

Essential oil of conifer turpentine on enterotoxigenic *Escherichia coli* strains isolated from pig gut

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Conifer turpentine is characterized as an important source of terpenes. These compounds belong to a group of natural substances that have considerable importance to plants for their antibacterial and antifungal properties. In animal production, an emerging search for antibiotic alternatives has focused on finding out natural compounds with antibacterial properties that could replace antibiotics in livestock (2). The aim of this work was to evaluate *in vitro* antimicrobial activity of a commercial conifer turpentine essential oil on 4 strains of enterotoxigenic *Escherichia coli* (ETEC) isolated from pig gut as an alternative to Antimicrobial Growth Promoter (AGP) utilization in swine production. ETEC strains were designated by their virulence factors as *E. coli* U21 (K88 LT/STb/F18/STa), *E. coli* U25 (LT/STb/F18/STa), *E. coli* U23 (K88 LT/STb/F18), *E. coli* U7 (K88/LT/STb). Also was performed a comparison with an *E. coli* ATCC 25922. Minimal Inhibitory Concentration (MIC) was performed with two-fold serial dilution from 14.8 to 0.12 mg/mL in 96-well microplate, using Tween 80 as an emulsifier. MIC was determined by absorbance measurement at the end of incubation period and resazurin test. Minimal Bacterial Concentration (MBC) was performed by plating an aliquot of wells that did not show apparent bacterial growth. Results to MIC and MBC, respectively, were 3.70 mg/mL on *E. coli* U21; 3.70 mg/mL to *E. coli* U25; 1.85 mg/mL to *E. coli* U23; 1.85 mg/mL to *E. coli* U7; 1.85 mg/mL to *E. coli* ATCC 25922. GC/MS analysis of conifer turpentine essential oil showed that cymene (20.29%), limonene (19.48%), 1,8-cineole (12.88%), α -pinene (10.44%) and camphene (8.03%) were the major compounds. Conifer turpentine essential oil showed antibacterial activity on strains of *E. coli* isolated from pig gut that could represent a possibility of its inclusion in animal diets to replace AGP. However, its utilization as an alternative to synthetic antibiotics should be verified by *in vivo* evaluations.

1.MERCIER, B.; PROST, J.; PROST, M. The essential oil of turpentine and its major volatile fraction (α - and β -pinenes): A review. **International Journal of Occupational Medicine and Environmental Health**, v. 22, n. 4, p. 331-342, 2009.

2.OETTING, L.L.; UTIYAMA, C.E.; GIANI, P.A.; RUIZ, U.S.; MIYADA, V.S. Effects of antimicrobials and herbal extracts on intestinal microbiology and diarrhea incidence in weanling pigs. **Revista Brasileira de Zootecnia**, v. 35, n. 5, p. 2013-2017, 2006.

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