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Propolis components accountable for bactericidal accomplishment and antibiofilm activity

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Mastitis, inflammation of the mammary gland, is considered the main disease causing milk yield reduction and consequently a prejudice source for dairy producers. Usually are caused by environmental and infectious pathogens, from which the most frequently found are bacteria of the genus *Staphylococcus*. Antibiotics are used to control bacterial infectious diseases, however bacteria show ability to circumvent antibiotic activity through different mechanisms. Bacterial resistance to antimicrobials is a global problem, which is exacerbated when bacteria are able to produce layered agglomeration, through the production of extracellular polysaccharide arrays, known as biofilms. The main etiologic agent of small ruminant mastitis is *Staphylococcus aureus*, usually very resistant to antimicrobials and often biofilm forming. The use of natural products with antibacterial and antibiofilm activity may be an alternative strategy to control bacterial diseases. *Appis mellifera* honeybees collect resins from various parts of plants and produce a mass, named propolis. This compound is known since Antiquity, is rich in phenolic compounds and, according to various reports, has antimicrobial and antibiofilm activities.

This study aimed at evaluating antimicrobial and antibiofilm activity of phenolic compounds present in propolis ethanol extracts (PEE). Seventy per cent ethanol extracts from seven types of propolis, one Green, two Red and four Brown collected in four Brazilian States were prepared and total phenolics, flavonoids, tannins and anthocyanins were assessed by high-performance liquid chromatography (HPLC). Minimum bactericidal concentration (MBC) and inhibitor effect on *Staphylococcus aureus* biofilm formation and capacity to disrupt established biofilms were assessed towards eight *S. aureus* isolates from milk of small ruminants with mastitis, one methicillin-resistant *S. aureus* (MRSA) and *S. aureus* ATCC 25923. To evaluate different propolis components accountability for bactericidal accomplishment and antibiofilm activity, the results were analysed by the non-parametric Spearman coefficient.

Results of phenolic compounds were 216,21 to 312,08 gallic acid milligram equivalent per extract gram (mg EGA/g) of total phenolics, 55,08 to 140,6 quercetin milligram equivalent per extract gram (mg EQ/g) of flavonoids, 118,51 to 3766,16 catechin milligram equivalent per extract gram (mg EC/g) of tannins and 1,03 to 8,39 milligram per extract gram (mg/g) of anthocyanins. Red1 and Red2 showed higher tannin contents, while Red2 exhibited superior amount of anthocyanins and total phenolics. Brown3 presented higher flavonoid quantity. Green, Red1 and Red2 PEE showed the lowest levels of flavonoids, but the higher antimicrobial activity. Most PEE inhibit bactericidal activity at a concentration of 1.6 mg/mL. Brown4 PEE showed the worst capacity to inhibit *S. aureus*. Green PEE showed to be the most efficient in both preventing and

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disrupting biofilm. All PEE studied exhibited a better inhibitory activity prior-to than post-biofilm formation. According to non-parametric Spearman correlation analysis, there seems to be a significant negative correlation between the ability to disrupt biofilm and both tannins and anthocyanins contents.

Keywords: Brazilian Propolis; Phenolics; Antibiofilm; Antimicrobial; Mastitis; *Staphilococcus aureus*