



ANTIBACTERIAL AND ANTIBIOFILM EFFECTS OF GREEN PROPOLIS EXTRACTS AGAINST BOVINE MASTITIS STRAINS: IN VITRO, IN VIVO AND ONE HEALTH PERSPECTIVES

Marilia dos Reis Nery Chen^{1*}, Débora Baptista Pereira², Carla Monteiro Leal¹, Otávio Augusto Leitão dos Santos¹, Carla Christine Lange³, Debora Foguel¹, Maria Isabel Sampaio dos Santos¹, Douglas Siqueira de Almeida Chaves²; Ivana Correa Ramos Leal¹.

marilia.nery@outlook.com

1. Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil. 2. Federal Rural University of Rio de Janeiro (UFRRJ), Rio de Janeiro, Brazil. 3. Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil.

Bovine mastitis is one of the main diseases that impairs dairy production, generating economic and social losses. Antibiotic therapy has become a growing concern due to bacterial resistance, which reinforces the need for safe, effective, and sustainable alternatives within the *One Health* framework. Brazilian green propolis (BGP) stands out for its biological potential, associated with the presence of phenolic and prenylated compounds. The objective of this study was to evaluate the antibacterial and antibiofilm potentials of BGP against multiresistant and sensitive strains isolated from bovine mastitis, comparing different extraction methods in order to develop a topical formulation for veterinary use. Ethanolic and methanolic extracts were obtained by maceration, percolation, and ultrasound, and were chemically characterized by HPLC-HRMS/MS and processed using MZmine 4.2. In parallel, feature-based molecular networking available on the GNPS2 platform were used to annotate substances present in the selected extracts. In addition, antimicrobial activity tests (MIC), antibiofilm activity, and scanning electron microscopy (SEM) analysis were also performed. The ethanolic extract obtained by percolation showed the highest yield (47.34%), while the methanolic extract achieved the best results in total phenolics (45.12 mg GAE/100 g). The chemical profile consisted in the annotation of 12 constituents, with artepillin C (*chemical marker*) reaching 33.062% and 30.839% in the percolated extracts, along with baccharin, drupanin, kaempferide, *p*-coumaric acid and other. All extracts demonstrated antibacterial activity against 11 out of 15 strains tested, including *Staphylococcus aureus* (MIC 256 µg/mL) and *S. auricularis* (MIC 64 µg/mL), being inferred as bactericide. Furthermore, all isolates were biofilm producers, with *Staphylococcus* and *Streptococcus* showing the strongest production (level 4). Ultrasound-treated methanolic extracts and ethanolic percolation extracts showed more than 80% inhibition of biofilm formations at subinhibitory concentrations, as evidenced by scanning electron microscopy through the formation of vesicles on the bacterial surface. In the *in vivo* assay stage, an infection model in the invertebrate *Tenebrio molitor* was established, determining sublethal concentrations of both extract and bacteria. To date, the results indicate a potent antibacterial, anti-biofilm, as well as good tolerance in larvae, supporting the continuation of the *in vivo* assays, now in the final stage, and the advancement of the topical nanoemulsion development. These findings strengthen green propolis as a promising candidate for the sustainable control of bovine mastitis, in line with the principles of *One Health*.

Keywords: Brazilian propolis, Natural product, One health, Biofilm, *Staphylococcus*, *Baccharis*.

References: 1. Morales-Ubaldo et al., 2023. (doi: 10.1016/j.vas.2023.100306); 2. Cella et al., 2023. (doi: 10.3390/pathogens12091074); Salatino, Salatino & Negri, 2021. (doi: 10.1007/s13592-021-00889-z).

