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Natural Disinfectant Emulgel Associated with Antimicrobial Photodynamic Therapy for Prevention Bovine Mastitis

A. S. Bispo[a], G. de Paula[a], J. B. da Silva[b], R. B. Balbinot[c], C. Nakamura[c], R. C. da Silva Junior[d], J. M. Bragatto[a], W. Caetano[d], & M. S. S. Pozza[a,*]

[a] Animal Science Department, Maringa State University (UEM), Maringá-PR, Brazil
[b] Pharmaceutical Science Department, Maringa State University (UEM), Maringá-PR, Brazil
[c] Biological Science Department (UEM), Maringa State University (UEM), Maringá-PR, Brazil
[d] Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), Maringa State University (UEM), Maringá-PR, Brazil
*Corresponding author: msspozza@uem.br
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ABSTRACT

Conventionally, disinfectants based on iodine, hypochlorite, or lactic acid are used in post-dipping for teat hygiene on dairy farms. As a natural alternative for the prevention of bovine mastitis, jurema-preta (*Mimosa tenuiflora*), copaiba oil *(Copaifera martii),* and *Chlorella vulgaris* have antimicrobial and anti-inflammatory properties. Associated with antimicrobial photodynamic therapy (PDT), these substances inhibit *Staphylococcus aureus*. This study developed two natural emulgels containing these extracts and evaluated their stability, bioadhesiveness, texture, and cytotoxicity for post-dipping to prevent mastitis. The emulgel was developed using Carbopol 934 P (0.25% w/w), jurema extract (12.5% w/w), and *Chlorella* extract (0.2% w/w), which was dissolved in copaiba oil (4% w/w). Twelve Holstein and Jersey cows were treated with: T1 - control with the use of iodine, T2 - application of emulgel with black jurema and copaiba oil without lighting, and T3 - application of emulgel with black jurema, copaiba oil, and *C. vulgaris* with lighting. The data obtained *in vitro* and *in vivo* were submitted to analysis of variance. For the texture parameters of the emulgel, the formulations showed stability, pseudoplastic behavior, and elastic nature, with no cytotoxic effect. The *in vitro* study showed that the treatments using light were effective in reducing *S. aureus* and the extracts were also effective in breaking up pre-formed biofilm by this bacterium. In the *in vivo* test, there was no significant difference between treatments for somatic cell counts and *Staphylococcus* in milk and teat swabs. The emugel has antimicrobial potential and can replace iodine for use as post-dipping without cytotoxic effects.

Keywords*: *dairy cows; natural extracts; photoinactivation; milk quality

INTRODUCTION

Therapeutic alternatives for mastitis prevention have been gaining prominence to reduce antibiotic dependence and bacterial resistance, making milk production more sustainable. *Mimosa tenuiflora* (jurema-preta) has antimicrobial properties (Santos *et al*., 2022). Copaiba oil (*Copaifera martii*), extracted from trees of the genus Copaifera, is known for its anti-inflammatory activities in the treatment of skin diseases (Símaro *et al*., 2020) and is also effective in the treatment of mastitis, with an antibiotic effect and tissue repair (Oliveira *et al*., 2020).

The microalgae *Chlorella vulgaris* has antioxidant, antimicrobial, and anti-inflammatory properties, inhibiting bacteria that cause mastitis (da Silva-Junior *et al*., 2020). Some pigments in microalgae, such as chlorophyll, may have photosensitizing properties, generating reactive species or singlet oxygen, which cause cell damage, in a treatment known as photodynamic therapy (Campanholi *et al*., 2022a).

Considering the problems caused by bovine mastitis, antimicrobial photodynamic therapy (aPDT) uses light-activated photosensitizing compounds to generate reactive oxygen species, inhibiting microorganisms and biofilms, contributing to milk quality (Teichert *et al*., 2002). *In situ* studies confirm the aPDT efficacy in post-dipping (da Silva Junior *et al*., 2019; Rodrigues *et al*., 2022). The present study evaluated a new and innovative formulation combined with photodynamic inactivation of microorganisms, proposing a photosensitive disinfectant for use as post-dipping, containing natural extracts incorporated into a polymeric matrix, aiming to reduce *Staphylococcus aureus* contamination in the milk and teats of animals.

