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Antimicrobial activity of the ethanolic extract of propolis against bacteria that cause mastitis in cattle

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Resumo

Atividade antimicrobiana do extrato etanólico de própolis contra bactérias causadoras de mastite em bovinos. A própolis é uma substância coletada por abelhas, especialmente pela *Apis mellifera*, a partir de resinas de brotos de plantas que possuem numerosas propriedades biológicas, como antimicrobiana e antiinflamatória. Atividades como a pecuária leiteira orgânica apresentam alta demanda por produtos naturais com propriedades como as presentes na própolis, uma vez que a contaminação do leite e a mastite são grandes problemas da indústria de laticínios. O objetivo deste trabalho foi avaliar a atividade antimicrobiana *in vitro* do extrato etanólico de própolis (EEP) em diferentes concentrações. Ação do EEP extraído de colmeias no sul do Brasil foi testada contra 13 diferentes gêneros de agentes bacterianos causadores de mastite em bovinos leiteiros. O experimento foi conduzido no laboratório de Parasitologia e Microbiologia da Universidade Federal do Pampa, Campus Dom Pedrito – RS, durante os meses de maio e junho de 2016. Seguiram-se as técnicas de difusão em ágar e microdiluição em placas. Através dos resultados do teste de concentração inibitória mínima (CIM), pode-se afirmar que o EEP possui atividade inibitória em 100% das bactérias testadas em concentrações acima de 10% (p/v). Tais resultados mostram que a própolis apresenta potencial antimicrobiano frente as principais bactérias envolvidas nos processos de mastite.

Palavras-chave: Antimicrobiano natural; Concentração inibitória mínima; Difusão de ágar; Mastite; Microbiologia; Própolis

Abstract

Propolis is a substance produced by bees, especially *Apis mellifera,* from resins of plant shoots. It possesses numerous biological properties, such as antimicrobial and anti-inflammatory. Activities such as organic dairy

farming show high demand for natural products with proprieties like those of propolis, since milk contamination and mastitis are major problems of the dairy industry. The objective of this study was to evaluate *in vitro* the antimicrobial activity of different concentrations of an ethanolic extract of propolis (EEP). Action of the EEP extracted from beehives in southern Brazil was tested against 13 different genera of bacterial agents that cause mastitis in dairy cattle. The experiment was carried out in the Laboratory of Parasitology and Microbiology at the Federal University of Pampa, Campus Dom Pedrito – RS, Brazil, during May and June 2016. Agar diffusion and microdilution plate methodologies were followed. Based on the results of the minimum inhibitory concentration test (MIC), the EEP had inhibitory activity on 100% of the bacteria tested at concentrations above 10% (w/v). These results show that propolis has antimicrobial potential against bacteria involved in the process of mastitis.

Key words: Agar diffusion; Mastitis; Microbiology; Minimum inhibitory concentration; Natural antimicrobial; Propolis

Introduction

Propolis is a substance collected by bees, especially *Apis mellifera,* from resins of plant shoots. In the hive, propolis is used to line the alveoli and chambers where the queens lay eggs. It is also used to seal the entrances and openings of the hive, besides serving as an emulsifier if the hive is invaded by an insect or animal that the bees cannot remove (WIELSE, 1995; BANKOVA et al., 2014). Propolis is composed of approximately 50% plant resin and balsam, 30% wax, 10% aromatic oils, 5% pollen, and 5% other substances (RUSSO et al., 2002). Hence, the composition of propolis reflects the flora of the area where the bees collect the resins (WIELSE, 1995; BANKOVA et al., 2014).

There are many active compounds with proven therapeutic action in propolis. Approximately 200 different components of propolis have been identified from different sources, among which, the main ones are flavonoids, aromatic, aliphatic and phenolic acids, aldehydes, esters and amino acids (MARCUCCI, 1995; VARGAS et al., 2004). Propolis possesses numerous biological properties, such as antimicrobial, antifungal, immunostimulant, antioxidant, anti-inflammatory and anticancer (REZENDE et al., 2006; LUSTOSA et al., 2008; HASHEMI, 2016). In a study by Astani et al. (2013), it was observed that a propolis extract presented high antimicrobial and antifungal potential in an *in vitro* analysis when applied to clinical isolates of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Enterococcus faecium* and *Candida albicans*.

