MICRO BIOTEC 25

CONGRESS OF MICROBIOLOGY
AND BIOTECHNOLOGY 2025

04/05/06 DECEMBER 2025

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ABSTRACT BOOK

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MB 25

ID496 | Integrating Traditional Antifungal Tests and Flow Cytometry to Evaluate the Activity of Essential Oils Against Malassezia furfur

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Background: Malassezia spp. are lipid-dependent yeasts that are an important part of the normal skin mycobiome in humans and other warm-blooded animals. Although generally harmless, they can, under certain conditions, contribute to skin disorders, with Malassezia furfur (M. furfur) being the main species linked to invasive infections. Current treatment mainly relies on topical or systemic antifungals, which, despite their effectiveness, are limited by side effects, drug interactions, species-specific resistance, and frequent recurrences. In this context, essential oils (EOs) are emerging as promising alternatives, as they show broad-spectrum antimicrobial activity and multi-target mechanisms that lower the risk of resistance. Thus, this study aimed to evaluate the antifungal activity of six EOs—rosemary, eucalyptus, lavender, basil, sage, and thyme—against M. furfur.

Methods: Antifungal activity was assessed through: (i) agar diffusion assay; (ii) determination of minimum inhibitory (MIC) and fungicidal concentrations (MFC); (iii) growth inhibition curves at MIC; (iv) anti-biofilm activity at MIC, ½MIC, and ¼MIC; (v) membrane integrity analysis by flow cytometry using propidium iodide (PI) and thiazole orange (TO) for the most active oils (eucalyptus, lavender, thyme); and (vi) fluorescence microscopy of yeasts exposed to eucalyptus EO at ¼MIC.

Results: Inhibition zones ranged from 20 ± 0.00 mm (rosemary) to 33 ± 2.00 mm (eucalyptus), with basil and thyme achieving complete inhibition. MIC and MFC values ranged from 0.08% to 2.5% (v/v), with thyme showing the lowest values. Growth inhibition curves confirmed MIC data. Thyme also displayed the strongest antibiofilm effect. Flow cytometry suggested the absence of viable cells due to a lack of TO staining, even in untreated controls. However, fluorescence microscopy revealed that cells remained viable but failed to show TO uptake, likely because the lipid-rich membrane interfered with staining.

Conclusions: This work demonstrates the strong antifungal activity of EOs, particularly thyme, eucalyptus, and lavender, against M. furfur, affecting both planktonic and biofilm forms. Importantly, it represents one of the few studies applying flow cytometry to this yeast, exposing critical limitations in conventional staining methods. The novelty of this study advances knowledge on EO efficacy and highlights the need for further research to refine flow cytometry protocols for reliable assessment of M. furfur viability