MATERIALS AND METHODS

Collection of Plant/Material and Extract Preparation

The copaiba oil-resin was collected in the Brazilian municipality of Bailão-PA. The copaiba oil-resin

harvesting method employed a traditional 1.0-inch hand drill for mechanical drilling in the middle of the trunk. The perimeter of the tree was greater than 1.20 m (about 40 cm in diameter). The rollers were drilled at a height of about 1-1.5 m from the ground. After drilling, the holes were kept closed. The product was registered in the National System of Authorization and Information on Biodiversity (SISBIO No. 72922-1) and in the National System of Management of Genetic Heritage (SISGEN No AOFOD20). The ground bark of black-jurema (*Mimosa tenuiflora*) and *Chlorella vulgaris* were purchased in local shops.

The jurema-preta extract was prepared by infusing 200 g of ground bark in 1000 mL of water at 60 °C, under stirring for 2 hours, followed by filtration. For quantification, 10 g of black jurema were infused in 50 mL of water and dried to a constant mass. The emulgel was developed using Carbopol 934 P (0.25% w/w) dissolved in 100 g of jurema extract (12.5% w/w), stirred for 5 hours, and adjusted to pH 7.0 with triethanolamine. *Chlorella* extract (0.2% w/w), rich in chlorophyll and sensitive to light, was dissolved in copaiba oil (4% w/w) and added to the polymer under agitation for 10 minutes. The formulation was stored at 4 °C. The emulgel subjected to illumination was named EJOC, while the non-illuminating emulgel was named EJO.

Emulgel Analysis

The formulations were submitted to previous stability tests, using centrifugation (BioTek/Elx808, Synergy), accelerated stability test, and shelf-life test (ANVISA, 2004). Texture profile analysis (TPA) was performed in the TAXTplus module (Stable MicroSystems, UK), using a 10 mm diameter polycarbonate probe. The probe was inserted twice into the sample, at a depth of 15 mm and at a rate of 2 mm/s^{-1}, with 15 s of rest between compressions (Borghi-Pangoni *et al*., 2016). The bioadhesive strength of emulgel has been proven using pig ear skin. The skin was introduced into a probe, which was pressed against the emulgel with a force of 0.1 N for 30 s. Next, the probe was raised to 1.0 mm/s, measuring the force required to detach the skin. Tests were performed at least six repetitions, using the texture analyzer TAXT plus (Stable Micro Systems, Surrey, United Kingdom) (Campanholi *et al*., 2018).

The determination of total phenols was performed using the Folin-Ciocalteu method, which used gallic acid as a standard. The measurement of total flavonoids in the extract was performed by UV-Vis spectrophotometry (Agilent Technologies/Cary 60) (Zhishen *et al*., 1999). For rheological analysis of continuous shear, the samples were analyzed in a MARS II rheometer (Haake, Newington, Germany), using a cone-plate parallel geometry of 35 mm in diameter and an angle of 2°, separated by 0.105 mm, at 38 ± 1°C. A shear gradient from 0 to 2000 s^{-1} was applied for 150 seconds. Rheological analysis of oscillatory shear was performed using the MARS II rheometer with cone-plate geometry. The linear viscoelastic region (LVR) was determined at 38 ± 1 °C, applying a voltage gradient of 0.01 to 20 Pa at 1 Hz. Then, a frequency gradient of 0.1 to 10 Hz was applied under constant voltage, with analysis of the oscillatory parameters in the RheoWin program (Campanholi *et al*., 2018).

