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SHORT COMMUNICATION

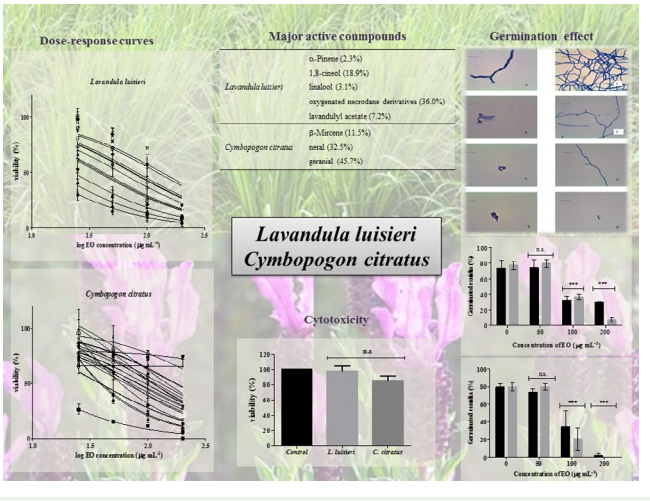
Oxygenated monoterpenes-rich volatile oils as potential antifungal agents for dermatophytes

N. Dias[a], M.C. Dias[b], C. Cavaleiro[c], M.C. Sousa[c,d], N. Lima[a] and M. Machado[b,c,e]

[a]CEB – Centre of Biological Engineering, University of Minho, Campus Gualtar, Braga, Portugal; [b]CESPU – Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal; [c]CEF – Centro de Estudos Farmacêuticos/Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; [d]CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; [e]CIBIO – Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus Agrário de Vairão, Vila do Conde, Portugal

ABSTRACT

Essential oils (EOs) extracted from *Lavandula luisieri* and *Cymbopogon citratus* were tested for their antifungal activity against ten clinical isolates of dermatophytes isolated from cases of tinea pedis. Inhibition of conidial germination and antifungal drug/EO combination assay were tested on two ATCC reference strains of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. EOs were characterised by high amount of oxygenated monoterpenes in their composition. Strong antifungal activity was observed for the majority of clinical strains, and fungicidal activity was demonstrated. Positive interaction between *L. luisieri* EO combined with terbinafine was observed against terbinafine-resistant strain (*Tr* ATCC MYA-4438). Significative reduction of the germination was observed above 100 µg mL^{-1}. Both oils were safe to macrophage mammalian cells at tested concentration. This study describes the antifungal activity of *L. luisieri* and *C. citratus* EOs against dermatophytes, which could be useful in designing new formulations for topical treatments.



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CONTACT N. Dias nidias@deb.uminho.pt

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1. Introduction

Dermatophytoses are among the most prevalent infectious diseases worldwide, which incidence has increased, mainly on urban environments. Occasionally, it can spread to other parts of the body, and it is highly contagious. High rate of recurrence following antifungal treatment limited use in some patients due to side effect, and medication interactions are common problems found when using conventional drugs (Vander Straten et al. 2003). Thus, clinical isolates of drug-resistant *Trichophyton rubrum* exhibiting high-level primary resistance to terbinafine have been reported (Favre et al. 2004). Medicinal plants are the richest natural source of bioactive phytochemicals and antioxidants. Among those, monoterpenes are main constituents of essential oils (EOs). Furthermore, EOs containing high amounts of oxygenated monoterpenes (OM) are related to broad spectrum antibacterial activities (Messaoud et al. 2012; Ait Said et al. 2015; Carrasco et al. 2016).

In this study, *Lavandula luisieri* and *Cymbopogon citratus*, which are known for their high content of OM, were tested for their potential use as topical therapeutic agents against dermatophyte strains involved in foot dermatophytoses.

2. Results and discussion

2.1. Analysis of oils, antifungal activities and viability assay

Major compounds are listed in Table S1. *L. luisieri* EO was predominantly composed by oxygenated necrodane derivatives, followed by 1,8-cineol as main compounds. The presence of necrodane derivatives has been reported as the characteristic and dominant compounds in *L. luisieri* EO, which could be used as a chemotaxonomic marker of this species (Zuzarte et al. 2012). Both EOs were characterised by high amounts of oxygen-containing monoterpenes (OM > 75%) totalizing 80.4% and 97.9% of monoterpenic compounds (MH and OM) for *L. luisieri* and *C. citratus*, respectively. In Table S2, a strong antifungal activity and a fungicidal mode of action with MFC = 200 µg mL^{-1} were observed for both EOs. Antifungal effect of both EOs was dose dependent for all dermatophytes (Figure S1), although *C. citratus* showed a mild effect on clinical strains of *T. mentagrophytes*. Strong antifungal activity has been positively correlated with the presence of OM (Mesa-Arango et al. 2009; Prusinowska et al. 2016). Besides, *L. luisieri* EO with the highest amount of necrodane compounds shown to be the most active against dermatophyte strains (Zuzarte et al. 2012).

