Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v47i1.71018

Syzygium aromaticum and propolis emulgel for preventing mastitis

Rogério Aleson Dias Bezerra[1], Natália Zampiroli Oliveira[1], Thainá Blasques Silva[1], Magali Soares dos Santos Pozza[1*], Ivanor Nunes do Prado[1], Fernando Rodrigues de Carvalho[2], Katiele da Silva Souza Campanholi[3] and Mônica Regina da Silva Scapim[4]

[1]Departamento de Zootecnia, Universidade Estadual de Maringá, Avenida Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. [2]Departamento de Tecnologia, Universidade Estadual de Maringá, Maringá, Paraná, Brasil. [3]Departamento de Química, Universidade Estadual de Maringá, Maringá, Paraná, Brasil. [4]Departamento de Engenharia de Alimentos, Universidade Estadual de Maringá, Maringá, Paraná, Brasil. *Autor para correspondência. E-mail: msspozza@uem.br

ABSTRACT. Several sanitary measures must be adopted during the milking process to minimize the transmission of mastitis-causing agents that can be transferred to the milk, depreciating its microbiological quality. The aim of this study was to evaluate the efficacy of an emulgel composed by propolis and clove essential oil in post-dipping for lactating cows. For the in vitro inhibition test against *Staphylococcus aureus*, was observed a greater effectiveness (88%) of clove essential oil (*Syzygium Aromatum*) comparing to the other components of the emulgel. For the field test, five animals were used for the control group (iodine) and five animals with emulgel (hydrated Carbopol® + propolis and essential oil of leaf clove) as post-dipping, applied daily to the teats of lactating cows with the aid of conventional applicators after milking. Milk samples were collected on days 1, 7, 14, 21 and 28, for the evaluation of milk composition and somatic cell count (SCC) and microbiological evaluation. No significant effects were observed for the variable fats and SCC ($p > 0.05$). However, for the protein variable, effects were significant for the interaction between the control group, the emulgel and the evaluated days ($p < 0.05$). The lactose variable showed significant effects for the evaluation days ($p < 0.05$). The staphylococcal count in milk was similar for both evaluated groups. The emulgel has promising characteristics for use as a post-dipping disinfectant.

Keywords: Natural antimicrobials; subclinical mastitis; post-dipping.

Received on January 1, 2024
Accepted on August 21, 2024

Introduction

Bacterial mastitis is one of the most economically important diseases in dairy cattle farming all over the world. (Fonseca et al., 2021; Franco et al., 2022; Kerslake et al., 2018). This disease not only reduces milk production, affecting its quality, as well as antibiotic residues from its treatment can also cause allergic reactions in consumers, putting human health at risk. The global prevalence of clinical mastitis and subclinical mastitis ranges from 15% to 50% (Fonseca et al., 2021; Krishnamoorthy et al., 2021).

Staphylococcus aureus (*S.* aureus) is one of the most common infectious agents that causes clinical and subclinical mastitis. (Alves et al., 2020). Due to concerns about bacterial resistance related to the continued use of antibiotics in dairy herds, researches about alternative products are needed. Prevention of mastitis basically consists of washing the teats with water and asepsis with chemical products with antimicrobial action such as chlorine, iodine or quaternary ammonium. (Ramalho et al., 2012)

Since there is no ideal disinfectant, some considerations must be taken into account when choosing the appropriate disinfectant, such as having a broad spectrum of action; being non-toxic and non-irritating to human and animal tissue; being stable on the skin and having an affordable cost. (Ramalho et al., 2012)

The use of natural products to prevent or control mastitis provides fewer side effects (Kher et al., 2019) and low resistance after prolonged exposure (Amarante et al., 2019) compared to conventional medicines, therefore giving to the natural compounds a great advantage. Staphylococcal mastitis is one of the most difficult problems to treat in dairy production, in most cases, this situation is prevented and/or treated with antibiotics and a livestock management program. However, over the years, *S.* aureus has developed virulence mechanisms that protect it not only from antibiotic treatment but also from the host's innate and adaptive immune responses. (Zaatout et al., 2020)

