

TITLE: *IN VITRO* EVALUATION OF ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS BY DIFFERENT MICROBIOLOGICAL TECHNIQUES

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Essential oils (EO) have been increasingly used in the health area due to their promising antimicrobial, antioxidant, and anti-inflammatory properties. The aim of this research was to evaluate the *in vitro* antibacterial activity of peppermint (*Mentha piperita*), clove (*Syzygium aromaticum*) and melaleuca (*Melaleuca alternifolia*) EO by two different microbiological techniques against standard bacteria: *Staphylococcus aureus* (ATCC 6538), Methicillin-resistant *Staphylococcus aureus* (ATCC 43300) – MRSA and *Escherichia coli* (ATCC 25922). The double-layer agar diffusion well technique was performed in triplicate, Petri dishes (90x15mm) with two layers of Muller Hinton Agar: base (12mL) and seed [8mL+standardized bacterial inoculum (10^6 UFC/mL)]. After the solidification of the culture medium, wells with a diameter of 3mm were made with sterilized straws and 20µL of the EO were added inside them. The plates were pre-incubated at room temperature for 2h and then incubated at 37°C for 24h. The technique for determining the maximum inhibitory dilution (MID) was performed in quintuplicate by microdilution in 96-well U-bottom polystyrene microplates with a final volume of 100µL of Mueller Hinton Broth+OE+5µL of standardized bacterial inoculum (10^6 CFU/mL) per well. Doubling dilutions of the EO were carried out from 25µL/mL to 0.0001µL/mL. Furthermore, the plates were incubated at 37°C for 24h. For both techniques, controls without and with antibacterial activities, sterilized class II purified water (CIIPW) and 0.12% chlorhexidine gluconate (CHX) were used in the experiments, respectively. The means of the EO inhibition halos (peppermint, clove, tea tree and CHX) were: *S. aureus* (8.7mm, 14.0mm, 21.0mm and 21.0mm), *E. coli* (5.7mm, 16.3mm, 17.3mm and 19.3mm) and MRSA (12.0mm, 13.0mm, 27.3mm and 20.3mm). In addition, the MID of the EO (peppermint, clove, tea tree and CHX) were: *S. aureus* (12.5µL/mL, 3.1µL/mL, 25µL/mL and 0.0005µL/mL), *E. coli* (12.5µL/mL, 6.3µL/mL, 12.5µL/mL and 0.002µL/mL) and MRSA (6.3µL/mL, 3.1µL/mL, 6.3µL/mL and 0.0005µL/mL). It should be noted that CIIPW did not show any antibacterial activity. In conclusion, peppermint, clove, and tea tree EO showed antibacterial activity against all bacterial strains evaluated in this study. Furthermore, the two microbiological techniques showed different results, being tea tree and clove EO with the highest inhibition halos and MID, respectively.

Keywords: microbiology, volatile oils, bacterias.

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