In a study with partially purified propolis extracts from hives in Argentina, the immunostimulator effect of propolis was tested by Sampietro et al (2016) and an *in vitro* effect was observed on chemotactic and phagocytic activities of neutrophils. Antioxidant and anti-inflammatory activity of propolis from Malaysia was observed *in vitro* and *in vivo* in a study with diabetic rats. After four weeks of treatment, fasting glycemia decreased in the rats of the ethanolic extract of propolis (EEP) experiment, in addition to an increase in pancreatic antioxidant enzymes and total antioxidant capacity (NNA et al., 2018).

Propolis has many bioactive components. Among them there is artepillin C (ARC), which according to an *in vitro* and *in vivo* study carried out with mice, by Bhargava et al. (2018), has potent anticancer activity. This is due to the potential to enter and annul mortalin-p53 complexes, causing the activation of p53 and stimulating cancer cells to stop growing.

Studies about the antimicrobial action of ethanolic extracts of propolis (EEP) have been conducted for many years and the main obstacle is the diverse composition of the extract, which shows variations according to region and vegetation available to bees, as well as the time period and selection of each hive (MARCUCCI et al., 2001; PARK et al., 2002).

More studies about the therapeutic action of propolis are necessary, especially because the composition varies according to the region where the propolis is produced. Despite this, propolis may be considered an alternative antimicrobial and anti-inflammatory.

Activities such as organic dairy farming show high demand for natural products with proprieties such as those present in propolis. Organic farming is

an expanding area with more consumers becoming interested, especially in developing countries. Organic products account for over 4% of food sales in the USA, and organic dairy products account for 15% of these products (USDA, 2017). In Brazil, it is believed that the idea of organic farming is also becoming more popular; however, market data to confirm this is scarce.

Milk is considered a medium favorable to bacterial growth, due to its high temperature immediately after milking (37°C), richness in nutrients and pH around 6.6 to 6.8 (KLOSS et al., 2010). Milk from healthy animals has a low bacterial count, absence of pathogenic microorganisms and low somatic cell count. The contamination of milk can happen through microorganisms present in the mammary gland, on the surface of the teats and udder and in equipment used for milking and milk storage. The contamination of milk in a bulk tank (milk collected from cooling tanks of dairy farms), by pathogens that cause mastitis, occurs due to the presence of bacteria in the lumen of the mammary gland (SILANIKOVE et al., 2014). Several studies report a correlation between mastitis pathogens and total bacterial count, which is reflected in a decrease in milk quality (ZADOKS et al., 2004; RYŠÁNEK et al., 2009; KATHOLM et al., 2012).

Bacterial contamination negatively interferes with milk quality, hindering industrialization processes, since it reduces the shelf life of fluid milk and its derivatives, in addition to affecting the final value paid by the industry to the farmer (BRITO et al., 2000; BRITO; BRITO, 2001; LANGONI, 2013). In Brazil there is a Normative Instruction (IN62) that establishes milk quality parameters. Based on these limits, enterprises who buy milk from dairy farmers stipulate the price paid by liter according to quality (CUNHA et al., 2010).

Mastitis is an inflammation of the mammary gland, which can be caused by microorganisms (bacteria, fungi, algae and viruses), physical trauma and chemical irritants, resulting in physical and chemical changes in milk composition and an increase in somatic cells. In addition to harming the mammary glandular tissue, it can lead to irreversible destruction of milk-secreting cells (LANGONI, 2000; TRONCO, 2003).

Mastitis is mainly caused by infections from different pathogens. According to Ranjan et al. (2006), the main agents involved in mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Escherichia coli*, which are responsible for approximately 80% of the cases. *Corynebacterium bovis*, *Pseudomonas* spp., *Mycobacterium* spp., *Nocardiaasteroides*, *Aspergillus* spp., *Candida* spp., *Serratia* spp. and *Prototheca* spp. account for less than 5% of the cases.

The disease is classified according to its clinical and subclinical symptoms. Clinical mastitis is evidenced by visible manifestations, such as edema, hyperthermia, induration and pain of the mammary gland and/or appearance of lumps and pus in the milk. The subclinical form is characterized by changes in milk composition, such as somatic cell count increase, increase in serum protein levels, and decrease in casein, lactose, fat and calcium levels in the milk (SANTOS; FONSECA, 2007). Subclinical mastitis is highly prevalent in Brazil compared to the clinical form. According to Costa et al. (2012), subclinical prevalence was 10%, and in the studies by Ribeiro et al. (2009) the prevalence was 48.64%; both studies were carried out in the Southeast Region of the country.