Over the past 20 years, more than ten different active compounds have been tested, such as teat disinfectants, including chlorhexidine, iodine compounds and quaternary ammonium salts. (Dore et al., 2019). In view of this, studies are necessary to reduce exposure to common disinfectants in dairy animals, so antimicrobial compounds derived from plants, animals and bacteria are used. A study developed by (Abboud et al., 2015) evaluated a phytotherapeutic bioadhesive emulgel using clove essential oil (*Syzygium aromaticum*) and propolis extract and this proved to be quite efficient in microbial control. According to the mentioned authors, the composition of clove essential oil contains eugenol, which in addition to being an excellent anesthetic, has antibacterial and fungicidal potential. Like essential oils, propolis has been widely used in both human and veterinary medicine. (Loguercio et al., 2006; Peixoto et al., 2012; Saeki et al., 2011). Propolis is a resinous substance produced by bees through their own secretions, when the bees collect resinous, gummy and balsamic substances of viscous consistency from tree bark and other plant tissues. It has a characteristic smell and the color can vary depending on the botanical origin, its chemical composition is complex and varied, including flavonoids, aromatic acids, terpenoids and phenylpropanoids, fatty acids and several other compounds.

Among the most used natural substances for their antibacterial, antifungal, anti-inflammatory and immune stimulating activity (Loguercio et al., 2006). The aim of this study was to evaluate the effectiveness of an emulgel containing clove essential oil and propolis as a post-dipping treatment for preventing mastitis.

Material and methods

The experiment was carried out at the Iguatemi Experimental Farm (FEI) of the State University of Maringá (UEM), in the Cattle Farming Sector and in the Milk Quality Analysis Laboratory. The analyses were carried out at the Milk Analysis Laboratory belonging to the Mesoregional Center of Excellence in Milk Technology (CMETL) Northwest region, located at the Iguatemi Experimental Farm - the State University of Maringá. Procedures involving animals were previously approved by the Animal Use Ethics Committee (CEUA) of the State University of Maringá (Protocol no. 3425280722).

Animals and experimental design

Ten animals were used in a crossover design, distributed into two groups: control (iodine 10%) and emulgel (based on propolis and clove essential oil Engetec™) repeated after 28 days with the animal inversion between groups. After milking, the milk was collected to evaluate the composition and SCC (somatic cell count) on the 1st, 7th, 14th, 21st and 28th days. For application, the animals' teats were washed with water and dried with paper towels. The products were administered using a non-returnable applicator cup, daily after milking.

Emulgel development

For the laboratory tests, two formulas were developed, called White, composed of hydrated polymer (Carbopol®) and Carb-PC, as the phytotherapeutic emulgel composed of hydrated Carbopol® polymer and combined with propolis and essential oil of leaf clove, as described in Table 1. The emulgel formulation was developed by hydrating carbopol (0.5%) in water (94.4%), after that the compound remained under mechanical agitation for five hours. After this period, it was added the mixture of clove essential oil (1%) and propolis (5%), under constant stirring, and then the pH was adjusted (7.0) using triethanolamine (Figure 1).

Table 1. In vitro microbiological evaluation of emulgel compounds against *Staphylococcus aureus* (log10)

Treatments	Log_{10}
Staphylococcus aureus ATCC	7.90 a
Ethanol	4.32 b
Emulgel	3.98 b
Propolis	1.73 bc
Clove Essential Oil (CEO)	0.90 c

CV = 27.39 DMS = 2.7730.

Determination of the emulgel physical characteristics

The texture profile determination (TPA) for the formulations was performed using the TAXTplus texture analysis module (Stable Micro Systems, Surrey, United Kingdom).(Jones et al., 2009). The analysis was performed at 5 and 35°C with three replicates for each sample. In this step, the following parameters were obtained: Hardness (0.094 N); Compressibility (0.023 N.s); Adhesiveness (0.10 N.s); Elasticity (0.67s); Cohesion (0.67) (Jones et al., 2009; Junqueira et al., 2016).