The cytotoxicity of the formulations was evaluated in murine fibroblasts (L-929 cells) using the cell viability assay based on MTT reduction (3-4,5-dimethylthiazole-2-2,5 diphenyltetrazolium bromide) (Mosmann, 1983). L-929 cells were cultured in DMEM medium with 10% fetal bovine serum, maintained at 37 °C and 5% CO_2 for 24 hours. After monolayer formation, the formulas were added at concentrations of 188 to 12,000 µg/mL and incubated for 48 hours. After treatment, the cells were washed and incubated with MTT for 4 hours. The formazan crystals were solubilized in dimethyl sulfoxide and absorbance was measured at 570 nm. The percentage of viable cells was calculated in relation to the control, and the CC50 values (50% cytotoxic concentration) were determined by nonlinear regression analysis, with the results expressed as mean ± standard deviation of at least three independent experiments (Campanholi *et al*., 2022b).

For the analysis of the minimum inhibitory concentration (MIC), each compound and the mix (jurema-preta + *Chlorella* + copaiba oil) were tested against *Staphylococcus aureus* (ATCC 25923) at concentrations of 100, 50, 25 and 12.5 mg/mL, without illumination and with illumination, using a 665 nm red LED light source for 30 minutes at a distance of 15 cm (Campanholi *et al*., 2022a).

The phototoxic potential of *C. vulgaris* as a photosensitizer (PS) was evaluated in 10% reconstituted skimmed milk (LDR 10%). After inoculation, the milk samples were refrigerated (4°C) in the dark for 10 min and were illuminated at a distance of 15 cm for 5, 10, and 15 min, sown in Baird Parker Agar. Controls were performed with inoculated milk and PS to evaluate the antimicrobial activity in the dark. The biofilm-breaking capacity of natural extracts was determined according to (Saini *et al*., 2023).

In Vivo Trial in Lactating Cattle Using Emulgel as Post-Dipping

In vivo assay in lactating cattle using emulgels as post-dipping: all experimental procedures were approved by the Ethics Committee for the Use of Animals in Experimentation (CEUA/UEM), under Protocol No. 6646060323. The experiment was carried out at the Iguatemi Experimental Farm, State University of Maringá, Paraná, Brazil. Before milking, the teats of the animals were washed with water and dried with disposable paper towels. After mechanical milking, the post-dipping procedure was performed. For the application, 12 Holstein animals in different stages of lactation were used, with an average weight of 500 kg and an average production of 20 liters/day, kept on pasture and supplemented with corn silage and corn grain concentrate, for 71 days. The animals were distributed in a 3x3 quadruple Latin square design, with 7 days of adaptation and 21 days of collection. The treatments were: T1-control, iodine application as

post-dipping; T2-application of EJO emulgel without illumination (jurema-preta and copaiba oil); T3-application of EJOC emulgel with illumination (jurema-preta, *Chlorella vulgaris,* and copaiba oil). The teats were irradiated with 665 nm red LED light, irradiance of 3.40 x 10^3 W cm^{-2}, at a distance of 3 cm for 1 min. Microbiological evaluations and milk analyses were performed on days 7, 14, 21, and 28. *Staphylococcus* spp. counts were performed by collecting swabs and milk at sowing on Baird Parker Agar (BP). For the somatic cell count (SCC), the Ekomilk Scan (CapLab) was used.

Blood samples were collected on days 0 and 28, after the first morning milking by means of jugular puncture using disposable needles and Vacutainer® tubes with the addition of ethylenediamine tetraacetic acid (EDTA). The blood samples were kept in a cooler with ice until they arrived at the laboratory, where, within 24 hours, the blood count was performed with the analysis of global red blood cell count, determination of cell volume, hemoglobin content, absolute hematimetric indexes, global leukocyte count, and differential leukocyte count (Viana *et al*., 2002).

The data obtained *in vitro* and *in vivo* were submitted to analysis of variance (ANOVA), and the significant difference between the means ($p<0.05$) was determined using Tukey's test using the SAS 9.3 Software (SAS, 2012).

RESULTS

The formulations were considered stable in the preliminary and accelerated stability studies, without phase separation and with adequate incorporation of copaiba oil. The emulgel EJO maintained structural stability, coloration, and absence of phase separation for up to eight months. The formulations demonstrated desirable viscosity, consistent appearance, physical stability, and satisfactory adhesion. For texture analysis, a significant difference was observed in the variables ($p<0.0001$) except for elasticity. The required strength for hardness (N), compressibility (N.mm), and adhesiveness (N.mm) was higher for the EJO emulgel. In the accelerated stability test, the EJOC showed instability, possibly due to the photodegradation of *C. vulgaris*.