Some of the microorganisms responsible for causing mastitis in cattle are also pathogenic to humans. Thus, the disease and the pasteurization processes should be monitored closely, given the importance to public health (FAGUNDES; OLIVEIRA, 2004). Mastitis is normally treated with chemical agents, which can cause environmental problems and, especially, problems related to pathogenic resistance to products (SADASHIV; KALIWAL, 2014).

Since there is an increasing concern related to food health, quality and safety by the population, organic food consumption is constantly increasing (VIEIRA et al., 2017). To fit in the organic system, organic farms differ from conventional ones mainly because they substitute the use of synthetic products, such as antibiotics, vermifuges and growth promoters, with biological agents, such as homeopathic and herbal medicines (HONORATO et al., 2014). Hence, propolis could be used in the organic control of mastitis and in

milking practices, such as pre and post-dipping, lowering contamination in the teats of cows, and replacing iodine, a chemical commonly used in conventional production systems. More *in vitro* studies showing the antimicrobial activity of propolis are needed.

Due to the high incidence of mastitis affecting cattle herds in Brazil, the recognized antimicrobial activity attributed to propolis and the fact that propolis is a natural product that can be used in organic systems, this study aimed to evaluate the *in vitro* action of an EEP against the main bacteria that cause intramammary infections in dairy cattle.

Material and Methods

The experiment was carried out in the laboratory of Parasitology and Microbiology of the Federal University of Pampa, Campus Dom Pedrito – RS, Brazil, during May and June 2016.

The propolis used in the experiment was extracted from hives of Africanized bees, located in the northwest region of Rio Grande do Sul State, in southern Brazil. The raw propolis was collected by scraping and was subsequently fragmented and macerated. To obtain the EEP, 96º GL ethyl alcohol was used at a proportion of 70%, thereby obtaining a 30% propolis extract. The extract was put in amber bottles for 42 consecutive days and shaken for 30 second each day, as described by Franco and Bueno (1999). At the end, the ethanolic extract of propolis was obtained by filtration. The reading of the final alcoholic level of the EEP was obtained with an alcoholmeter, resulting in 67º GL.

To evaluate the *in vitro* antimicrobial activity and minimum inhibitory concentration (MIC) of the EEP, bacterial strains of *Escherichia coli* derived from the American Type Culture Collection (ATCC) 8739 and *Staphylococcus aureus* (ATCC 25923), as well as other bacteria obtained by isolation from field collections of bovine milk and swabs of dairy cow teat surfaces, were used. The bacteria isolated from field collections were the following: *Staphylococcus warneri; Staphylococcus lugdunensis*; *Klebsiella* sp., *Pseudomonas* sp., *Citrobacter* sp., *Enterobacter* spp., *Salmonella* sp., *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp. and *Streptococcus* spp.

The antimicrobial activity of the EEP at a concentration of 30% (w/v) and 0.2% iodine was measured by the agar diffusion method on BHI agar. An analysis of the antimicrobial activity of 67º alcohol was also conducted to check the solvent effect. As a control treatment, plates were prepared with culture medium inoculated with the bacterial suspensions.

To prepare the bacterial inoculums, strains of microorganisms were isolated in Brain Heart Infusion (BHI) agar and diluted in 0.85% sterile saline until achieving a turbidity of 0.5 on the McFarland nephelometric scale, which equals approximately 1.5 x 10^8 bacteria/mL. The inoculums were prepared following the methodology of the Clinical and Laboratory Standards Institute – CLSI (2006), according to standard M7-A6. Then, 100 µL of suspension was spread on the agar surface with the aid of a Drigalski strap. After that, 100 µL of EEP, at different concentrations (30, 25, 20, 15, 10 and 5% [w/v]), was also spread on the agar surface with the aid of a Drigalski strap. All analyses were performed in duplicate.

The plates were incubated at 35°C for 48 h to observe bacterial growth and to count the colony forming units/mL (CFU/mL). Bacteria whose growth was not observed in the culture medium were considered susceptible to the treatment (EEP, iodine and alcohol).