The bioadhesion strength was determined using the texture analyzer in stress mode (Bruschi et al., 2007) using pig the ear skin of animals from the Iguatemi Experimental Farm (FEI/UEM) slaughterhouse.

Figure 1. Stirring the emulgel before adjusting the pH.

Assessment of antimicrobial capacity

First, 1 mL of sterile distilled water and 3 g of phytotherapeutic emulgel were used. For the other samples in eppendorf, 200 µL (propolis and essential oil of leaf clove), 100 µL of 95% ethyl alcohol and 700 µL of sterile distilled water were mixed, and then distributed into twelve-well plates. In the sequence, in each well, the mixture was homogenized using a sterile tip and 10 µL of the *Staphylococcus aureus* suspension (ATCC 25923) was added, leaving it to rest for thirty minutes. Subsequently, 1 mL was collected from the wells and added to Petri plates containing agar. Mueller Hinton (Campanholi et al., 2022).

Collections and laboratory analyses

Milk samples were collected directly from the gallon and then sent weekly by the Paraná Association of Dutch Cattle Breeders (APCBRH) for composition and somatic cell count (SCC) analyses. The microbiological analysis of the milk occurred at the beginning (day 1) and at the end (day 28) of the experimental period. The samples were collected in sterile bottles using the serial dilution method, with 1 mL of milk in 9 mL of sterile peptone water. At the end of the dilutions, 100 µmL of each dilution was pipetted into petri plates containing Mueller Hinton Agar. The plates remained for 24 hours in a B.O.D. type incubator at 35°C for the growth of staphylococcal colonies, after that the counts were carried out.

Statistical analysis

The statistical analysis of the studied variables was performed using the SAEG program (System for Statistical and Genetic Analysis), developed by the Federal University of Viçosa (UFV, 1997), with the average of the groups (control x emulgel) being compared by the Tukey test at 5% of probability.

Results and discussion

Evaluation of antimicrobial capacity

It was observed greatest effectiveness ($p = 0.00008$) (88%) of clove essential oil (*Syzygium aromaticum*), followed by propolis (78%) and emulgel (50%). These results (Table 1) demonstrate the real effectiveness of the evaluated components in reducing *Staphylococcus aureus* (ATCC-25923) colonies. Similar results were described by Sethunga et al. (2023) when evaluating in vitro the inhibition halos of clove, cinnamon and ginger essential oil and their combinations, being effective against the food pathogens *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger*.

Antimicrobials from natural origin are effective and economical alternatives because they are obtained from aromatic plants and spices rich in essential oils characterized by remarkable antimicrobial activity. (Brixner et al., 2022). The MIC values of red propolis against the Gram-positive bacterial strain *S. aureus* were higher, ranging from 64 mg mL^{-1} to 101.6 mg mL^{-1} and 512 mg mL^{-1} to 1024 mg mL^{-1} (Regueira Neto et al., 2017). Similar results were reported for *S.* aureus in red propolis and were found in other studies (Bueno-Silva et al., 2013).

The mechanism of antibacterial action is related to the flavonoids found in propolis. These mechanisms were summarized as: (1) Inhibition of the nucleic acid synthesis (DNA and RNA); (2) DNA gyrase inhibition mechanism; (3) inhibition of cytoplasmic membrane function (plasma membrane damage due to reduced fluidity); (4) Inhibition of energy metabolism (the exchange of nutrients and metabolites is disturbed due to damage to the cytoplasmic membrane, thus inhibiting energy supply) (5) Inhibition of adhesion and biofilm formation (Freires et al., 2016; Xie et al., 2015).

The clove essential oil has a significant effect on both Gram positive and Gram-negative bacteria (*Escherichia coli*). These results may be due to the lipophilic properties of clove essential oil, which promotes interaction with lipids in bacterial cell membranes, increasing the permeability (Radünz et al., 2019).