There was a significant difference for the variables analyzed regarding bioadhesion in the *ex vivo* skin ($p<0.0001$). The bioadhesiveness of the EJOC was higher, demonstrating greater compatibility with cutaneous adhesiveness (Table 1).

The mean values for total polyphenols were 16.976 mg EAG/L for jurema-preta and 16.1571 mg EAG/L for *C. vulgaris*. The flavonoid values were 983.941 µg/mL equivalent quercetin and 134.2084 µg/mL equivalent quercetin, respectively.

The emulgels showed pseudoplastic behavior and nonlinear viscosity (non-Newtonian, $n<1$) of the thixotropic type, presenting a small hysteresis area of 714.60 Pa.s (EJO) and 4414.33 Pa.s (EJOC), characterized by the ability to increase the material viscosity after application of tension. EJO presented the yield stress. There was a significant difference for the K and n variables ($p<0.05$) (Figure 1). The emulgels showed G'> G'' behavior, presenting an elastic nature, conferring viscoelastic properties, and presenting themselves as

Table 1. Mechanical characterization of the type of post-dipping agent copaiba (*Copaifera martii*), black jurema (*Mimosa tenuiflora*), *Chlorella* oil emulgels, and bioadhesiveness on *ex vivo* skin

Properties	Type of post-dipping agent			
	EJO	EJOC	IODINE	p
Hardness (N)	0.155 ± 0.003[a]	0.067 ± 0.000[b]	0.034 ± 0.013[c]	<0.0001
Compressibility (N.mm)	1.305 ± 0.086[a]	0.363 ± 0.069[b]	0.079 ± 0.011[c]	<0.0001
Adhesiveness (N.mm)	0.736 ± 0.044[a]	0.232 ± 0.010[b]	0.000 ± 0.000[c]	<0.0001
Elasticity (mm)	0.995 ± 0.001	0.998 ± 0.000	0.980 ± 0.06	>0.9534
Cohesiveness (dimensionless)	1.218 ± 0.072[a]	0.000 ± 0.000[a]	1.005 ± 0.133[b]	<0.0001
Bioadhesiveness				
Maximum strength (N)	0.063 ± 0.009[a]	0.069 ± 0.005[b]		<0.0001
Bioadhesion work (N.mm)	0.009 ± 0.000[a]	0.011 ± 0.000[b]		<0.0001

Note: EJO= jurema-preta and copaiba oil, EJOC= jurema-preta, copaiba oil and *Chlorella* vulgaris. Values indicate significance ($p<0.01$) by Tukey's test at 1% probability.

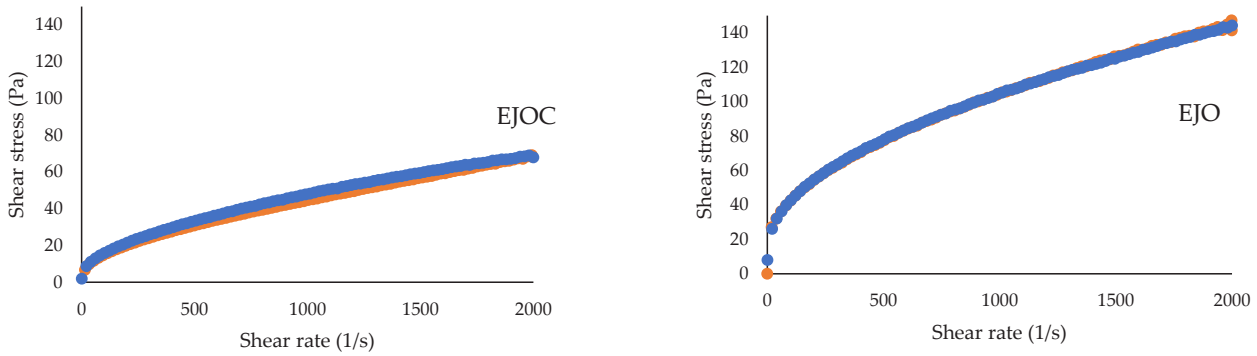

Figure 1. Continuous shear rheology of the emulgel systems. EJOC= jurema-preta, copaiba oil, *Chlorella vulgaris*; EJO= jurema-preta, copaiba oil at 38 °C. The orange symbol represents the outward curve and the blue symbol the return curve.

