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Lavandula luisieri essential oil as a source of antifungal drugs

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ABSTRACT

This work reports the antifungal activity of *Lavandula luisieri* essential oils against yeast, dermatophyte and *Aspergillus* strains responsible for human infections and food contamination. The oil's cytotoxicity and its effect on the yeast-mycelium transition in *Candida albicans*, an important virulence factor, were also evaluated.

Analyses by GC and GC/MS showed a peculiar composition of irregular monoterpenes. Significant differences between the samples occurred in the amounts of 1,8-cineole, fenchone and *trans*-α-necrodyl acetate. The oil with higher amounts of irregular monoterpenes was the most effective. The influence of the oils on the dimorphic transition in *C. albicans* was also studied through the germ tube inhibition assay. Filamentation was completely inhibited at concentrations sixteen times lower than the minimal inhibitory concentration.

The results support the use of *L. luiseiri* essential oils in the development of new phytopharmaceuticals and food preservatives and emphasise its antifungal properties at concentrations not cytotoxic or with very low detrimental effects on mammalian cells.

1. Introduction

Fungi are responsible for serious pathogenic infections that have increased during the last decades, particularly among high-risk patients (Pfaller, Pappas, & Wingard, 2006). Although conventional antifungals are available, the increased resistance to these compounds and their side-effects varying from mild reactions to hepatotoxicity, neurotoxicity, nephrotoxicty and haematologic reactions are frequently responsible for unsuccessful treatments (Del Rosso, 2000; Gupta & Cooper, 2008). Moreover, the effective lifespan of classical antifungals is limited due to their repeated use as antifungals and immunosuppressive drugs as well as in organ transplantation, lymphomas, and HIV secondary infections.

Foods, commodities and raw materials are also vulnerable to contamination by fungi, in particular with *Aspergillus* spp., a major cause of food spoilage in tropical countries, due to the production of powerful mycotoxins (Whitfield, 2004). These fungi are responsible for food decomposition and the production of allergenic compounds which may occur before the fungal growth is detectable (Pirbalouti, Hamedi, Abdizadeh, & Malekpoor, 2011). Thus, and taking into account the increasing worldwide incidence of fungal infections, the search for more effective and less toxic antifungals as an alternative to synthetic ones is reasonable.

Aromatic plants have been used in traditional medicine due their antimicrobial properties. Nowadays, their essential oils have become particularly widespread in screening bioactivity assays (e.g., Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Reichling, Schnitzler, Suschke, & Saller, 2009). Although many studies have reported the antifungal activity of essential oils, the interactions between the oils and microorganisms, which are lately responsible for its activity, remain poorly understood. Furthermore, studies aiming to understand the action mechanisms and the toxicological safety of the oils are currently missing, hence hampering its potential utilisation for industrial and commercial purposes.

Lavandula spp. essential oils, in particular those of *L. angustifolia*, *L. latifolia*, *L.* x *intermedia* and *L. stoechas*, have been used in perfumery, cosmetics, food processing, and more recently in aromatherapy, since ancient times (Upson & Andrews, 2004). The lavender scent is very popular in pillows, bath care, home and pet products, and provides a unique taste to many beverages, sweets, jellies, jams, marmalades, honey and condiments.

The oils have also been claimed to possess antibacterial, antifungal, carminative, antidepressive and sedative properties and are well known as effective against burns and insect bites (Cavanagh & Wilkinson, 2002). However, many of these putative properties are based only on traditional beliefs rather than on scientific evidence.

L. luisieri (Rozeira) Rivas Mart. is one of the five spontaneous species of the genus *Lavandula* occurring in Portugal. It is endemic

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to the Iberian Peninsula, being very common throughout Portugal and in the Southwest of Spain (Amaral Franco, 1984; Upson & Andrews, 2004).Traditionally this species has been used as an expectorant in chest problems and colds, against headaches and migraines and for its anti-spasmodic, laxative and stimulant properties. It is also used as a disinfectant, to perfume linen and protect it against moths (Upson & Andrews, 2004).

During the last years, several studies have shown interesting properties of *L. luisieri* essential oils foreseeing many other applications. The biological activities so far reported for these oils include: antifeedant effects against *Leptinotarsa decemlineata, Myzus persicae*, *Rhopalosiphum padi*, and *Spodoptera littoralis* (González-Coloma, Delgado, Rodilla, Silva, Sanz, & Burillo, 2006; González-Coloma, Martín-Benito, Mohamed, García-Vallejo, & Soria, 2011); nematicidal activity against *Bursaphelenchus xylophilus* (Barbosa et al., 2010); antioxidant ability (Matos et al., 2009); and antimicrobial activity on methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (Roller, Ernest, & Buckle, 2009) and *Candida albicans, Bacillus subtilis, Bacillus cereus, Enterobacter cloacae, Enteroccocus hirae, Enteroccocus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Serriata marcescens, Staphylococcus aureus, Staphylococcus epidermis* and *Staphylococcus pyogenes* (Baldovini, Lavoine-Hanneguelle, Ferrando, Dusart, & Lizzani-Cuvelier, 2005).