In studies conducted by Zhao et al. (2021), the MIC and MBC values of clove essential oil against *Escherichia coli* were 0.64 and 1.28 mg ml^{-1}, respectively, which were higher than those against *S.* aureus (0.52 and 1.04 mg ml^{-1}, respectively). Gram-positive bacteria have a cell wall composed only of a monolayer of peptidoglycan, so their ability to prevent the invasion of antibacterial agents is considered weak. However, the structure of the Gram-negative bacteria cell wall is more complex.

Physicochemical and microbiological analysis of milk

There was no difference between the products applied regarding physical-chemical ($p > 0.05$) (Table 2) and microbiological (Table 3) composition. The values referring to the physical-chemical composition met the minimum recommended by current legislation (Brasil, 2018). For the protein variables, there was an effect for the interaction of products (iodine or emulgel) and Time, and for lactose, there was an effect for the days of evaluation, however, these parameters can be altered depending on the lactation curve of the animals. The physical-chemical composition of milk can be influenced by intrinsic factors to the animal, such as: production level, birth order, lactation stage, breed, diet, age, etc.(Cabral et al., 2016).

Table 2. Percentage of chemical composition and somatic cell count (SCC mL^{-1}) of milk from lactating cows using emulgel post-dipping

Treatment[1]	Day	Variables			
		FAT	PROT	LAC	SCC
Control	0	4.75	4.75	4.75	4.75
	7	3.54	3.54	3.54	3.54
	14	3.98	3.98	3.98	3.98
	21	5.23	5.23	5.23	5.23
Propolis + CEO	0	4.70	4.70	4.70	4.70
	7	3.43	3.43	3.43	3.43
	14	4.13	4.13	4.13	4.13
	21	5.10	5.10	5.10	5.10
Standard Error		1.22	0.45	0.55	0.49
CV (%)[3]		25.49	13.02	12.87	9.58
p-value[4]					
Treatment		0.2953	0.1330	0.2074	0.2581
Day		0.7838	0.2548	0.0002**	0.9499
Treatment*Day		0.8509	0.0076**	0.2367	0.0806

[1]Control commercial product; Propolis + CEO = emulgel made of propolis and clove essential oil (*Syzygium aromaticum*);[2]FAT = % fat; PROT = % protein; LAC = % lactose y= -0.0003 x + 4.2684; SCC = somatic cell count (log 10); [3]CV (%) = coefficient of variation; [4]p-valor = **0.0001, * 0.05.

Table 3. Microbiological evaluation of milk from cows treated with emulgel made of propolis and clove essential oil

Treatment[1]	Staphylococcus milk (log_{10}) [2]	
	Start	End
Control	2.795	2.518
Propolis + CEO	2.985	2.963
CV (%)[3]	23.44	
SD[4]	0.66	
p-value[5]		
Treatment	0.1845	
Period	0.5290	
Treatment*Period	0.5891	

[1]Control = Iodine; Propolis + CEO = emulgel made of propolis and clove essential oil (*Syzygium aromaticum*); [2]Start = evaluation carried out at the beginning of each period; End = it was performed at the end of each period; [3]CV (%) = coefficient of variation; [4]SD = standard deviation*;* p-value = ** 0.0001, * 0.05.

For SCC, the recommended values are 500.000 cells mL^{-1} (IN 76/2018) (5,70 log_{10}). The average SCC values of the present study were 5.17 log_{10}. The effectiveness of using propolis as a pre- and post-dipping has already been verified by some authors. Fiordalisi et al. (2016) found that propolis has the potential to be used in the prevention of subclinical mastitis or during dry cow therapy when the bacterial count is comparable to the clinical picture. Schelles et al. (2021) when evaluating the use of propolis and iodine as pre- and post-dipping for lactating cows for 28 days, they found no significant difference between the groups evaluated in the concentration of protein, lactose and fat in the milk. The values for staphylococcus count in milk did not show any significant difference between the groups evaluated, which indicates the same efficiency of emulgel when compared to iodine in maintaining low staphylococcus counts in raw milk (Table 3). Reports of staphylococcal poisoning are frequently associated with the ingestion of the minimum toxic dose of 100 ng of its heat-stable toxin and foods with staphylococcal counts between 105-106 CFU g^{-1} or mL^{-1} of food; in this study, counts of the order of 102 CFU mL^{-1} were obtained in milk, for both groups of animals tested.