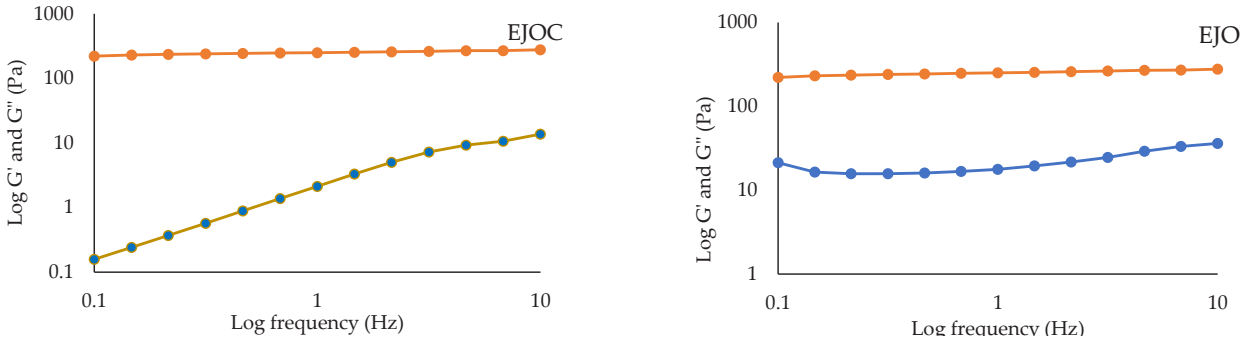

Figure 2. Elastic modulus (G', orange) and viscous modulus (G", blue) as a function of the frequency of the emulgel systems. EJOC= jurema-preta, copaiba oil, *Chlorella vulgaris*; EJO= jurema-preta, copaiba oil at 38 °C.

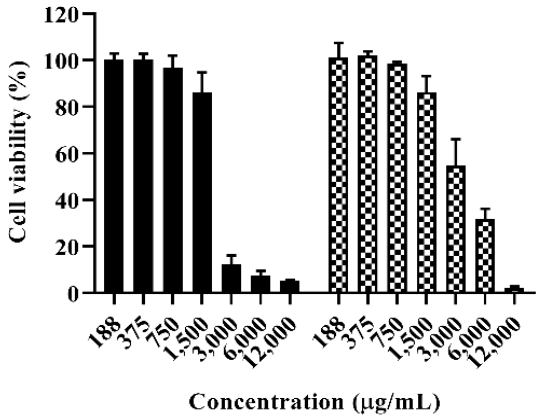

Figure 3. Evaluation of the cytotoxicity of the formula against L-929 fibroblasts. ■ EJOC= Jurema-preta emulgel, copaiba oil, and *Chlorella vulgaris*; ▩ EJO= jurema-preta emulgel and copaiba oil.

a structured system, with interactions between their constituents (Figure 2). A dose-dependent effect was observed in citotoxicity analysis (Figure 3).

Regarding the photoinactivation of these isolates in milk, the results showed significant differences between the strains ($p<0.0001$) and for the exposure times ($p<0.0001$) (Figure 4), with the light exposure time of 10 minutes being more efficient for isolates 1, 4, 9, and 10.

For the *in vitro* evaluation against *S. aureus*, the mix of extracts reduced 100% microbial count at a concentration of 25 mg using light. The standard strain (*S. aureus* ATCC 25923) submitted and not submitted to illumination presented values of 7.90 and 8.00 Log CFU/mL, respectively. When testing different concentrations of *C. vulgaris* and jurema-preta extract, the one that most inhibited growth was 12.5 mg with light, and the decimal reduction value of the control strain was 5.33 Log CFU/mL and 5.34 Log CFU/mL for both compounds. For copaiba oil, the concentration of 100 mg with light had the greatest reduction, with 4.92 Log CFU/mL. *C. vulgaris* has great antimicrobial potential, varying with the type of solvent and the bacterial species tested.