The MIC was determined by the dilution method on microplates according to the methodology described by Duarte et al. (2003), with modifications. The 96-well inert polystyrene microtiter plates for Elisa/RIA (Falcon®) were filled with 20 µL of EEP at different concentrations (30, 25, 20, 15, 10 and 5% [w/v]), 10 µL of bacterial inoculum and BHI broth. As a positive control, only the culture medium (BHI) with inoculum was used, and each test also included verification of the effect of the extract solvent, 67º GL alcohol, on microbial growth.

The microplates were incubated at 35°C for 48 hours. After incubation, readings were made with the revealing colorant 2,3,5-triphenyltetrazolium chloride (TTC), at a concentration of 2%. To do this, 20 µL of

TTC was added to each well of the microplate. After 2 h, reading was performed by checking the color of the wells; those that remained colorless had no microbiological growth, while the wells that stained red-purple indicated microbial growth. Tests were performed in duplicate. Inhibition of microbial growth was evidenced by the absence of growth in the medium being considered the MIC, which is the lowest concentration that the EEP is capable of inhibiting bacterial growth.

A statistical analysis was done to compare the different concentrations of EEP and alcohol (67°GL) using the Fisher Exact test in the software BioEstat 5.3 (SPRENT, 2011).

Results

In vitro results from the EEP inhibition test at a concentration of 30% (w/v) against the mastitis causing bacteria can be observed in Table 1.

The EEP at a concentration of 30% (w/v) inhibited the growth of all Gram-negative bacteria tested, including *Klebsiella* sp., *Pseudomonas* sp., *Citrobacter* sp., *Enterobacter* spp., *Salmonella* sp. and *Escherichia coli*.

Among the Gram-positive bacteria, four species showed growth inhibition (*Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Bacillus* spp. and *Streptococcus* spp.) and three (*Corynebacterium* spp., *Micrococcus* spp., and *S. warneri*) presented an uncountable number of CFU.

The 67º GL alcohol showed no antimicrobial activity against all tested bacteria, which indicates that the antimicrobial activity observed in the EEP treatments was not due to the alcohol used as solvent in the preparation of the extract, but to the propolis itself. The low efficiency of the 67º GL alcohol as an antimicrobial may be due to the concentration. According to Andrade et al. (2002), the recommended concentration that results in high antibacterial efficiency is 70º GL alcohol.

Only the populations of the strains of *S. aureus* and *Bacillus* spp. could be counted, which exhibited a growth of 110 CFU/mL and 200 UFC/mL, respectively.

TABLE 1: Colony forming unit count of bacteria that cause mastitis, according to the 30% (w/v) ethanolic extract of propolis, 67º GL alcohol and 0.2% iodine.

	Bacteria	0.2% Iodine CFU/mL	67º GL Alcohol CFU/mL	30% (w/v) EEP CFU/mL
Gram-positive	*S. aureus* 25923	Uncountable*	110	0
	S. lugdunensis	Uncountable	Uncountable	0
	Bacillus spp.	150	200	0
	Corynebacterium spp.	Uncountable	Uncountable	Uncountable
	Micrococcus spp.	Uncountable	Uncountable	Uncountable
	S. warneri	Uncountable	Uncountable	Uncountable
	Streptococcus spp.	Uncountable	Uncountable	0
Gram-negative	*E. coli* 8739	Uncountable	Uncountable	0
	Citrobacter sp.	Uncountable	Uncountable	0
	Enterobacter spp.	Uncountable	Uncountable	0
	Klebsiella sp.	Uncountable	Uncountable	0
	Pseudomonas sp.	Uncountable	Uncountable	0
	Salmonella sp.	Uncountable	Uncountable	0

* Uncountable = dishes with counts above 300 CFU.

Results of the MIC test for the EEP at different concentrations, in relation to the Gram-positive and Gram-negative bacteria, can be observed in Table 2. When comparing the different concentrations of EEP and alcohol (67°GL) by the Fisher Exact test, there was a statistical difference (p=0.0001) between the 30, 25, 20, 15 and 10% concentrations and the alcohol without propolis. The 5% concentration was statistically different from the 30, 25, 20, 15 and 10% (p = 0.0391) concentrations and the alcohol (p = 0.0016).

According to the results obtained, it can be seen that the growth of all the bacteria was inhibited by the EEP at concentrations of 30, 25, 20, 15 and 10%.