Nascimento et al. (2022) tested a natural antiseptic product containing 1% propolis in a 10% hydroalcoholic solution for pre-dipping, and 10% glycerin with 0.2% citronella oil added for post-dipping. The results obtained showed efficiency in terms of total bacterial reduction, indicating great antibacterial activity against the bacteria most commonly associated with bovine mastitis.

In the study *in vivo* Abboud et al. (2015) it was observed the effectiveness of the antibacterial activity of the essential oils of Thymus vulgaris and Lavandula angustifolia against *Staphylococcus spp. and Streptococcus spp.*. Their mixture promoted a significant reduction in the bacterial count in milk samples after four consecutive days of application of the evaluated products. However, more researches need to be carried out to better evaluate the effect of exposure to essential oils and its implications on animal health.

Conclusion

The tested emulgel showed in vitro antimicrobial activity against *Staphylococcus aureus*. The efficiency of the natural antiseptic was satisfactory comparing to iodine, presenting itself as a natural solution with antimicrobial efficiency for use as a post-dipping disinfectant product.

Acknowledgements

We appreciate and acknowledge the collaborative efforts between the Department of Zootechnics, the Postgraduate Program in Zootechnics of the State University of Maringá. This study was funded in part by the National Institute of Science and Technology of the Milk Production Chain (INCT-LEITE/UEL/UEM-PR), Londrina/Maringá, Paraná, Brazil.

References

Abboud, M., El Rammouz, R., Jammal, B., & Sleiman, M. (2015). In vitro and in vivo antimicrobial activity of two essential oils Thymus vulgaris and Lavandula angustifolia against bovine Staphylococcus and Streptococcus mastitis pathogen. *Middle East Journal of Agriculture Research*, *4*(4), 975–983.

Alves, B. F. C., Chaves, A. J. S., Vieira, N. F., Chaves, G. V., França, P. M., Barezani, A. S. A., & Soares, P. H. A. (2020). Sensitivity of *Staphylococcus aureus* to the antimicrobials used in the treatment of bovine mastitis: review. *PUBVET*, *14*(4), 1–6. https://doi.org/10.31533/pubvet.v14n4a557.1-6.

Amarante, J. F., Ribeiro, M. F., Costa, M. M., Menezes, F. G., Silva, T., Amarante, T. A. B., Gradela, A., & Moura, L. (2019). Chemical composition and antimicrobial activity of two propolis extracts against Staphylococcus spp. isolates and multiresistant bacteria. *Brazilian Veterinary Research*, *39*(9), 734–743. https://doi.org/10.1590/1678-5150-PVB-6128

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. (2018). *Instrução Normativa nº 76, de 26 de novembro de 2018: Regulamentos técnicos que estabelecem as características de identidade e qualidade que devem ser apresentadas pelo leite cru refrigerado, leite pasteurizado e leite pasteurizado tipo A*. Diário Oficial da União.

Brixner, B., da Silva, C. de M., Pollo, L. D., & Renner, J. D. P. (2022). Antimicrobial activity of chitosan associated with essential oils in biomedical application: an integrative review. *Research, Society and Development*, *11*(14), e447111436563. https://doi.org/10.33448/rsd-v11i14.36563

Bueno-Silva, B., Alencar, S. M., Koo, H., Ikegaki, M., Silva, G. V. J., Napimoga, M. H., & Rosalen, P. L. (2013). Anti-inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian red propolis. *Journal of Agricultural and Food Chemistry*, *61*(19), 4546–4550. https://doi.org/10.1021/jf305468f

Cabral, J. F., Silva, M. A. P., Carvalho, T. S., Brasil, R. B., Garcia, J. C., & Nascimento, L. E. C. (2016). Relation of chemical composition of milk with the level of production stage of lactation and parity order cows crossbred. *Revista Do Instituto de Laticínios Cândido Tostes*, *71*(4), 244–255. https://doi.org/10.14295/2238-6416.v71i4.536

