Oregano Essential Oil: An Effective and Non-Toxic Approach for prevent o treat Resistant *Candida* Species

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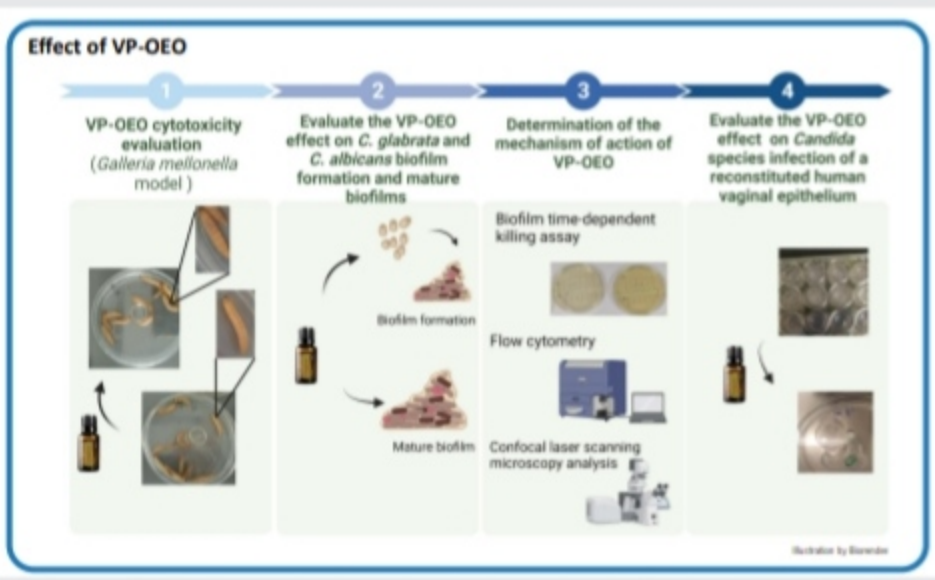
Introduction

Vulvovaginal candidiasis (VVC) is a prevalent vaginal infection, with drug-resistant *Candida* strains representing a growing treatment challenge[1,2]. *Candida albicans* and *Candida glabrat* particularly resistant to azoles, are the most frequently found species[3]. Traditional antifungal agents like azoles, polyenes and echinocandin are becoming less effective, making the discove of effective alternative therapies crucial[1]. In this sense, oregano essential oil (OEO) has emerged as a promising alternative due to its antimicrobial properties, with the ability to inhib *Candida* biofilms, a key factor in resistance[4]. Recent studies have shown that the vapor phase of essential oils (VP-EOs) has greater antimicrobial activity than the liquid phase[5]. Howeve essential oils (EOs) effectiveness is influenced by factors such as photosensitivity, high volatility, low water miscibility, and degradability at higher temperatures, reducing bioavailability.

Goal of the study

- Evaluation of the vapor phase of oregano EO (**VP-OEO**) effect on biofilms of antifungal-resistant *Candida* species (*C. albicans* and *C. glabrata*) and determine their mode of actio Furthermore, a reconstituted human vaginal epithelium (RHVE) was used to mimic vaginal conditions and evaluate the effect of this therapy on *Candida* infection.
- *In vitro* and *in vivo* evaluation of the effect of microparticles (KMPs) loaded with oregano essential oil (**OEO-KMP´s**) for the *C. albicans* infection treatment.

Methods



Results

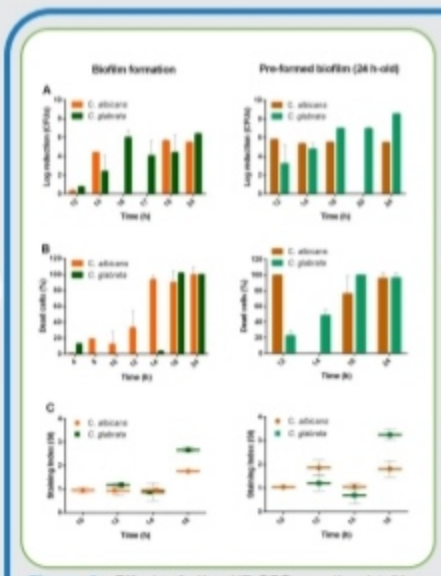


Figure 1. Effect of the VP-OEO on the biofilm formation and pre-formed biofilm of *C. albicans* and *C. glabrata* , evaluated from **(A)** time-killing assay, **(B)** cell viability and **(C)** metabolic activity (SI).

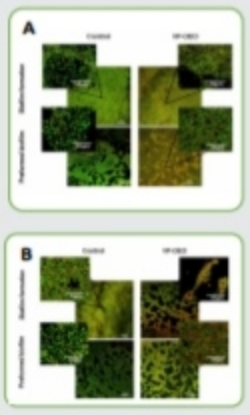


Figure 2. Confocal Laser Scanning Microscopy images of **(A)** *C. albicans* biofilms and **(B)** *C. glabrata* biofilms after 12 h exposed of VP-OEO. CLSM images show the staining pattern for live cells (green) and dead cells (red).

