

IS PROPOLIS A PROMISING CANDIDATE ON BACTERIAL BOVINE MASTITIS' TREATMENT?

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ABSTRACT

In the production and industrialization of cow's milk, an important factor that reduces the quality and quantity of the product is bovine mastitis. The dairy cattle mastitis leads to the use of antibiotics, which indiscriminate and prolonged use is progressively increasing the number of resistant pathogens. In this context, the natural products used to combat pathogenic microorganisms have become an interesting alternative. Among those, propolis stands out on different studies, presenting bacteriostatic and bactericidal properties, being a possible alternative on mastitis control and treatment. To determine that, a series of studies on propolis itself and its compounds properties must be analysed. This work aims to answer if the propolis represents a natural alternative on bovine mastitis' treatment and control, based on scientific studies analyzing its use against different bacterial species, both *in vitro* and *in vivo*.

Keywords: Natural compound. Antibacterial activity. Alternative treatment.

INTRODUCTION

In the production and industrialization of cow's milk, an important factor that reduces the quality and quantity of the product is bovine mastitis. The loss generated by the disease accounts for approximately 25% of all diseases of economic importance in dairy production (BUSATO et al., 2000; ZANETTE et al., 2010). The dairy cattle mastitis leads to the use of antibiotics (ANDRADE, 2018), which indiscriminate and prolonged use is progressively increasing the number of resistant pathogens (ORTEGA et al., 2011).

In this context, the natural products used to combat pathogenic microorganisms have become an interesting alternative, including propolis, which stands out both for its bacteriostatic and bactericidal properties, as for enabling its use in the therapeutic pharmaceutical industries (LIMA et al., 2019; TRONCARELLI et al., 2013). Propolis is a resinous substance produced by bees from the exudate of various plant parts, such as shells and flower buds (BANKOVA et al., 2000).

Several studies have been published demonstrating the biological properties and the chemical composition of propolis, showing the interest of researchers in the substance and its potential for the development of new drugs (MIYATA et al., 2020; ORYAN et al., 2018; SFORCIN; BANKOVA, 2011).

BOVINE MASTITIS

Bovine mastitis is characterized as an inflammation of the mammary gland, usually of an infectious character, and is classified as clinical or subclinical mastitis (BHUTIA et al., 2019). The pathogens responsible for bovine mastitis can be classified in two ways: environmental and contagious (OLIVEIRA et al., 2011).

In mastitis due to environmental factors, the causing agents are usually found in the environment. Meanwhile, contagious mastitis is classified by the survival of microorganisms within the mammary gland, excretions of the infectious agent, and capacity of transmission to another animal (QUINN et al., 2002).

Table 1 - Different bacterial species identified in different mastitis classification.

Classification	Main species identified	
Environmental mastitis	Coliform bacteria	<i>Escherichia coli</i>
		<i>Klebsiella sp.</i>
		<i>Enterobacter aerogenes</i>
	<i>Streptococcus</i>	<i>Streptococcus uberis</i>
		<i>Streptococcus dysgalactiae</i>
		<i>Streptococcus bovis</i>
	<i>Enterococci</i>	<i>Enterococcus faecium</i>
<i>Enterococcus faecalis</i>		
Contagious mastitis	<i>Staphylococcus</i>	<i>Staphylococcus aureus</i>
	<i>Streptococcus</i>	<i>Streptococcus agalactiae</i>
		<i>Streptococcus dysgalactiae</i>

(MARTINS et al., 2010; VICARIO et al., 2009).

Although there is great disparity among the pathogens causing bovine mastitis, *S. aureus*, *S. agalactiae*, *S. dysgalactiae*, *S. uberis* and *E. coli* are responsible for about 80% of cases of the disease in herds (RANJAN et al., 2006). Schlegelová et al. (2003) report that *S. aureus* particularly is one of the most found agents in mammary infections and in Brazil this bacterium was the main causal agent of bovine mastitis, with isolation rates varying between 8.3% and 49.23% of cows in different herds (LAFFRANCHI et al., 2001).