Campanholi, K. S. S., Silva Júnior, R. C. S., Jaski, J. M., Silva, J. B., Oliveira, M. C., Santos, R. S., Pozza, M. S. S., Castro-Hoshino, L. V., Baesso, M. L., & Cardozo Filho, L. (2022). Thermo and photoresponsive emulgel loaded with copaifera reticulata Ducke and Chlorophylls: Rheological, mechanical, photodynamic and drug delivery properties in human skin. *Pharmaceutics*, *14*(12). https://doi.org/10.3390/pharmaceutics14122798.

Dore, S., Ferrini, A. M., Appicciafuoco, B., Massaro, M. R., Sotgiu, G., Liciardi, M., & Cannas, E. A. (2019). Efficacy of a terpinen-4-ol based dipping for post-milking teat disinfection in the prevention of mastitis in dairy sheep. *Journal of Essential Oil Research*, *31*(1), 19–26. https://doi.org/10.1080/10412905.2018.1523069.

Fiordalisi, S. A. L., Honorato, L. A., Loiko, M. R., Avancini, C. A. M., Veleirinho, M. B. R., Machado Filho, L. C. P., & Kuhnen, S. (2016). The effects of Brazilian propolis on etiological agents of mastitis and the viability of bovine mammary gland explants. *Journal of Dairy Science*, *99*(3), 2308–2318. https://doi.org/10.3168/jds.2015-9777.

Fonseca, M. E. B., Mourão, A. M., Chagas, J. D. R., Ávila, L. M., Marques, T. L. P., Baêta, B. de A., Moraes, R. F. F., & Roier, E. C. R. (2021). Mastite bovina: Revisão. *PUBVET*, *15*(2), 1–18. https://doi.org/10.31533/pubvet.v15n02a743.1-18.

Franco, A. B., Mourão, A. C., Gouveia, F. M., & Freitas, T. M. S. (2022). Bovine mastitis and its consequences for public health. *PUBVET*, *16*(10), 1–10. https://doi.org/10.31533/pubvet.v16n10a1233.1-10.

Freires, I. A., Alencar, S. M., & Rosalen, P. L. (2016). A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases. *European Journal of Medicinal Chemistry*, *110*, 267–279. https://doi.org/10.1016/j.ejmech.2016.01.033.

Jones, D. S., Bruschi, M. L., Freitas, O., Gremião, M. P. D., Lara, E. H. G., & Andrews, G. P. (2009). Rheological, mechanical and mucoadhesive properties of thermoresponsive, bioadhesive binary mixtures composed of poloxamer 407 and carbopol 974P designed as platforms for implantable drug delivery systems for use in the oral cavity. *International Journal of Pharmaceutics*, *372*(1–2), 49–58. https://doi.org/10.1016/j.ijpharm.2009.01.006.

Junqueira, M. V, Borghi-Pangoni, F. B., Ferreira, S. B. S., Rabello, B. R., Hioka, N., & Bruschi, M. L. (2016). Functional polymeric systems as delivery vehicles for methylene blue in photodynamic therapy. *Langmuir*, *32*(1), 19–27. https://doi.org/10.1021/acs.langmuir.5b02039.

Kerslake, J. I., Amer, P. R., O'Neill, P. L., Wong, S. L., Roche, J. R., & Phyn, C. V. C. (2018). Economic costs of recorded reasons for cow mortality and culling in a pasture-based dairy industry. *Journal of Dairy Science*, *101*(2), 1795–1803. https://doi.org/10.3168/jds.2017-13124.

Kher, M. N., Sheth, N. R., & Bhatt, V. D. (2019). In vitro antibacterial evaluation of Terminalia chebula as an alternative of antibiotics against bovine subclinical mastitis. *Animal Biotechnology*, *30*(2), 151–158. https://doi.org/10.1080/10495398.2018.1451752

Krishnamoorthy, P., Goudar, A. L., Suresh, K. P., & Roy, P. (2021). Global and countrywide prevalence of subclinical and clinical mastitis in dairy cattle and buffaloes by systematic review and meta-analysis. *Research in Veterinary Science*, *136*, 561–586. https://doi.org/10.1016/j.rvsc.2021.04.021.

