

**TITLE:** ACTIVITY OF ETHANOLIC AND SUPERCRITICAL EXTRACTS OF BRAZILIAN RED PROPOLIS ON *C. pseudotuberculosis* DIFFERENT STRAINS

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**ABSTRACT:**

Caseous lymphadenitis is an infectious and contagious disease caused by the bacteria *Corynebacterium pseudotuberculosis*. It is characterized by the development of granulomas in superficial lymph nodes and in some organs, and causes significant economic losses due to the decrease of wool and meat production. The treatment of the disease is not effective since the antibiotics are not able to penetrate the granulome's capsule, and the current treatment is based on the surgical remove of the lesions. Propolis has been extensively studied, since many interesting compounds are been described in its composition and it has several activities, markedly an antibiotic action on many different microorganisms. This study aimed to evaluate the resistance/susceptibility of *C. pseudotuberculosis* different strains to ethanolic and supercritical extracts of Brazilian red propolis. The red propolis extracts were obtained by conventional ethanolic extraction and by extraction with CO<sub>2</sub> supercritical fluid on a specific equipment. Three *C. pseudotuberculosis* strains were used: the 1002 strain, used as a standard in the genome sequencing of the bacteria; the pathogenic VD57 strain and the viscerotropic N1 strain. The susceptibility pattern of each strain was obtained using the broth microdilution method using different dilutions of the ethanolic and supercritical extracts. In addition, supercritical extracts obtained with different conditions of temperature and pressure were tested. All extracts obtained with the different supercritical extraction conditions were able to inhibit the bacterial growth of the 1002 strain, but the extract obtained with 40°C temperature and 300 bar pressure conditions showed the highest inhibition pattern (100%). The red propolis supercritical extract was able to fully inhibit N1 and VD57 *C. pseudotuberculosis* strains growth at the concentration of 4mg/mL, while the ethanolic extract was not able to inhibit 100% of N1 and 1002 strains growth at the highest concentration tested (8mg/mL). In conclusion, the red propolis supercritical extract presented a higher antimicrobial capacity than the ethanolic extract and showed this profile on all the tested strains. This extract can be used in further studies on the control and treatment of caseous lymphadenitis.

**Keywords:** antimicrobial activity, caseous lymphadenitis, propolis, small ruminants

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