J. Dairy Sci. 99:1–11
http://dx.doi.org/10.3168/jds.2015-9777

The effects of Brazilian propolis on etiological agents of mastitis and the viability of bovine mammary gland explants

Samira A. L. Fiordalisi,* Luciana A. Honorato,† Márcia R. Loiko,† César A. M. Avancini,‡ Maria B. R. Veleirinho,§ Luiz C. P. Machado Filho,# and Shirley Kuhnen#[1]
*Federal University of Santa Catarina, Florianópolis, Brazil, 88034-001
†Desidério Finamor Veterinary Research Institute, Eldorado do Sul, Rio Grande do Sul, Brazil, 92990-000
‡Federal University of Rio Grande do Sul, Porto Alegre, Brazil, 91540-000
§Biological Sciences Centre, Federal University of Santa Catarina, Florianópolis, Brazil, 88034-001
#Department of Animal Sciences, Agrarian Sciences Centre, Federal University of Santa Catarina, Florianópolis, Brazil, 88034-001

ABSTRACT

The objective of this study was to evaluate in vitro the antimicrobial activity of Brazilian propolis from Urupema, São Joaquim, and Agua Doce (Santa Catarina State) and green propolis from Minas Gerais State, and the effects of propolis on bovine mammary gland explant viability. The propolis samples differed in flavonoid content and antioxidant activity. Green propolis showed the highest content of flavonoids, followed by the sample from São Joaquim. The propolis from Urupema showed the lowest flavonoid content along with the lowest antioxidant activity. The total phenolics were similar across all studied samples. Despite phytochemical differences, the propolis samples from Minas Gerais, São Joaquim, and Urupema presented the same level of antimicrobial activity against *Staphylococcus aureus* strains. The reduction in *S. aureus* growth was, on average, 1.5 and 4 $\log_{10}$ times at 200 and 500 μg/mL, respectively. At concentrations of 1,000 μg/mL, all propolis reduced bacterial growth to zero. On the other hand, when the propolis were tested against strains of *Escherichia coli*, the samples presented weak antimicrobial activity. Mammary explants were maintained in culture for 96 h without a loss in viability, demonstrating the applicability of the model in evaluating the toxicity of propolis. The origin and chemical composition of the propolis had an effect on mammary explant viability. We encountered inhibitory concentrations of 272.4, 171.8, 63.85, and 13.26 μg/mL for the propolis from Água Doce, Urupema, São Joaquim, and Mina Gerais, respectively. A clear association between greater antimicrobial activity and toxicity for mammary explants was observed. Of all propolis tested, the Urupema sample was noteworthy, as it showed antimicrobial activity at less toxic concentrations than the other samples, reducing bacterial growth to an average of 9.3×10^2 cfu/mL after 6 h of contact using 200 μg/mL of extract. The results demonstrate the potential for Brazilian propolis in the treatment of mastitis, although effectiveness is dependent on geographical origin and concentration. The results from the mammary gland explant assays are promising for the investigation of other natural products with antimicrobial and anti-inflammatory properties that can be used in the intramammary treatment of subclinical mastitis and during dry cow therapy.

Key words: phytotherapy, antimicrobial activity, toxicity, mammary gland explants

INTRODUCTION

Mastitis is the most significant disease in dairy herds, causing reductions in milk production and quality, increases in treatment costs, and a reduction in animal welfare (Nielsen et al., 2010). Despite being disease with many etiologies, the majority of mastitis cases are caused by bacteria (Watts, 1988). *Staphylococcus aureus* and *Escherichia coli* have been documented as the principal etiological agents of bovine mastitis (Passey et al., 2008; Rall et al., 2014).

The prevalence of mastitis in dairy herds has resulted in the extensive use of antibiotics (Bradley, 2002). However, treatment with antibiotics during lactation not only has limited efficacy, but also leads to increased residues found in commercial milk samples (Mukherjee et al., 2005). On the other hand, the use of dry cow therapy, which consists of an antibiotic preparation infused into each quarter immediately after the last milking of a lactation, has helped to cure recurrent infections and prevent new infections (Bradley, 2002; Robert et al., 2006). In such cases, the greatest risk lays in the development of antibiotic-resistant bacteria, but it also presents an obstacle for organic milk production (USDA, 2015).