Among the measures taken to reduce rates of mastitis are the adoption of hygienic measures during milking, the elimination of possible sources of infection within the herd and the use of antimicrobials (BHUTIA et al., 2019). However, the prolonged and inappropriate use of antimicrobials may cause the emergence of resistant strains, compromising the efficiency of the treatment and causing the appearance of traces of antibiotics in milk, impacting human health (MENDONÇA et al., 2012), which reinforces the search for alternatives on mastitis treatment.

PROPOLIS

The chemical composition of propolis is a complex reflection of flora that bees use for its preparation, the technique used for the extraction, as well as the genus and species of bees (BANKOVA et al., 2000; SFORCIN, 2016). Considered a very heterogeneous substance (BASTOS et al., 2011), over 300 distinct compounds have been identified on propolis composition throughout the years (ELNAKADY et al., 2017; FROZZA et al., 2013; PRZYBYLEK, KARPIŃSKI, 2019).

The vast biodiversity of Brazil, as well as the biochemical ability of bees to modify the native composition or add their own components to propolis (PEREIRA et al., 2002) may explain Bastos et al. (2011) and Cruz et al. (2020), that samples of Brazilian propolis may have divergent biological properties and chemical compositions according to different geographic origins. This characteristic was also demonstrated by Przybylek and Karpiński (2019) when comparing the chemical composition of propolis extracts from different countries, such as Brazil, Turkey, Korea, Australia and Chile, and also comparing the antibacterial activity of different samples. As a result, the authors found different results regarding efficacy against *S. aureus* and *E. coli* between different samples of propolis, in addition to showing action of propolis against other gram-positive and gram-negative bacteria.

Through the chemical profiles obtained by absorption spectrophotometry in the UV-visible region, high-performance liquid chromatography in reverse phase (HPTLC) and high-performance liquid chromatography (HPLC), Park et al. (2000) described the Brazilian propolis in 12 distinct varieties. Later, Trusheva et al. (2004) identified a new type, named red propolis, which hydroalcoholic extract showed an important antioxidant and antitumor activities on tumour (Hep-2 and HeLa) and non-tumour (Hek-283) established cell lines by MTT assay (FROZZA et al., 2013).

Fiordalisi et al. (2016) evaluated action of four propolis samples from distinct regions of Brazil (brown propolis from Urupema, São Joaquim and Água Doce, Santa Catarina, and green propolis from Minas Gerais) on agents of bovine mastitis and the viability of bovine mammary gland cells when exposed to these propolis samples. They obtained difference in chemical composition between samples, however, all samples (except from Água Doce), showed the same level of antimicrobial activity against strains of *S. aureus*. The samples showed no

significant antimicrobial activity against strains of *E. coli*. As a result of cell viability, there were no changes in cell structure, demonstrating the applicability of this model to evaluate the toxicity of propolis.

Among the chemical substances isolated from different samples of propolis, the flavonoids predominate in holding the primary responsibility for pharmacological properties (PINTO et al., 2001). Phenolic compounds also stand out on propolis composition and Artepillin C (3,5-diprenyl-p-coumaric acid) was identified on ethanolic extracts that showed antibacterial activity against MRSA *S. aureus* (VEIGA et al., 2017) and that caused bubble in membrane on the anaerobic bacteria *Porphyromonas gingivalis* (YOSHIMASU et al., 2018), reinforcing the requirement on in-depth studies on specific propolis compounds and different extracts.

The propolis mechanism of action on bacteria occurs mainly in the permeability of the inner membrane to ions, thus causing the dissipation of the membrane potential (PRZYBYLEK, KARPIŃSKI, 2019). According to Saeki et al. (2011), if the electrochemical gradient of protons across the membrane is essential for the microorganism to maintain ATP synthesis, and transport through the plasma membrane and motility, it also can contribute to a large part of the cytotoxic action of propolis, decreasing the resistance of bacterial cells to other compounds. This fact justifies the synergistic action of propolis and some antibiotics (KROL et al., 1993).

