin-resistant S. aureus (MRSA).

The reductions observed with glycolic extract of S. cumini in this study ranged from 12% to 55.7% in biofilms formed by isolates of coagulase-positive staphylococci, and 7.9% to 71.5% in biofilms of coagulase-negative staphylococci. According phytochemical investigations, the leaves of S. cumini are rich in phenolic compounds, quercetin, myricetin, sitosterol, betulinic acid, terpenoids, alkaloids and lignans [15]. The phenolic compounds inhibit bacterial and fungal enzymes, or associate with the substrates of these enzymes, altering metabolism, acting on their cell membranes or complexing with metal ions essential for microbial metabolism [16]. Results of a study showed the antibacterial activity of S. cumini against planktonic cultures of Escherichia coli, Salmonella typhi, and Porphyromonas aeruginosa [17].

The extracts R. officinalis and S. cumini showed great antibiofilm activity against S. xylosus, reduced more than 50% of this biofilm. S. xylosus is a commensal bacteria that is found occasionally in humans [18], and reports describe opportunistic infections in humans with this bacteria [19]. For S. aureus, only R. officinalis extract presented more than 50% of antibiofilm activity. S. aureus is an opportunistic pathogenic microorganism that has developed antibiotic resistance to penicillin by beta-lactamase plasmid, and causes a wide range of infections, including acute, chronic, and toxin-mediated disease [20].

Among the CNS, S. capitis biofilms showed the greatest reduction with the extracts, more than 70%. These results are very important findings, because it shows antimicrobial activity of the extracts utilized to this species, that, consistent with previous study [21], S. capitis isolates showed a high level of oxacillin resistance. The extracts also inhibited more than 50% of S. epidermidis biofilms, bacteria that demonstrated, in this study, strong production of slime, evidencing its potential virulence. S. epidermidis is a recognized opportunistic pathogen, responsible for nosocomial infections of indwelling medical devices [22], may also cause peritonitis, otitis, urinary tract infections, and septicemia [23].

In the present study was used as positive control 0.12% CHX digluconate, and it can be observed that effects similar or smaller to those showed in the extracts analyzed. The reductions media ranged from 13.4 to 49.1% to coagulase-positive staphylococci. When we compared the results obtained with the extracts, only for the S. scheleiferi biofilms CHX showed greater efficacy. To coagulase-negative staphylococci, CHX obtained more effective action than extracts for biofilms formed by S. haemolyticus (84.1%) and S. capitis (81.7%). In vivo experiments with the antibiofilm properties revealed that the different concentration of R. officinalis essential oils was significantly ($p < 0.001$) more effective than CHX in biofilm of Streptococcus mutans and Streptococcus pyogenes [24].

CHX occupies a prominent role among the antiseptics used for chemical control of biofilm in dentistry, unfortunately, the long-term use of CHX is limited by the adverse effects related: staining of teeth, soft tissues, restorations and prostheses; excess formation of supragingival calculus, soft-tissue lesions in young patients, allergic responses, dysgeusia, parotid enlargement, and desquamation of the oral mucosa; urticaria; and, rarely reversible swelling lips or glands parotid [25]. These factors have encouraged the search for other antimicrobial agents.

To illustrate the effects of different treatments on biofilms, two species were selected, S. aureus and S. epidermidis. These species are respectively the most prevalent

coagulase-positive and coagulase-negative staphylococci isolated from the oral cavity of individuals with denture stomatitis [26]. In the SEM analysis, could be observed in the untreated controls biofilms of the both species large cocci surrounded by extracellular matrix. However, in biofilms exposed to extracts or CHX, few cells were observed. These data suggest that extracts or CHX showed antibiofilm activity, reducing the cells of the biofilms.

Another important data of this work was the use of glycol extracts without alcohol addiction. Most mouthwashes with anti-biofilm properties (essential oil and some CHX mouthwashes) contain denatured alcohol as a delivery vehicle. Although the antimicrobial action of alcohol in these solutions can be null, its use has been questioned because, according to some authors, the alcohol used for long-term may have carcinogenic potential [27], increasing the risks of cancer of the mouth, pharynx, esophagus and liver [28]. Currently, there is concern for the removal of alcohol in mouthwashes, so that many companies are producing alcohol free mouthwashes. Aiming for a possible clinical applicability of R. officinalis and S. cumini extracts in mouthwashes or root canal irrigation, it was decided in this study the use of alcohol-free extracts.

CONCLUSION

Based on the current results, it can be concluded that R. officinalis and S. cumini glycol extracts showed effective antibiofilm activity against the isolates tested in this study that showed slime production, an important virulence factor of *Staphylococcus*. This finding might mean an alternative to control biofilms and treatment of diseases occasioned through them in the oral cavity.