2.2. In vitro antifungal drug/EO combination assay

The combination between EOs and conventional antimicrobial drugs has been referred as a strategy to bring about the maximum of therapeutic efficacy by additive or synergistic effect (Wagner & Ulrich-Merzenich 2009). Although combination therapy has been already recommended to improve monotherapy results, few studies (Shin & Lim 2004; Khan & Ahmad 2011) have reported on the antifungal effect against dermatophytes. Table S3 shows a positive interaction of *L. luisieri* EO with terbinafine (TER) against the terbinafine-resistant *Tr* ATCC MYA-4438 in all fixed-ratio concentrations suggesting a synergistic activity between the drug and EO, as the effect of the combined substances is greater than the sum of the individual effects (Burt 2004). Consequently, a minor dose of terbinafine would be necessary to obtain the same antifungal effect when used in combination with *L. luisieri* EO. In this

study, the best antifungal effect was obtained for the ratios 1:1 and 3:1. At TER/EO ratio 1:3, combined activity was apparently additive. In this perspective, combined therapy of EO with conventional drugs may lead to a reduction of drug doses by decreasing possible adverse side effects or increasing therapeutic effects (Khan & Ahmad 2011).

2.3. Effect of EOs on conidial germination and morphology

Both oils proved to be effective in delaying germination of conidia on reference strains. Results on the percentage of conidia germinated after 24 h of treatment with *L. luisieri* and *C. citratus* EOs are displayed in Figure 1. Significant reduction of the germination ($p < 0.001$) was observed for strains treated with EO concentrations above 100 µg mL^{-1}, and most conidia were non-germinated at 200 µg mL^{-1}. The effect of *L. luisieri* EO on the morphology of conidia and hyphae after 24 and 48 h of treatment, respectively, is shown in Figure S2. *L. luisieri* EO induced morphological alterations on *T. rubrum* conidia. Germination alterations were clearly visible after 24 h of exposure at sub-MIC concentrations (A–D). Thus, growth of the germinative tube was severely impaired at 50 µg mL^{-1}. At 100 µg mL^{-1}, alterations on the morphology of conidia were clearly observed. After 48 h of incubation (E–H), the mycelium structure became irregular with the loss of ramifications. No mycelium growth was observed for most cells, at 200 µg mL^{-1}. Inhibition of DNA synthesis or disruption of membranes is mechanism of phytotoxicity found in OM, which lead to anatomical and physiological changes (De Martino et al. 2010). Therefore, it was expected that impaired germ tubes originated distorted hyphae compromising cell morphogenesis and further growth. In a previous work, Zuzarte et al. (2012) reported on the capacity of inhibition of the germ tube of *Candida albicans* by *L. luisieri* at sub-MIC values.

2.4. Cytotoxicity of EOs

Careful use of lavender oil in highly diluted forms, when applied to the skin, has been recommended by Prashar et al. (2004). In our study, EOs did not cause a significant alteration

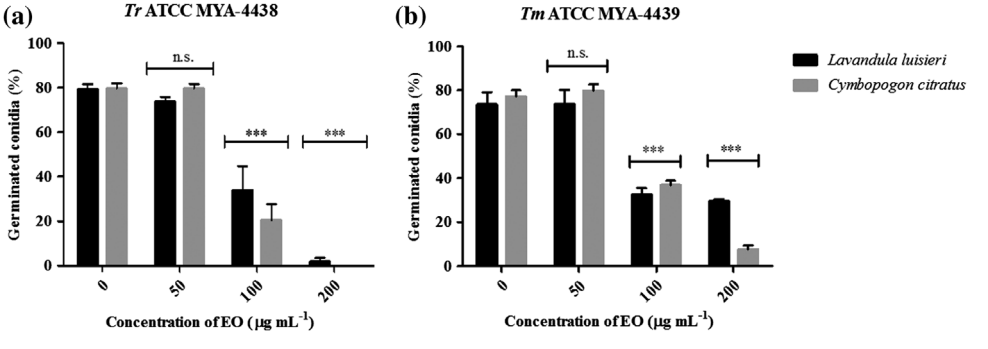

Figure 1. Effect of *L. luisieri* and *C. citratus* on the germination of conidia of reference strains of dermatophytes *T. rubrum* ATCC MYA-4438 (a) and *T. mentagrophytes* ATCC MYA-4439 (b). Fungal cultures were treated for 24 h with different concentrations of the oils ranging from 0 to 200 µg mL^{-1}. Results are expressed as percentage of germinated conidia on untreated and treated cultures of dermatophytes. Values are expressed as mean ± SEM from three independent experiments. ***$p < 0.001$ significant statistical differences compared to control culture. n.s. = not significative.

on the viability of treated mouse macrophage cells when compared to control cells (Figure S3). No induction of alterations on the morphology of cells was observed under light microscopy (data not shown). In addition, a very low detrimental effect was found on mouse macrophage cells at concentrations of *L. luisieri* EO up to 320 µg mL^{-1} (Zuzarte et al. 2012).

3. Conclusion

The chemical composition of the tested EOs, mainly the OM fraction, suggests an association with the activity profile of EOs against dermatophytes. In addition to fungistatic and fungicidal activity, the EOs revealed an important inhibitory effect on germination of conidia at sub-MIC concentrations, thus reinforcing their potential use in topical therapy. This study also proposes *L. luisieri* as a suitable oil for combined treatment against terbinafine-resistant fungal strains, although further *in vivo* experiments are needed to evaluate the therapeutic efficacy and safety of the EO in combination with antifungal drugs.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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