The extract of jurema-preta and *C. vulgaris* promoted bactericidal activity against the isolates in preformed biofilm (Table 2). These compounds inhibit the synthesis of polysaccharides, altering the integrity of the bacterial membrane and causing the rupture of the preformed biofilms. This strategy may be promising for the development of natural treatments against bacterial infections in biofilms, especially in veterinary settings. Highlight for the lowest concentrations of tested extracts (0.78125 µg), which were efficient in breaking 70.36% and 66.62% of preformed biofilm. It was observed that the milk parameters evaluated did not present a statistically significant difference, except for the *Staphylococcus* count in the teats (Table 3).

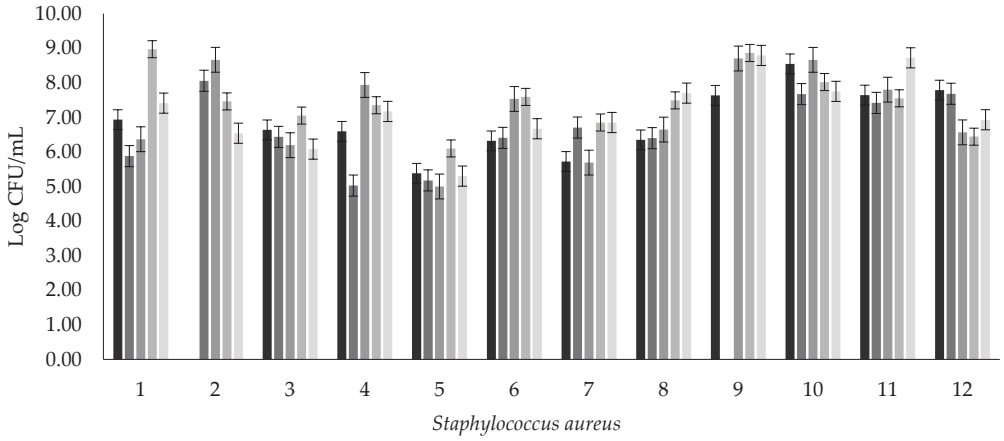

Figure 4. Photodynamic inactivation of 12 strains of *Staphylococcus aureus* in milk- strains count (log_{10}) after times of exposure to light: 5 min, 10 min, and 15 min. Strains count in the dark (without using photoinactivation): with and without *Chlorella*. ■ 5 min ■ 10 min ■ 15 min ■ With Chlorella ■ Without Chlorella

Table 2. Bactericidal activity of jurema-preta and *Chlorella vulgaris* extract on *Staphylococcus aureus* isolates present in biofilm (%)

Bacteria	Biofilm inhibition (%)			
	Jurema-preta extract (μg)		*Chlorella vulgaris* extract (μg)	
	100	0.78125	100	0.78125
CP	73.24 ± 0.018	61.60 ± 0.313	73.14 ± 0.082	61.19 ± 0.356
Sv1	76.78 ± 0.026	64.88 ± 0.331	78.36 ± 0.061	65.36 ± 0.369
Sv3	74.96 ± 0.004	68.94 ± 0.016	75.40 ± 0.009	66.62 ± 0.042
Sv5	72.78 ± 0.061	70.36 ± 0.161	71.04 ± 0.005	52.97 ±0.007

Note: CP: standard strain of *Staphylococcus aureus* (ATCC 25923); Sv1, Sv3, and Sv5 are strains of *S. aureus*; the highest concentration of extract tested is 100 and the lowest is 0.78125; values represent the mean (± standard deviation).

