Title ASSESSING THE ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST SKIN MICROORGANISMS USING FLOW CYTOMETRY **Code** 36

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Topic Microbiology

Abstract

Introduction The skin microbiome is a dynamic ecosystem vital for skin health, comprising bacteria like Staphylococcus spp. and Cutibacterium acnes, and fungi such as Candida and Malassezia spp. Dysbiosis, or microbial imbalance, can lead to conditions like acne and dermatitis. Current treatments, including anti-inflammatory drugs and antimicrobials, help manage dysbiosis but have limitations, such as microbiome disruption and antimicrobial resistance. Consequently, interest in natural alternatives, particularly essential oils (EOs), is increasing. Rich in bioactive terpenes and terpenoids, EOs target multiple cellular structures, reducing microbial adaptation and resistance. Flow cytometry, a powerful analytical tool, enables precise assessment of antimicrobial activity by distinguishing live and dead microorganisms using fluorescent staining. This study evaluates the antimicrobial effects of eucalyptus, lavender, and thyme EOs, offering insights into their potential as microbiome-friendly therapeutic agents.

Methods A flow cytometry assay was used to assess the antimicrobial activity of eucalyptus, lavender, and thyme EOs against seven skin-associated microorganisms: methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant S. aureus (MRSA), Staphylococcus epidermidis (S. epidermidis), Cutibacterium acnes (C. acnes), Candida albicans (C. albicans), Candida tropicalis (C. tropicalis), and Malassezia furfur (M. furfur). Firstly, minimum inhibitory (MIC) and bactericidal (MBC) concentrations were determined. Then, to further explore EOs antimicrobial effects, microbial cells were exposed to MIC, ½MIC, and ¼MIC of each oil, followed by staining with propidium iodide (PI) and thiazole orange (TO). Flow cytometry was then used to assess viability, membrane integrity, and cell death. Additionally, fluorescence microscopy was performed on yeasts exposed to eucalyptus oil at ¼MIC, providing complementary validation of the flow cytometry findings.

Results The MIC and MBC values of the tested EOs ranged from 0.06% to 2.5% (v/v), with thyme EO showing the lowest values for all microorganisms, indicating its strongest antimicrobial activity. Among bacteria, S. epidermidis had the highest MIC/MBC values, suggesting greater resistance, while C. acnes had the lowest, indicating higher susceptibility. Among yeasts, C. albicans exhibited the highest values, whereas C. tropicalis and M. furfur were more susceptible. Flow cytometry confirmed these findings, validating thyme EO as the most effective, with the highest percentages of dead and injured cells. It also demonstrated that EOs antimicrobial activity is concentration-dependent. S. epidermidis had the highest percentage of live cells, reinforcing its resistance, while C. acnes had the lowest, confirming its susceptibility. C. albicans was more resistant than C. tropicalis. For M. furfur, fluorescence microscopy clarified an apparent lack of TO staining in flow cytometry, confirming viable but unstained cells.

Conclusion The selected EOs exhibited antimicrobial activity against all tested microorganisms. Thyme EO showed the strongest effect. Flow cytometry confirmed the MIC/MBC results, revealing that thyme EO induced the highest percentages of dead and injured cells at all tested concentrations. Among bacteria, S. epidermidis was the most resistant, while C. albicans was the most resistant yeast. For M. furfur, flow cytometry suggested an absence of live cells due to the lack of TO staining, even in untreated samples. However, fluorescence microscopy confirmed that M. furfur cells remained viable but did not stain green like Candida spp. This discrepancy likely results from the yeast's lipid-rich membrane interfering with TO uptake, highlighting potential staining limitations. This work reinforces flow cytometry as a powerful tool to validate EO's antimicrobial effects and support the potential of thyme, eucalyptus, and lavender EOs as natural alternatives for managing skin dysbiosis while preserving microbiome balance.

Conflict of Interest No.