The MIC for the *Citrobacter* sp., *Bacillus* spp., *Streptococcus* spp., *Klebsiella* sp., *Staphylococcus warneri*, *Staphylococcus aureus 25923*, *Staphylococcus lugdunensis* and *Corynebacterium* spp. strains was obtained with an EEP equal or less than 5%. The *Micrococcus* spp., *E. coli 8739*, *Pseudomonas* sp., *Enterobacter* spp. and *Salmonella* sp. strains had a MIC of 10% EEP.

As can be seen, the EEP inhibited the growth of 6 out of 7 of the tested Gram-positive bacteria (86%) at a 5% concentration of the extract, while at the same concentration, the EEP inhibited the growth of 2 out of 6 (33%) of the Gram-negative strains. The other five strains showed inhibition for the extract at a concentration of 10%. The lowest MIC with 5% EEP (w/v) was more effective against Gram-positive bacteria than Gram-negative bacteria.

Based on the results in Table 1 and Table 2, it can be seen that all strains in the microdilution test did not show growth for the 10% EEP (w/v); however, *Corynebacterium* spp., *Micrococcus* spp. and *S. warneri* showed no growth inhibition for the 30% EEP (w/v) in the test of antimicrobial activity on the agar dilution.

Discussion

Propolis has the ability to inhibit bacterial growth by preventing cell division, disrupting the cytoplasm, plasma membrane and the cell wall, which causes

TABLE 2: Minimum inhibitory concentration of ethanolic extracts of propolis at different concentrations.

	Bacteria	EEP						Alcohol
		30%	25%	20%	15%	10%	5%	67º GL
Gram-positive	*Micrococcus* spp.	NG	NG	NG	NG	NG	G	G
	S. aureus 25923	NG	NG	NG	NG	NG	NG	G
	S. lugdunensis	NG	NG	NG	NG	NG	NG	G
	Bacillus spp.	NG	NG	NG	NG	NG	NG	G
	Corynebacterium spp.	NG	NG	NG	NG	NG	NG	G
	S. warneri	NG	NG	NG	NG	NG	NG	G
	Streptococcus spp.	NG	NG	NG	NG	NG	NG	G
Gram-negative	*E. coli* 8739	NG	NG	NG	NG	NG	G	G
	Pseudomonas sp.	NG	NG	NG	NG	NG	G	G
	Enterobacter spp.	NG	NG	NG	NG	NG	G	G
	Salmonella sp.	NG	NG	NG	NG	NG	G	G
	Klebsiella sp.	NG	NG	NG	NG	NG	NG	G
	Citrobacter sp.	NG	NG	NG	NG	NG	NG	G
	Fisher Exact test	a	a	a	a	a	b	c

* NG = No growth; G = Growth. Different letters for the Fisher Exact Test represent a statistical difference ($p < 0.05$).

partial bacteriolysis, besides inhibiting protein synthesis (TAKAISI-KIKUNI; SCHILCHER, 1994). Sforcin et al. (2000) verified that strains of *Staphylococcus aureus* are inhibited by an EEP at a concentration of 0.4% (v/v). These results corroborate those found in the present study.

For the results of the 0.2% iodine treatment, there was bacterial growth for all tested strains, which clearly indicates the iodine did not inhibit the major bacteria that cause mastitis in dairy herds. The populations of the microorganism could only be counted (150 CFU/mL) in the tests with *Bacillus* spp.; nevertheless, there was no inhibition of growth.

Silva et al. (2015), in an *in vitro* experiment evaluating the sensitivity of *S. aureus* strains to different commercial disinfectants, noted that concentrations of iodine at 1 and 2% inhibited microbial growth, whereas a concentration of 0.5% iodine did not result in inhibition. In a study by Flachowsky et al. (2007), it was observed that a 0.3% iodine solution used to disinfect cows before milking was responsible for an increase of 54µg iodine/kg of milk. O'Brien et al. (2013) reported that the use of iodinated solution in pre- and post-dipping cattle represents a substantial risk of transferring iodine to milk. Due to the low concentration, the 0.2% iodine concentration had little antimicrobial effect against the bacteria tested in this study. According to Pedrini and Margatho (2003), 2 and 1% iodine solutions exhibit good antimicrobial performance, but these concentrations are very high and leave residues in the milk.

