

P2269 Flow cytometry assay of antifungal activity and mechanism of action of selected essential oils on *Candida albicans* clinical strains

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Background: *Candida* is the most common yeast causing worldwide fungal infections, with an increasing number of resistant isolates to currently available antifungal drugs recognized worldwide, raising an acute need for alternative/complementary strategies to solve the infections due to resistant yeast strains. The purpose of present study was to assess the potential of flow cytometry (FC) to evaluate the antifungal activity of three vegetal essential oils, as well as to identify some of the mechanisms responsible for their antimicrobial activity against *Candida albicans* strains.

Materials/methods: The essential oils extracted from *Origanum vulgare*, *Syzygium aromaticum* and *Thymus vulgaris* were tested, using Fluconazol (FCZ) as positive control. The fungal strains were grown in liquid medium for 24 h in the presence of different concentrations of the tested oils and of FCZ. After incubation, the cultures were centrifuged, washed and adjusted to a density corresponding to 0.5 McFarland standard. Heat-treated cells (90°C for 15 min) were used as a positive staining control. Both samples and control were stained with oxonol DiBAC4(3) viability marker. FC measurements were performed using a Becton Dickinson (BD) FACS Calibur™ flow cytometer with CELLQuest™ software for data acquisition. Live/dead bacteria were differentiated by a side scatter threshold. The samples positive for DiBAC4(3) were considered dead, whereas viable cells were negative. All results were compared with those obtained by culture-based standard assays.

Results: All essential oils tested in this study exhibited a high antifungal activity against both reference and clinical *C. albicans* strains, comparable or even higher than the positive control (FCZ). The FC assay identified the efflux pump inhibition as one of the main mechanisms of the antifungal action of the tested oils.

Conclusions: Our results proved the anti-infectious potential of *Origanum vulgare*, *Syzygium aromaticum* and *Thymus vulgaris* essential oils which are valuable as alternatives for the development of novel antifungal strategies.