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REFERENCES

1. Vuong C, Otto M. *Staphylococcus* epidermidis infections. Microbes Infect. 2002 Apr;4(4):481-9.
2. Arciola CR, Campoccia D, Gamberini S, Cervellati M, Donati E, Montanaro L. Detection of slime production by means of an optimised Congo red agar plate test based on a colourimetric scale in *Staphylococcus* epidermidis clinical isolates genotyped for ica locus. Biomaterials. 2002 Nov;23(21):4233-9.
3. Bai N, He K, Roller M, Lai CS, Shao X, Pan MH, et al. Flavonoids and phenolic compounds from *Rosmarinus officinalis*. J Agric Food Chem. 2010 May 12;58(9):5363-7.
4. Baliga MS. Anticancer, chemopreventive and radioprotective potential of black plum (Eugenia jambolana lam.). Asian Pac J Cancer Prev. 2011;12(1):3-15.
5. Querido SM, Back-Brito GN, Dos Santos SS, Leao MV, Koga-Ito CY, Jorge AO. Opportunistic microorganisms in patients undergoing antibiotic therapy for pulmonary tuberculosis. Braz J Microbiol. 2011 Oct;42(4):1321-8.
6. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989 Aug;42(8):872-4.
7. Pfaller M, Davenport D, Bale M, Barrett M, Koontz F, Massanari RM. Development of the quantitative micro-test for slime production by coagulase-negative staphylococci. Eur J Clin Microbiol Infect Dis. 1988 Feb;7(1):30-3.
8. Pereira CA, Romeiro RL, Costa AC, Machado AK, Junqueira JC, Jorge AO. Susceptibility of Candida albicans, *Staphylococcus* aureus, and Streptococcus mutans biofilms to photodynamic inactivation: an in vitro study. Lasers Med Sci. 2011 May;26(3):341-8.
9. Sakarya S, Oncu S, Ozturk B, Tuncer G, Sari C. Neuraminidase produces dose-dependent decrease of slime production and adherence of slime-forming, coagulase-negative staphylococci. Arch Med Res. 2004 Jul-Aug;35(4):275-8.
10. Gao M, Teplitski M, Robinson JB, Bauer WD. Production of substances by Medicago truncatula that affect bacterial quorum sensing. Mol Plant Microbe Interact. 2003 Sep;16(9):827-34.
11. Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, et al. The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). J Appl Microbiol. 2000 Jan;88(1):170-5.
12. Turi M, Turi E, Koljalg S, Mikelsaar M. Influence of aqueous extracts of medicinal plants on surface hydrophobicity of Escherichia coli strains of different origin. APMIS. 1997 Dec;105(12):956-62.
13. Doolaege EH, Raes K, Smet K, Andjelkovic M, Van Poucke C, De Smet S, et al. Characterization of two unknown compounds in methanol extracts of rosemary oil. J Agric Food Chem. 2007 Sep 5;55(18):7283-7.
14. Quave CL, Plano LR, Pantuso T, Bennett BC. Effects of extracts from Italian medicinal plants on planktonic growth,

biofilm formation and adherence of methicillin-resistant *Staphylococcus* aureus. J Ethnopharmacol. 2008 Aug 13;118(3):418-28.

15. Srivastava S, Chandra D. Pharmacological potentials of *Syzygium cumini*: a review. J Sci Food Agric. 2013 Jul;93(9):2084-93.
16. Scalbert A, Mila I, Expert D, Marmolle F, Albrecht AM, Hurrell R, et al. Polyphenols, metal ion complexation and biological consequences. Basic Life Sci 1999;66:545-54.
17. Shad AA, Ahmad S, Ullah R, AbdEl-Salam NM, Fouad H, Ur Rehman N, Hussain H, Saeed W. Phytochemical and biological activities of four wild medicinal plants. ScientificWorldJournal. 2014;2014:857363. doi: 10.1155/2014/857363.
18. Nagase N, Sasaki A, Yamashita K, Shimizu A, Wakita Y, Kitai S, et al. Isolation and species distribution of staphylococci from animal and human skin. J Vet Med Sci. 2002 Mar;64(3):245-50.
19. Koksal F, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative *staphylococcus* strains isolated from blood cultures of septicemic patients in Turkey. Microbiol Res. 2009;164(4):404-10.
20. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus* aureus in the antibiotic era. Nat Rev Microbiol. 2009 Sep;7(9):629-41.
21. Cui B, Smooker PM, Rouch DA, Daley AJ, Deighton MA. Differences between two clinical *Staphylococcus* capitis subspecies as revealed by biofilm, antibiotic resistance, and pulsed-field gel electrophoresis profiling. J Clin Microbiol. 2013 Jan;51(1):9-14.
22. Otto M. *Staphylococcus* epidermidis--the 'accidental' pathogen. Nat Rev Microbiol. 2009 Aug;7(8):555-67.
23. Kumari N, Rai A, Jaiswal CP, Xess A, Shahi SK. Coagulase negative Staphylococci as causative agents of urinary tract infections-prevalence and resistance status in IGIMS, Patna. Indian J Pathol Microbiol. 2001 Oct;44(4):415-9.
24. Rasooli I, Shayegh S, Taghizadeh M, Astaneh SD. Phytotherapeutic prevention of dental biofilm formation. Phytother Res. 2008 Sep;22(9):1162-7.
25. Quirynen M, Avontroodt P, Peeters W, Pauwels M, Coucke W, van Steenberghe D. Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. J Clin Periodontol. 2001 Dec;28(12):1127-36.
26. Pereira CA, Toledo BC, Santos CT, Pereira Costa AC, Back-Brito GN, Kaminagakura E, et al. Opportunistic microorganisms in individuals with lesions of denture stomatitis. Diagn Microbiol Infect Dis. 2013 Aug;76(4):419-24.
27. Carretero Pelaez MA, Esparza Gomez GC, Figuero Ruiz E, Cerero Lapiedra R. Alcohol-containing mouthwashes and oral cancer. Critical analysis of literature. Med Oral. 2004 Mar-Apr;9(2):120-3, 116-20.
28. Marques LA, Eluf-Neto J, Figueiredo RA, Gois-Filho JF, Kowalski LP, Carvalho MB, et al. Oral health, hygiene practices and oral cancer. Rev Saude Publica. 2008 Jun;42(3):471-9.

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