Targeting Skin-Associated *Candida*: Antifungal Efficacy of Selected Essential Oils

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ABSTRACT (250-300 words) – 299 words

Background: *Candida* spp. are commensal yeasts that inhabit the skin and mucous membranes and are the leading cause of invasive fungal infections. Mucocutaneous candidiasis is a common condition, often treated with azoles and polyenes, which may cause side effects and foster resistance. Essential oils (EOs), natural compounds with antimicrobial activity, present a promising alternative due to their multi-target action, reducing the likelihood of resistance.

Objective: To study the antimicrobial activity of three EOs – eucalyptus, lavender, and thyme – against two species of *Candida* (*C. albicans* and *C. tropicalis*).

Methods: The antimicrobial potential of the EOs against the selected yeasts was assessed using the following tests: (i) agar diffusion assay; (ii) determination of minimum inhibitory (MIC) and fungicidal concentrations (MFC); (iii) growth inhibition curves at MIC, 2×MIC, and ½MIC; (iv) anti-biofilm activity at MIC, ½MIC, and ¼MIC; (v) membrane integrity analysis by flow cytometry using propidium iodide (PI) and thiazole orange (TO); and (vi) fluorescence microscopy of yeasts exposed to eucalyptus EO at ¼MIC.

Results: *C. albicans* inhibition zones were 5.13 mm (eucalyptus), 5.0 mm (lavender), and complete inhibition with thyme EO. All EOs fully inhibited *C. tropicalis*. MIC and MFC values ranged from 0.08% to 2.5% (v/v), with thyme EO having the lowest values for both yeasts. Inhibition curves confirmed MIC results. Thyme EO also showed the greatest inhibition of biofilm formation. Flow cytometry revealed membrane damage in both species, with *C. tropicalis* showing a higher proportion of dead and damaged cells. Thyme EO consistently induced the highest levels of cell damage across all concentrations tested. Fluorescence microscopy supported flow cytometry findings, revealing live (green), injured (yellow), and dead (red) cells following EO exposure.

Conclusion: All tested EOs displayed antifungal activity, with thyme EO being the most effective. *C. albicans* was more resistant than *C. tropicalis*, highlighting species-specific EO sensitivity.

BIOGRAPHY (100-150 words)

Ana Isabel Lopes holds a BSc in Biology from Faculdade de Ciências da Universidade do Porto (FCUP) and an MSc in Applied Microbiology from Escola Superior de Biotecnologia – Universidade Católica Portuguesa (ESB-UCP). After finishing her master's degree, she did an internship at Centro de Genética Médica Doutor Jacinto de Magalhães (Porto), where she acquired skills in the field of medical genetics. From December 2020 to June 2023, she was a research fellow at Centro de Biotecnologia e Química Fina (CBQF-ESB), working on the project BIOMA. In this project, she was responsible for the production and characterization of antimicrobial edible films with plant extracts and essential oils and their application on fruits in order to expand their shelf life. Currently, she is a PhD student at ESB-UCP, developing her PhD thesis under the supervision of Professor Freni Tavaria and Professor Manuela Pintado.

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