Concerning the chemical composition of *L. luisieri* essential oils, several studies have pointed out the presence of irregular monoterpenes, namely necrodane derivates, although chemical variability seems to be very common among populations (Baldovini et al., 2005; González-Coloma et al., 2011; Lavoine-Hanneguelle & Casabianca, 2004; Sanz, Soria, & García-Vallejo, 2004).

Considering the peculiar composition of this lavender and the lack of information about its antifungal activity, the aim of this study was to evaluate the antifungal activity of two oils with distinct chemical profiles against several pathogenic fungi responsible for human and animal infections (candidosis, meningitis, dermatophytosis and aspergillosis), and a common contaminant of food (*Aspergillus* spp.). Moreover, the effect of the oils on the yeast-mycelium transition in *C. albicans* was also studied. Furthermore, in order to find appropriate doses of the oil showing both antifungal activity and very low detrimental effect to mammalian cells, cytotoxicity assays were also performed.

2. Materials and methods

2.1. Plant material

Aerial parts of two samples of *L. luisieri* were collected from field-growing plants at the flowering stage in the centre (A- *Piódão* region) and South (B- *Cabo São Vicente* region) of Portugal. Voucher specimens were deposited at the Herbarium of the Department of Life Sciences of the University of Coimbra (COI).

2.2. Reference compounds

Fluconazole was kindly provided by Pfizer (pure powder) and amphotericin B by Sigma (80.0% purity).

2.3. Isolation and analyses of essential oils

The essential oils were isolated by hydrodistillation for 3 h from air-dried plant material, using a *Clevenger*-type apparatus, according to the procedure described in the European Pharmacopoeia (Council of Europe, 1997). The oils were stored in dark vials at 4 °C for further assays. Analyses of the volatile compounds were carried out by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), using fused silica capillary columns with two different stationary phases (SPB-1 and SupelcoWax-10), as previously reported (Cavaleiro, Salgueiro, Miguel, & Proença da Cunha, 2004).

The different components were identified by their retention indices and mass spectra. Retention indices, calculated by linear interpolation relative to retention times of a series of *n*-alkanes, were compared with those of authenticated samples from the database of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Coimbra. Mass spectra were compared with reference spectra from a home-made library and literature data (Adams, 1995; Joulain & König, 1998). Relative amounts of individual components were calculated based on GC peak areas without FID response factor correction.

2.4. Fungal strains

The antifungal activity of the essential oils was evaluated against several pathogenic strains: four clinical *Candida* strains isolated from recurrent cases of vulvovaginal and oral candidosis (*Candida albicans* D5, *Candida albicans* M1, *Candida krusei* H9 and *Candida guillermondii* MAT23); three *Candida* type strains from the American Type Culture Collection (*Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803, and *Candida parapsilopsis* ATCC 90018); one *Cryptococcus neoformans* type strain from the Colección Espanõla de Cultivos Tipo (*C. neoformans* CECT 1078); one *Aspergillus* clinical strain isolated from bronchial secretions (*Aspergillus flavus* F44) and two *Aspergillus* type strains from the American Type Culture Collection (*Aspergillus niger* ATCC 16404 and *Aspergillus fumigatus* ATCC 46645); three dermatophyte clinical strains isolated from nails and skin (*Epidermophyton floccosum* FF9, *Microsporum canis* FF1, and *Trichophyton mentagrophytes* FF7), and four dermatophyte type strains from the Colección Espanõla de Cultivos Tipo (*Microsporum gypseum* CECT 2908, *Trichophyton mentagrophytes* var. *interdigitale* CECT 2958, *Trichophyton rubrum* CECT 2794, *Trichophyton verrucosum* CECT 2992). All strains were stored in Sabouraud dextrose broth with 20% glycerol at −80 °C and subcultured in Sabouraud dextrose agar (SDA) or Potato dextrose agar (PDA) before each test, to ensure optimal growth conditions and purity.

2.5. Antifungal activity

Due to the distinct chemical composition of the oil samples, the antifungal activity was carried out in both oil types. A macrodilution broth method was used to evaluate the minimal inhibitory concentrations (MICs) of the oils according to the Clinical and Laboratory Standards Institute (CLSI) reference protocols M27-A3 (CLSI, 2008a) and M38-A2 (CLSI, 2008b), for yeasts and filamentous fungi, respectively. Briefly, inoculum suspensions were prepared at appropriate densities in RPMI 1640 broth (with L-glutamine, without bicarbonate, and the pH indicator phenol red) from SDA or PDA cultures and distributed into 12 × 75 mm glass test tubes. Inoculum densities were confirmed by viability counts on SDA. Serial twofold dilutions of the oils were prepared in dimethyl sulphoxide (DMSO) and added to the cell suspensions to prepare several test concentrations (final DMSO concentrations never exceeded 2% v/v). Oil-free growth controls and DMSO control tubes, were also included. The test tubes were incubated aerobically at 35 °C for 48 h/72 h (*Candida* spp. and *Aspergillus* spp./*Cryptococcus neoformans*) or at 30 °C for 7 days (dermatophytes). MIC values were determined as the lowest concentration of the oil causing full growth inhibition. Quality control was performed by testing fluconazole and amphotericin B with the reference strains *C. parapsilopsis* ATCC 22019 and *C. krusei* ATCC 6258 and the results were within the predetermined limits. To evaluate minimal lethal concentrations (MLCs), aliquots (20 µl) of broth were taken from each negative