Table 3. Somatic cell count, physicochemical and microbiological composition of milk and teats of cows subjected to the application of emulgels

Variables	T1 (Iodine)	T2 (Emulgel)	T3 (Emulgel + Light)	SE	p - value		
					T1 x T2	T1 x T3	T2 x T3
SSC Log10	5.27	5.28	5.16	0.1331	0.979	0.554	0.536
F	4.84	4.44	4.61	0.1902	0.158	0.413	0.537
ST	9.69	9.64	9.82	0.2105	0.874	0.672	0.553
D	1.0334	1.0335	1.0342	0.6949	0.884	0.417	0.503
P	3.55	3.52	3.62	0.0798	0.777	0.519	0.357
L	5.22	5.18	5.30	0.0961	0.798	0.588	0.427
SL	0.80	0.79	0.82	0.0163	0.701	0.490	0.287
Swab_Log10	2.02	2.46	2.18	0.0822	0.001**	0.212	0.025*
Milk_Log10	2.28	2.78	2.66	0.2272	0.137	0.254	0.713
Milk production	15.16	16.54	15.73	2.0093	0.636	0.846	0.779

Note: SCC= somatic cell count; Treatment 1= iodine; Treatment 2= emulgel; Treatment 3= emulgel light; F= Fat (%); ST= deffated solids; D= Density (g/mL); L= Lactose (%); SL= minerals (%); P= Protein (%); Swab log10= staphylococcal count on the ceiling; Milk_Log10= staphylococcal count in milk; Milk production= milk production/L. SE= average standard error. *Values indicate significance ($p<0.05$) by Tukey's test at 5% probability; **Values indicate significance ($p<0.01$) by Tukey's test at 1% probability.

DISCUSSION

In the accelerated stability test, the EJOC formulation showed instability due to photodegradation. Light radiation can alter the color and odor of the emulgel components, and evaporation by heating results in a more fluid texture, which is reversed with cooling.

For texture analysis, adhesiveness favors the emulgel permanence in the teats. The emulgel cohesiveness affects the ability to maintain its integrity; however, adhesion to the skin depends more on the adhesiveness of the emulgel than on its cohesiveness. Therefore, the EJOC lack of cohesiveness does not necessarily preclude its adhesion. Cohesiveness indicates greater viscosity and structure (de Oliveira *et al*., 2021). EJOC has higher bioadhesive properties compared to EJO, which are related to its mechanical characteristics and ability to interact with the skin surface. This ensures that the active ingredients are released at the application site, increasing contact time and, consequently, efficacy. Studies about bioadhesive gels with herbal principles, using polymers such as Carbopol and natural bioactive substances, such as oils and chlorophyll, show great therapeutic potential (Campanholi *et al.,* 2022a).

The high concentration of flavonoids and tannins in jurema-preta confers antimicrobial properties, inhibiting the growth of several bacteria, highlighting its potential as a natural antimicrobial agent (Santos *et al*., 2022). The values reported in the literature were: jurema-preta extract 5.4989 μg/mL of flavoids, a value lower than the present study, for total phenolics of 50.584 μg/mL (Silva *et al*., 2021), and for *C. vulgaris* of 24.95 mg/100 g for total phenolics (Miranda *et al*., 2001).

The materials analyzed showed pseudoplastic behavior, with adequate viscosity for topical application, facilitating spreadability and adhesion to the skin. They also showed thixotropic behavior. In oscillatory rheology, the emulgels showed an elastic predominance over viscous ($G' > G''$) at low frequencies, indicating adequate mechanical stability. The transition from elastic to viscous behavior was observed at a critical frequency. These results suggest that the formulation has cohesive and stable structures reinforced by the presence of bioactive compounds, such as polysaccharides and phenolic compounds, which contribute to its efficacy and stability (Narvaes *et al*., 2023).

Regarding toxicity, a dose-dependent effect was observed, i.e., with the increase in the concentration of the extracts, there was a decrease in cell viability. However, this decrease only occurred more prominently at concentrations greater than 1,500 μg/mL (Figure 3). Copaiba oil is recognized for its anti-inflammatory and healing properties, and is generally safe in moderate concentrations, but high doses can cause cytotoxicity in some cells. The addition of *C. vulgaris* to the EJOC emulgel seems to have reduced the toxic effects, increasing cell viability at intermediate concentrations,

which is in line with the evidence that *C. vulgaris* can increase the stability of formulations and offer cellular protection due to the presence of chlorophyll and polysaccharides, which promote tissue repair and reduce oxidative stress (Latif *et al*., 2021). EJOC emulgel shows advantages in cell viability at higher concentrations, making it suitable for topical applications with low toxicity. These results highlight the importance of combining plant extracts with synergistic activities, such as copaiba oil and *C. vulgaris*, to optimize the efficacy and safety of formulations.