PROPOLIS USE AGAINST BACTERIA CAUSING BOVINE MASTITIS: *IN VITRO* AND *IN VIVO* STUDIES

Langoni et al. (1996) study using propolis extract showed 100% growth inhibition on *S. aureus* and *Corynebacterium bovis* strains, and between 90% and 91% inhibition of *S. agalactiae* and *E. coli*, respectively, increasing researchers' interest on propolis antimicrobial potential. However, Bankova et al. (1999) evaluated the effects of three samples of essential oils of propolis from stingless bees against *S. aureus* and *E. coli*, using the disk diffusion method. The propolis' essential oils did not show significant activity against *S. aureus* and no activity against *E. coli*.

Furthermore, Farnesi (2007) evaluated the antimicrobial activity of propolis produced by honeybees and stingless bees by microdilution technique and concluded that propolis from

honeybees was more effective than from the stingless bees against the microorganisms *S. aureus*, *Micrococcus luteus*, *E. coli* and *Pseudomonas aeruginosa*.

The primary components found on honeybees' propolis, regarding their chemical composition, were: caffeic acid and p-coumaric acid, 3-prenyl p-coumaric, p-coumaric 3-5diprenil and lesser compounds such as flavonoids. About the stingless bees' propolis, components with different polarities were encountered, possibly phenolic acids, phenylpropanoid or flavonoid (FARNESI, 2007). The chemical composition may justify the results of antimicrobial activity, since the primary compounds identified in honeybees' propolis are known for their effective action against bacteria (MARCUCCI et al., 1996; MARCUCCI, 1996; PINTO et al., 2001; SANTANA et al., 2012).

Loguercio et al. (2006) analysed the propolis antibacterial activity against coagulase-positive isolates of *Staphylococcus* and *Streptococcus*. As a result, 90.5% presented sensitivity to propolis extract. The average sensitivity of coagulase-positive *Staphylococcus* isolates (94.4%) was higher than the isolates of *Streptococcus* (85.2%). Similarly, Saeki et al. (2011) evaluated brown propolis extract activity against 38 *S. aureus* strains and 35 (92.1%) presented effectiveness. The chemical composition was identified as 55.5% flavonoids, which could be responsible for the antimicrobial activity (SANTANA et al., 2012).

Ethanollic extracts have shown interesting results in *in vitro* studies, as Table 2 summarizes. Through time, different activities have been demonstrated against bacterial agents and encourages studies on propolis extracts antibacterial action.

Table 2 - Different propolis extracts and its *in vitro* activity against bacteria.

Extract	Activity	Bacteria species	Reference
Ethanolic	Cytoplasmic disorganization, defects in the cell wall structure and stopping cell division.	<i>S. agalactiae</i>	Takaisi-Kikuni, Schilcher, 1994
Ethanolic	Growth inhibition	<i>S. aureus</i>	Langoni et al., 1996
		<i>Corynebacterium bovis</i>	
		<i>S. agalactiae</i>	
		<i>E. coli</i>	
Essential oil (from stingless bees)	Low or zero activity	<i>S. aureus</i>	Bankova et al., 1999
		<i>E. coli</i>	
Ethanolic	Sensitivity	<i>Staphylococcus</i>	Loguercio et al., 2006
		<i>Streptococcus</i>	
Cereal alcohol	Sensitivity	<i>S. aureus</i>	Saeki et al., 2011
Ethanolic	Antibacterial	MRSA <i>S. aureus</i>	Veiga et al., 2017
Ethanolic	Caused bubble in membrane	<i>Porphyromonas gingivali</i>	Yoshimasu et al., 2018
Not informed	Growth inhibition	<i>S. aureus</i>	Lima et al., 2019

Despite the correlation between flavonoids and phenolic compounds with better bactericidal activity (CASTRO et al., 2007), Amarante (2011) evaluated two propolis alcoholic extracts, one (A) from Southeast and another (B) from Northeast Brazil against *Staphylococcus* spp. isolated from bovine mastitis. The bacteria isolates were sensitive to both extracts, 90.9% to A and 83.1% to extract B, being the total flavonoids and phenolic compounds 126.22 mg (A) and 73.12 mg (B).