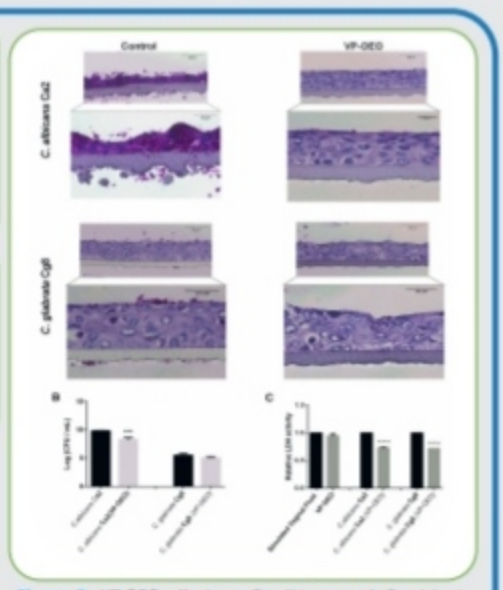


Figure 3. VP-OEO effect on *C. albicans* and *C. glabrata* species infection of the RHVE after 24 h **(A)** RHVE images showing *Candida* infection and VP-OEO effect; **(B)** *Candida* infection of the RHVE (log CFU per milliliter).; **(C)** Relative lactate dehydrogenase (LDH) activity measured in the RHVE supernatant after incubation with the *Candida* strains compared to the untreated epithelium and effect of VP-OEO compared to simulated vaginal fluid. * indicate a statistically significant reduction in comparison with the respective control (*** P<0,001, ****, P<0.0001).

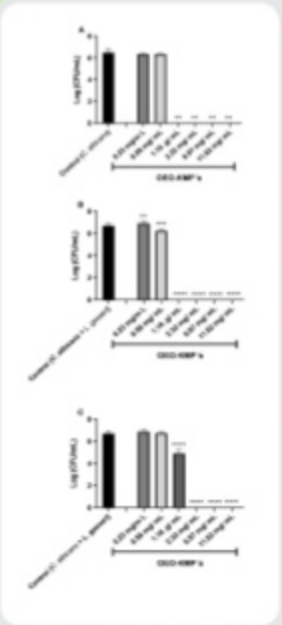


Figure 4. Effect, *in vitro*, of the OEO-KMP´s on **(A)** single and mixed infection of **(B)** *C. albicans* with **(C)** *Lactobacillus gasseri*. * indicate statistical reduction of biofilms cell cultivability in comparison with the respective control (**p<0.01, *** p< 0.001, **** p< 0.0001).

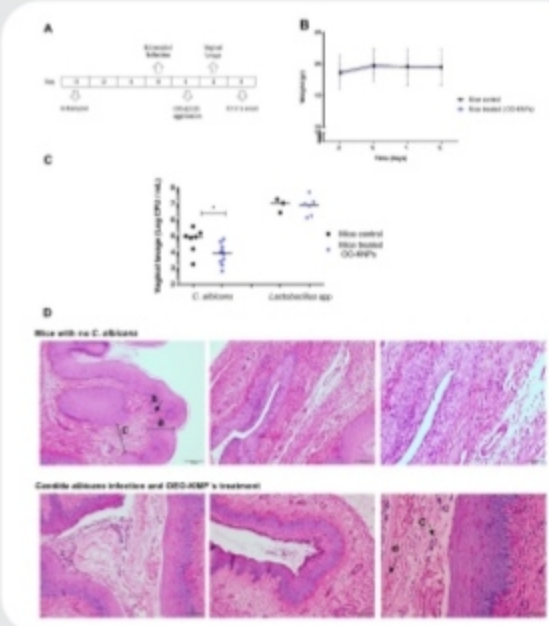


Figure 5. Mice model of vaginal candidiasis. **(A)** Timeline of *albicans* infection and OEO-KMP's treatment of mice models; **(B)** M weight according to the timeline of infection; **(C)** *C. albicans* a *Lactobacillus* species in mice vaginal lavage fluid (*p < 0.05); Histopathology of vaginal tissue from mice with no *C. albicans* and a *C. albicans* infection and OEO-KMP's exposure. (a) layer of superfi squamous epithelial cells, (b) basal membrane, (c) submucosa, (d) *albicans* cell and (e) neutrophils. The results were registered under and 20× magnification.

The study showed potent antifungal activity of the **VP-OEO** against two drug-resistant *Candida* isolates:

- ✓ Flow cytometry analysis indicated that VP-EO's mechanisms of action involve altering membrane integrity and decreasing metabolic activity.
- ✓ The epithelial model confirms the VP-OEO's, with LDH activity levels showing no significant effect on RHVE compared to the control.

The **OEO-KMP´s** effectiveness, *in vitro* and *in vivo*, against *C. albicans* infecti were confirmed:

- ✓ This therapy keeps the microflora intact, specifically in terms *Lactobacillus* species.

Main conclusion

These studies highlight the promising efficacy of **Oregano essential oil** as an alternative for VVC treatment. Both approaches, **VP-OEO and OEO-KMP´s**, showed effective antifungal activity against drug-resistant strains while preserving vaginal health. These therapeutic options not only combat antifungal resistance, but also potentially propose a safer option for women's health due to their natural characteristics.

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