Loguercio, A. P., Groff, A. C. M., Pedrozzo, A. F., Witt, N. M., Silva, M. S., & Vargas, A. C. (2006). In vitro activity of propolis extract against bacterial agents of bovine mastitis. *Pesquisa Brazilian Agriculture*, *41*(2), 347–349. https://doi.org/10.1590/s0100-204x2006000200021.

Nascimento, G. M., Cardozo, M. V., Valmorbida, M. K., Pereira, N., Barbosa, J. C., Júnior, F. R. F., & Ávila, F. A. (2022). Propolis in the control of bacterial bovine mastitis: a tool for the production of organic milk. *Semina: Ciências Agrárias*, *43*(2), 869–882. https://doi.org/10.5433/1679-0359.2022v43n2p869.

Peixoto, E. C. T. M., Jardim, J. G., Heinzen, E. L., Domingues, P. F., Padovani, C. R., & Orsi, R. de O. (2012). Própolis no controle da mastite bovina. *Archives of Veterinary Science*, *17*(4), 43–52. https://doi.org/10.5380/avs.v17i4.25393.

Radünz, M., Trindade, M. L. M., Camargo, T. M., Radünz, A. L., Borges, C. D., Gandra, E. A., & Helbig, E. (2019). Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum*, L.) essential oil. *Food Chemistry*, *276*, 180–186. https://doi.org/10.1016/j.foodchem.2018.09.173.

Ramalho, A. C., Soares, K. D. A., Silva, D. F. da, Barros, M. R. C., Pinheiro Júnior, J. W., de Oliveira, J., Mota, R. A., & Medeiros, E. S. de. (2012). In vitro efficacy of commercial disinfectants used in pre- and post-dipping against Staphylococcus spp. isolated from dairy herds. *Brazilian Veterinary Research*, *32*, 1285–1288.

Regueira Neto, M. S., Tintino, S. R., Silva, A. R. P., Costa, M. S., Boligon, A. A., Matias, E. F. F., Balbino, V. Q., Menezes, I. R. A., & Coutinho, H. D. M. (2017). Seasonal variation of Brazilian red propolis: Antibacterial activity, synergistic effect and phytochemical screening. *Food and Chemical Toxicology*, *107*, 572–580. https://doi.org/10.1016/j.fct.2017.03.052.

Saeki, E. K., Mello Peixoto, E. C. T., Matsumoto, L. S., Marcusso, P. F., & Monteiro, R. M. (2011). Bovine mastitis caused by *Staphylococcus aureus*: sensitivity to antimicrobial drugs and propolis alcoholic extract. *Acta Veterinaria Brasilica*, *5*(3), 284–290. https://doi.org/ 10.21708/avb.2011.5.3.2172

Schelles, J. L., Rodrigues, B. M., Pozza, M. S. S., & Lima, L. S. (2021). Use of propolis extract as an antiseptic agent for pre and post dipping in dairy cows. *Agrarian*, *14*(51), 95–101. https://doi.org/10.30612/agrarian.v14i51.9164.

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current Medicinal Chemistry*, *22*(1), 132–149. https://doi.org/10.2174/0929867321666140916113443.

Zaatout, N., Ayachi, A., & Kecha, M. (2020). *Staphylococcus aureus* persistence properties associated with bovine mastitis and alternative therapeutic modalities. *Journal of Applied Microbiology*, *129*(5), 1102–1119. https://doi.org/10.1111/jam.14706

Zhao, M., Bai, J., Bu, X., Yin, Y., Wang, L., Yang, Y., & Xu, Y. (2021). Characterization of selenized polysaccharides from Ribes nigrum L. and its inhibitory effects on α-amylase and α-glucosidase. *Carbohydrate Polymers*, *259*. https://doi.org/10.1016/j.carbpol.2021.117729.