There are few reports on the use of propolis extracts and/or derivatives for treating or preventing mastitis in cattle. Some are highlighted on Table 3. The first study (MIROLYUBOV, BARSKOV, 1980) evaluated the effectiveness of curing bovine mastitis (milk production recover) by using propolis alcoholic extracts on different concentrations. In animals that presented serous and catarrhal mastitis, an ointment with 2% ethanolic extract was applied intramammary and for haemorrhagic and/or purulent mastitis, an ointment with 5% propolis extract. Milk production was recovered in 97% of animals with serous mastitis, 96%, 72% and 83% of animals with catarrhal, haemorrhagic and/or purulent mastitis, respectively. Thus, the authors concluded that the use of propolis ointment for the treatment of various types of bovine mastitis results in a therapeutic effect by acting directly on the healing process.

Table 3 - Different propolis extracts and its *in vivo* activity against bacteria.

Extract	Activity	Percentage	Bacteria species or mastitis characterization	Reference
Not informed	Clinical and microbiological cure	84.8% of treated teats	<i>Prototheca zopfii</i>	Langoni et al., 1995
Alcoholic	Complete recovery	100% of cases	<i>Candida albicans</i>	Meresta et al., (1989)
		85% of cases	<i>E. coli</i>	
		91% of cases	<i>Staphylococcus</i> sp.	
		84,3% of cases	<i>Streptococcus</i> sp.	
Alcoholic	Milk production recovery	97% of animals	Serous mastitis	Mirolyubov; Barskov, 1980
		96% of animals	Catarrhal mastitis	
		72% of animals	Haemorrhagic mastitis	
		83% of animals	Purulent mastitis	

On naturally infected herds, Langoni et al. (1995) tested the antibacterial efficacy of a propolis extract as an option to control mastitis caused by *Prototheca zopfii*. Utilizing propolis formulation at 30%, prepared in solution of Dimethyl Sulfoxide (DMSO) to 20% to facilitate the diffusion in the mammary parenchyma, the study presented clinical and microbiological cure in 84.8% of treated teats.

Andrade (2010) tested the antimicrobial activity of a water-soluble propolis extract against 443 bacterial isolates: the most frequent microorganisms were *Staphylococcus* spp. (33.2%), *S. aureus* (19.86%) and *S. agalactiae* (13.7%). As a result, propolis extract showed a minimum inhibitory concentration (MIC) of 155.46 mg/mL, and significant inhibitory effect on strains of *S. aureus* when compared with the control group (glycerine iodine 0.5%).

Several studies have reported that the combination of propolis extracts with commercial antimicrobial agents can allow a reduction of the clinical dose of certain antibiotics, decreasing the incidence of side effects while enhancing the treatment of infections where bacterial resistance becomes a determining factor (PINTO et al., 2001; SANTANA et al., 2012; SILVA, 2010; VIGNOTO et al., 2014). Kedzia and Holderna (1986), in an experiment using *S. aureus* resistant to antimicrobials when tested against the mixture of propolis and antibiotic extract, showed that about 70% of the tests found lower MIC than observed with antibiotics alone.

Santana et al. (2012) studied the effect of an ethanol extract of green propolis against strains of *S. aureus* derived from bovine mastitis. The authors aimed to analyse a possible selection of bacterial cells resistant to propolis when exposed to sub-inhibitory concentrations and the influence of propolis on the bacterial cell. The results showed that the alcoholic extract

presented bacteriostatic effects, but no bactericidal effect, and the dose must be at least 20 times higher for the elimination of the agent. Cultures of *S. aureus* did not undergo selection when exposed to at least 60 generations with sub-lethal doses, thus not causing significant action on the sensitivity of the cell. Finally, they observed, via microscope, changes in morphology and cell size of the microorganism exposed to the propolis extract (0.5 mg/mL). The authors suggest, however, that the use of propolis extract, combined or not, with other antimicrobial agents, may be useful for the control of mastitis *in vivo*.