In the MIC test, higher inhibition of Gram-positive bacteria was found compared to Gram-negative bacteria. Similar results were obtained by Sfoncin et al. (2000), Fernandes Júnior et al. (2006) and Bispo Júnior et al. (2012), who showed susceptibility of Gram-positive bacteria to low concentrations of EEP, whereas the inhibition of Gram-negative bacteria required higher extract concentrations. The inhibition of Gram-positive bacteria, in the works performed by the aforementioned authors, was reached at EEP concentrations of 3% 0.4% and 0.1%, respectively. In the treatments with the Gram-negative bacteria, growth inhibition occurred at EEP concentrations of 9%, 8% and 1%, respectively. Grange and Davey (1990), when analyzing the antimicrobial activity of 5% EEP, observed that this concentration completely inhibited the group with Gram-positive strains, but this result was not as efficient in tests with strains of Gram-negative bacteria, which exhibited partial growth.

In addition, according to Sforcin et al. (2000), a higher MIC for Gram-negative bacteria can be explained by the chemical complexity of the cell wall of these bacteria. The lipid bilayer of the cell wall is less sensitive to antibiotics, whether natural or chemical (BUZZATO et al., 2011). The Gram-negative cell wall contains a larger fraction of lipids. Also, Gram-negative bacteria have a component called lipopolysaccharide that is not present in Gram-positive bacteria. According to Pinto et al. (2001), lipopolysaccharides are responsible for determining the antigenicity, toxicity and pathogenicity of these microorganisms. Due to the chemical constitution of the cell wall of Gram-negative bacteria, it is believed that these characteristics are probably the ones that establish the lower sensitivity of the bacteria belonging to these genera to the EEP (MARCUCCI et al., 2001; REZENDE et al., 2006; VARGAS et al., 2004).

For the alcohol treatment in this experiment, the growth observed for all strains demonstrates its low antimicrobial activity. Dos Santos et al. (2003), in a study evaluating the antimicrobial efficiency of EEP impregnated in filter paper discs, observed that when using 96º alcohol there was no sensitivity to a strain of *S. aureus*. Loguercio et al. (2006), when evaluating the *in vitro* activity of EPP against strains of *Staphylococcus* sp. coagulase-positive and *Streptococcus* sp., observed that for 96° GL ethyl alcohol all isolates were able to grow in the control medium. In another experiment developed by Casquete et al. (2016), the control group used 95º GL ethanol and, at the end of 62 days of storage, sausages experimentally contaminated with *Listeria* sp. innocua 2030c still presented microbial counts of this species, whereas in the group treated with EEP (0.28 g/mL), the *Listeria* population was reduced to below the detection limit after 8 days of storage.

When analyzing the results of the two *in vitro* tests, it was observed that *Corynebacterium* spp., *Micrococcus* spp. and *S. warneri* showed growth for the EEP at a concentration of 30% (w/v), for the agar diffusion

method, while in the MIC test at the same concentration of 30% (w/v), no growth was observed for all the bacteria tested (Tables 1 and 2). The presence of microbial growth when using the agar diffusion method and absence of growth of the same genera of microorganisms when using the MIC method has also been observed by other researchers (OTHMAN et al., 2011; DE-BONA et al., 2014). Microbial growth when using the agar diffusion method does not specifically mean that the extract is inactive for the microorganism tested because, according to Moreno et al. (2006), the diffusion of the antimicrobial compounds evaluated may not have been complete, especially for less polar compounds, which diffuse more slowly in the culture media.

The EEP at a concentration of 10% (w/v), in *in vitro* tests, exhibited antimicrobial activity against the tested bacteria responsible for mastitis in cattle. The solvent used in the preparation of the ethanolic extract of propolis showed no antimicrobial activity against the bacteria tested, demonstrating that the antimicrobial action found in the experiments was due only to propolis. Iodine at a concentration of 0.2% was not effective at inhibiting microbial growth.

The EEP at the concentrations used in this experiment has potential as an antimicrobial when applied to the bacteria tested in this work and, thus, could be used in new *in vitro* tests with other types of infections. The results observed in the study are promising, since propolis is a natural product whose antimicrobial use in the tested bacteria is still little explored and, therefore, resistance of microorganisms to this product is low or zero. Hence, the use of different applications of propolis in healthcare must be further researched.

Considering that bovine mastitis bacteria were analyzed *in vitro*, an *in vivo* experiment would be of great interest to better understand the response of the animal against the use of EEP as an antimicrobial.

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