In vitro antibacterial activity of individual and blended essential oils on pathogenic and probiotic gut bacterial microbiota of poultry and pigs.

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Due to the risk of transmission of antibiotic resistant bacteria to humans through food chain, in 2006 the European Community prohibited the use of antibiotics as growth promoters in poultry and pig production. Therefore, many studies to find alternatives to substitute antibiotics as growth promoters have been carried out. Some essential oils have shown antimicrobial properties and may be an excellent alternative. The aim of this research was to evaluate, in vitro, the antibacterial activity of different essential oils, individually and in binary blends, on pathogenic and probiotic bacteria of pig and poultry microbiota. A screening was performed with five essential oils (EO) obtained from *Eucalyptus globulus*, *E. exserta*, *Pimenta pseudocaryophylllus*, and two EO's which are by-products of orange juice production: Orange oil phase essence, and Citrus terpens. The EO's and blends were tested by disk diffusion method on five pathogenic bacteria: *Salmonella Enteritidis, Escherichia coli, Staphylococcus aureus, Listeria innocua* and *Enterococcus faecalis*, and on three probiotic bacteria*: Lactobacillus plantarum, Lactobacillus rhamnosus* and *Bacillus subtilis*. Analysis of variance showed a significant difference in the antibacterial activity between the evaluated products, and Tukey's test ($p \leq 0.05$) allowed distinguishing them. Thus, orange oil phase essence, and the binary blend of essential oils (BEO), constituted of *E. globulus* and *P. pseudocaryophylllus*, were selected based on their highest activity on pathogenic bacteria and their lowest activity on probiotic bacteria, and also according to the availability of oils that were used in this study. These two oils were tested by microdilution method to determine the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) on the most resistant pathogenic bacterium, *E. faecalis*, and the least resistant probiotic bacterium, *L. rhamnosus*. Two-fold serial dilutions from 14.80 to 0.116 mg/mL were tested. MICs were determined by construction of growth curves and by the resazurin test. Hence, the MIC of orange oil phase essence was 14.80 mg/mL for both bacteria, *E. faecalis* and *L. rhamnosus,* by growth curves. By the resazurin test, the MIC was not observed in this oil for *E. faecalis,* because, at all concentrations, a change in resazurin color was observed. The MICs of BEO for *E. faecalis* and *L. rhamnosus* was 14.80 mg/mL and 7.40 mg/mL, respectively. The BEO was bactericidal at 14.80 mg/mL (MBC) for *L. rhmanosus*. These results showed that, by microdilution methods, it was not possible to observe a selective effect from the oils tested on probiotic bacteria, in contrast with disk diffusion method. Nevertheless, it is important to emphasize the potential antibacterial activity of the oils tested. Limonene was detected as a major compound in orange oil phase essence (87.2%) by GC/MS. In the case of oils that made up the blend, Chavibetol was detected as a major compound in *P. pseudocaryophylllus* (29.2%), and 1,8-cineole as major compound in *E. globulus* (83.7%).

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