All 12 isolates amplified the *nuc* gene, only two isolates for the *hla* and *hlb* genes, and three for the *sea* gene. The presence of the *nuc* gene is used to characterize the species *S. aureus.* The presence of the *sea* gene is essential to produce toxins and contributes to their virulence. The genes *hla* and *hlb* encode hemolysins that increase the ability of *S. aureus* in the host to aggravate infections such as mastitis.

Formation of biofilms by *S. aureus* is a challenge in the treatment of infections due to the resistance of Gram-positive bacteria. Thus, studies aiming at the bactericidal activity of different compounds against preformed biofilms become necessary. The fractions of the *C. urucarana* extract eradicated *S. aureus* in biofilms in a manner equivalent to the antibiotic vancomycin, where the extract (5 mg/mL) was able to inhibit 88.94% of the formation of the bacterial matrix of *S. aureus* (Nader *et al*., 2018). These studies demonstrate the importance of several natural extracts in the inhibition and eradication of *S. aureus* biofilms, indicating the development of new antibacterial agents.

It was observed that the milk parameters evaluated did not present a statistically significant difference. This suggests that the different treatments applied did not influence the composition or physicochemical quality of the milk, since the animals did not present mastitis during the application of the products. In contrast analysis, it was observed that the control treatment showed significantly lower levels of *Staphylococcus* contamination in the teats compared to the emulgel without illumination ($p<0.001$). The contrast between emugel treatments proves the efficacy of photodynamic therapy in reducing the microbial load, since the application of light generated singlet oxygen on the surface of the teats, contributing to the reduction of microorganisms ($p=0.025$). The SCC values obtained were lower than the limits established by IN 77 (Ministério da Agricultura, Pecuária e Abastecimento, 2020), which establishes a maximum count of 5.70 $\log_{10}$ (500.000 cells/mL) (Table 3). As in the present study, da Silva Junior *et al*. (2019) demonstrated that the use of an SF hydrogel (photosensitizer) was equivalent to iodine, ensuring microbiological quality and low SCC in milk. The emulgels acted as an antiseptic product, providing adequate teat coverage and as a physical barrier. The addition of carbopol provided better adhesiveness, stability, and consistency, since it is a synthetic hydrophilic polymer derived from polyacrylic acid (Campanholi *et al*., 2022c).

For the blood analysis, there was no significant difference for the parameters evaluated ($p>0.05$). However, at 28 days, the animals showed a significant reduction in the values of eosinophils ($p<0.024$) and for the Days x Treatment interaction for the eosinophil variable (%), the lowest count was for the emulgel containing copaiba oil (EJOC) (6.67^{b}), followed by EJO (12.67^{a}) and control (9.00^{a}). In the present study, the values obtained were in accordance with the reference limits for eosinophils; lower values may indicate stress, acute infections, use of corticosteroids, pregnancy, lactation, or severe allergic reactions. In cases of mastitis, the immune response tends to prioritize neutrophil activation, resulting in decreased eosinophil count (Braun *et al*., 2021).

CONCLUSION

The emulgels tested showed adequate characteristics of stability, bioadhesiveness, and pseudoplastic behavior, making them a promising option for therapeutic applications. The association of natural extracts combined with photodynamic therapy proved to be effective in reducing *Staphylococcus aureus*, without presenting cytotoxic effects and with anti-biofilm capacity, reinforcing its potential to fight bacterial infections. In the context of post-dipping application, the tested formulations have efficacy similar to iodine in the SCC maintenance and staphylococci control, evidencing their potential as an antiseptic alternative to prevent bovine mastitis.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

No AI tools were used in this work.

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