Received May 4, 2015.
Accepted November 8, 2015.
[1]Corresponding author: shirley.kuhnen@ufsc.br

For mastitis, the search for an antimicrobial product that possesses a wide spectrum of biological properties is directly related to the fact that the bacterial infection is accompanied by an inflammatory response. As such, therapeutic agents with anti-inflammatory, antioxidant, and immunostimulatory properties are needed to improve the overall health of not only the mammary gland, but also the animal. With this goal in mind, plant extracts from magnolol, oregano, macela, and cranberry have been investigated in the treatment of mastitis (Sperotto et al., 2012; Diarra et al., 2013; Wei et al., 2015). Similarly, propolis extracts, which are recognized for their antimicrobial, anti-inflammatory, immunostimulatory, and antioxidant properties (Sforcin, 2007; Bachiega et al., 2012), have also been studied in the treatment of mastitis (Santana et al., 2012). Despite reports of curing mastitis with intramammary propolis use (Mirolyubov and Barskov, 1980; Meresta et al., 1989), Romvary et al. (1993) noted the occurrence of inflammation in the gland after its use. Contradictory results in relation to the use of propolis stems from fact that the product has a highly variable chemical composition that is mainly the result of the flora in the region where the propolis originates and the season. Thus, it has been suggested that the therapeutic properties of propolis are subject to change (Silva et al., 2006; Velazquez et al., 2007).

The study of new therapeutic agents for the treatment of mastitis must begin with the screening of a potential product for its antimicrobial effects on the principal etiological agents causing the disease. Such analysis has been done using in vitro tests with standard bacterial strains and samples of mastitic milk (Silva et al., 2006; Santana et al., 2012); studies have also been done on animal models, such as rats, goats, and cows (Chen et al., 2013; Wei et al., 2015). However, because of the ethical concerns of using animal models in research, the implementation of more effective in vitro models is necessary. Recently, Rabot et al. (2006) assessed the use of bovine mammary explants (alveolar tissue sections) in the study of inflammatory reactions and gland immunity. The results were similar to those found during in vivo studies, suggesting that this model is suitable for the analysis of inflammatory reactions of the mammary gland.

Considering the risks associated with intramammary application of a product that has not been tested for possible tissue damage, the current study investigates both the antimicrobial activity of Brazilian propolis as well as the effects of propolis on the viability of in vitro mammary explants. Our study demonstrates a need to consider the results of in vitro antimicrobial tests in conjunction with tests on mammary explants in selecting potential new substances as substitutes for antibiotics for the intramammary treatment of bovine mastitis or during dry cow therapy. In the current study, we evaluated in vitro the effects of Brazilian propolis from different locations (typical of Santa Catarina and Minas Gerais States) on standard strains and wild-types of *S. aureus* and *E. coli*, as well their effects on the viability of bovine mammary tissue.

MATERIALS AND METHODS

Origins of the Propolis Samples

Propolis samples from the municipalities of Urupema, São Joaquim, and Água Doce in Santa Catarina State, southern Brazil, and green propolis from Minas Gerais were collected during the winter of 2011 (Figure 1). The municipality of Água Doce, located in western Santa Catarina (Figure 2) at approximately 970 m above sea level, is characterized by a humid mesothermal climate, without a dry season, and with cool summers and cold winters, often with frosts and snow. The vegetation is dense and several large tree species, such as *Araucaria angustifolia*, *Parapiptadenia rigida*, *Mimosa scabrella*, and *Cinnamomum zeylanicum*, are typical of the region. Urupema and São Joaquim are neighboring municipalities located in Santa Catarina's mountain range, at approximately 1,500 m above sea level (Figure 2). The climate of the region is classified as humid temperate, with cool summers and harsh winters, and the region is considered one of the coldest in Brazil. The flora of the region is a transition zone between forest interspersed with angiosperms, mainly of the genera *Pinus* spp. and *Araucaria* spp., and natural grasslands, characterized by several grass species of the genera *Avena* spp., *Hemarthria* spp., *Axonopus* spp., and *Trichocline* spp., among others (IBGE, 2012).

Propolis from Minas Gerais (green propolis) was included in our study because it has a unique chemical composition. Green propolis is recognized worldwide for its diverse biological properties and, as such, the majority of its production is destined for the export market. Previous studies have attributed the diverse biological activities of green propolis to the presence of the compound Artepillin C, a prenylated cinnamic acid derivative whose main source is the plant known as field rosemary (*Baccharis dracunculifolia*; Park et al., 2002; Silva et al., 2006; Sforcin, 2007; Bachiega et al., 2012).

Propolis Extraction

For extraction, crude propolis samples from each location were ground with 70% (vol/vol) ethanol (Synth, São Paulo, Brazil) at a ratio of 1:10 (wt/vol). The ex-