Antimicrobial activity of essential oils against periodontal pathogens

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Introduction

Periodontal diseases are inflammatory disorders caused by pathogenic bacteria accumulation around the teeth, forming a dental plaque that affects the tissues that surround and support teeth and, in a more advanced stage, can lead to tooth loosening and eventually its loss. [1] The main problem with the common administration of antimicrobial agents to treat the infection, alongside known side effects, is the growing bacterial resistance to these drugs, which is considered by WHO as one of the top global challenges. [2]

Essential oils (EO) are biosynthesized by plants and consist of a mixture of compounds whose synergistic effects result in antimicrobial activities of great interest, so they have been studied as a natural medicine alternative for these treatments. [3] [4]

Aim

The main objective of this project is to assess the efficacy of the eucalyptus EO as a therapeutic agent by:

- Studying the antimicrobial activity of the EO against planktonic Candida albicans ATCC 10231 , Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923.
- Evaluating their cytotoxicity using fibroblast cells L929.

Methodology

Antimicrobial activity assays (according to what was defined by the Clinical and Laboratory Standards Institute)

- C.albicans, E.coli and S.aureus grown overnight at 37°C in TSB.
- Preparation of a suspension with 10⁶ CFU/mL.
- Incubation of the exponential microorganism cultures with different concentrations of the EO on 96-well plates at 37°C, 150 rpm for 24h.
- Quantification of the planktonic population by the colony-forming units (CFUs) method.
- Determination of the minimum inhibitory concentration (MIC) as the lowest concentration of EO at which no visible growth was detected and the minimum lethal concentration as the lowest concentration which killed the microorganism.

Cytotoxicity assay (based on the standard cytotoxicity assessment set by the International Standardization Organization)

- Seeding of the fibroblast cells at 1x10⁶ cells/mL on a 96-well plate in α-MEM supplemented with 10% SBF and 1% P/F for an incubation of 24h at 37°C in a humidified atmosphere of 5% CO₂.
- Incubation of the cells with different EO concentrations for 24h in the standard conditions.
- MTT assay to assess cell metabolic activity.

Results

Antimicrobial Activity

Figure 1. Log₉₉ planktonic concentration in CFUs/mL of E.coli, C.albicans and S.aureus incubated with the different concentrations (v/v) of the eucalyptus essential oil for 24 hours, quantified by the CFUs method.

For the range of concentrations tested, the eucalyptus EO showed antimicrobial activity for the concentrations of 50% against E.coli, 1% for C.albicans and 10% for S.aureus. Regarding the MIC, it was determined as 0.5% for C.albicans, as the lowest EO concentration capable to inhibit the planktonic population of about 99%. Related to E. coli and S. aureus, the used EO concentrations were not able to reduce the bacterial growth, being impossible to define the MICs for both bacteria.

Cytotoxicity

Figure 2. Values of fibroblast cell viability in percentage of control after 24 hours of incubation in the presence of the different concentration of the eucalyptus essential oil, obtained by MTT assay.

As depicted in figure 2, a reduction in cell viability of about 40% was observed when the cells were exposed to the EO at a 50% concentration, as compared to the control. For the remaining concentrations evaluated, no cytotoxicity was observed.

Conclusion

- Minimum antimicrobial lethal concentrations of the eucalyptus EO at 50%, 1% and 10% were obtained for E.coli, C.albicans and S.aureus, respectively.
- A MIC of 0.5% was determined for C.albicans. For E.coli and S.aureus the MIC will be further assessed.
- Eucalyptus EO showed cytotoxicity on fibroblast cells for 50% concentration and no cytotoxicity for the remained concentrations.
- Together, these findings indicated that this agent is ought to be further investigated as a potentially reliable antimicrobial agent.

References