Meresta et al. (1989) tested a protocol for treatment of bovine mastitis with an alcoholic extract of propolis. As a result of the treatment, complete recovery was obtained in 86.6% of the cows which had acute mastitis, in 100% of cases of infection caused by *Candida albicans*, 85% by *E. coli*, 91% by *Staphylococcus* sp. and 84,3% by *Streptococcus* sp. Given these results, the authors concluded that propolis is effective in mastitis therapy caused by microorganisms resistant to conventional antibiotics. Furthermore, despite the propolis activity against one of the most common agents in mastitis, *S. aureus*, Lima et al. (2019) found significant growth inhibition using propolis in comparative with commercial antibiotics. These results are important, especially considering that *S. aureus* is a very common mastitis agent, and has been becoming increasingly resistant to commercial antibiotics, being propolis a potential substitute.

CONCLUSION

Propolis has antibacterial effects *in vitro* and *in vivo* against causative agents of bovine mastitis and has potential as an alternative to conventional antimicrobial treatment of this disease. However, although satisfactory results have been achieved in scientific studies, there are few reports in the literature regarding the efficacy, using both models (*in vitro* and *in vivo*), safety, and waste released into the milk. More *in vivo* studies should be developed, as there is the need for clinical trials to be able to determine the dosage and the actual effect of different types of propolis in the mammary gland of cattle.

A PRÓPOLIS É UMA CANDIDATA PROMISSORA AO TRATAMENTO DA MASTITE BACTERIANA BOVINA?

RESUMO

Na produção e industrialização do leite bovino, um importante fator que reduz a qualidade e quantidade do produto é a mastite bovina. A mastite em gado leiteiro leva ao uso de antibióticos, que realizado de forma indiscriminada e prolongada leva progressivamente ao crescimento do número de patógenos resistentes. Nesse contexto, produtos naturais usados no combate de microrganismos patogênicos tornaram-se uma alternativa interessante. Entre eles, a própolis se destaca em diferentes estudos, apresentando propriedades bacteriostáticas e bactericidas, sendo uma possível alternativa no controle e tratamento da mastite. Para determinar isso, uma série de estudos aprofundados a respeito da própolis em si, assim como seus componentes, precisam ser analisados. O presente trabalho objetivou responder se a própolis representa uma alternativa natural no tratamento e controle da mastite bovina, baseado em estudos científicos, analisando o seu uso contra diferentes espécies de bactérias, tanto *in vitro* como *in vivo*.

Palavras-chave: Composto natural. Atividade antibacteriana. Tratamento alternativo.

¿ES EL PROPÓLEO UN CANDIDATO PROMETEDOR EN EL TRATAMIENTO DE LA MASTITIS BACTERIANA BOVINA?

RESUMEN

En la producción e industrialización de la leche de vaca un factor importante que reduce la calidad y cantidad del producto es la mastitis bovina. La mastitis del ganado lechero conduce al uso de antibióticos, cuyo uso indiscriminado y prolongado está aumentando progresivamente el número de patógenos resistentes. En este contexto, los productos naturales utilizados para combatir los microorganismos patógenos se han convertido en una alternativa interesante. Entre ellos se destaca el propóleo en diferentes estudios, que presenta propiedades bacteriostáticas y bactericidas, siendo una posible alternativa en el control y tratamiento de la mastitis. Para determinarlo, es necesario analizar una serie de estudios profundos sobre el propio propóleo y las propiedades de sus compuestos. Este trabajo tiene como objetivo responder si el propóleo representa una alternativa natural en el tratamiento y control de la mastitis bovina, a partir de estudios científicos que analizan su uso frente a diferentes especies bacterianas, tanto *in vitro* como *in vivo*.

Palabras clave: Compuesto natural. Actividad antibacteriana. Tratamiento alternativo.

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