Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form

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The antifungal activity of the essential oil of *Lavandula angustifolia* Mill. (lavender oil) and its main components, linalool and linalyl acetate, was investigated against 50 clinical isolates of *Candida albicans* (28 oropharyngeal strains, 22 vaginal strains) and *C. albicans* ATCC 3153. Growth inhibition, killing time and inhibition of germ tube formation were evaluated. The chemical composition of the essential oil was determined by gas chromatography and mass spectrometry.

Lavender oil inhibited *C. albicans* growth: mean minimum inhibitory concentration (MIC) of 0.69% (vol./vol.) (vaginal strains) and 1.04% (oropharyngeal strains); mean MFC of 1.1% (vaginal strains) and 1.8% (oropharyngeal strains). Linalool was more effective than essential oil: mean MIC of 0.09% (vaginal strains) and 0.29% (oropharyngeal strains); mean MFC of 0.1% (vaginal strains) and 0.3% (oropharyngeal strains). Linalyl acetate was almost ineffective.

Lavender oil (2%) killed 100% of the *C. albicans* ATCC 3153 cells within 15 min; linalool (0.5%) killed 100% of the cells within 30 s. The essential oil inhibited germ tube formation (mean MIC of 0.09%), as did the main components (MIC of 0.11% for linalool and 0.08% for linalyl acetate). Both the essential oil and its main components inhibited hyphal elongation of *C. albicans* ATCC 3153 (about 50% inhibition at 0.016% with each substance).

Lavender oil shows both fungistatic and fungicidal activity against *C. albicans* strains. At lower concentrations, it inhibits germ tube formation and hyphal elongation, indicating that it is effective against *C. albicans* dimorphism and may thus reduce fungal progression and the spread of infection in host tissues.

**Keywords** antifungal activity, *Candida albicans*, germ tube, *Lavandula angustifolia*, linalool, linalyl acetate

**Introduction**

Essential oils are well known in traditional medicine as antiseptics and antimicrobial agents and they are characterized by a wide spectrum of activity, including that against bacteria and fungi [1,2]. When topically applied, these substances can represent a valid alter-native to synthetic drugs, although they are generally not suitable for systemic use, owing to their chemical and physical characteristics and their potentially irritating effects. The essential oil of *Lavandula angustifolia* Mill. (Lamiaceae) (lavender oil) is predominantly used in aromatherapy as a relaxant and as a carminative and sedative agent [3]. It was traditionally used as an antiseptic agent for wounds, burns and insect bites and in veterinary practice to kill lice and other animal parasites [4]. However, only in the last few years have in-depth investigations on the antimicrobial activity of lavender oil been carried out. Some studies have described its *in vitro* activity against different bacteria, including antibiotic-resistant strains [5], and its anti-
fungal activity, including the inhibition of conidium germination and of germ tube growth for the fungus *Botrytis cinerea* [6].

The objective of the present study was to evaluate the activity of lavender oil against *Candida albicans*, an important human opportunistic pathogen. To this end, we tested the essential oil’s antimicrobial activity against clinical isolates of *C. albicans*, *C. albicans* ATCC 3153, and, as a quality control strain, *Candida parapsilosis* ATCC 22019. Both the essential oil and the pure form of its main chemical components (i.e. linalool and linalyl acetate, determined by gas chromatography and mass spectrometry) were tested for their ability to inhibit fungal growth, to kill fungal cells, and to inhibit the formation of germ tube, which is a crucial step for the dissemination of *C. albicans*.

**Materials and methods**

**Essential oil and pure substances**

Lavender oil (batch JL055000) was supplied by Janousec (Muggia, Trieste, Italy). Linalool (97% pure) and linalyl acetate (97% pure) were supplied by Sigma-Aldrich (St Louis, MO, USA). For the biological assays, the essential oil and its two pure components were dissolved in ethanol then diluted in culture media (concentrations expressed as %vol./vol.). Miconazole, ketoconazole (Janssen Pharmaceutica, Rome, Italy), and fluconazole (Pfizer, Rome, Italy) were dissolved in dimethylsulfoxide (DMSO) at 10 mg/ml and serially diluted in RPMI 1640 broth (Sigma-Aldrich) at 10 mg/ml and serially diluted in RPMI 1640 broth (Sigma-Aldrich). All pure substances used as gas chromatography reference standards were supplied by Sigma-Aldrich.

**Organisms**

We evaluated the antifungal activity of lavender oil on *C. albicans* strains responsible for oropharyngeal and vaginal infections, which can be treated with topical therapy. Fifty strains (28 oropharyngeal, 22 vaginal) were freshly isolated at the Microbiology Centre, Umberto I Hospital, Rome, Italy, from patients suffering from oropharyngeal or vaginal candidiasis. All of the organisms were identified to the species level by Microscan panels (Baxter, Milan Italy). As a reference strain, we used *C. albicans* ATCC 3153, given that more than 90% of the cells of this strain form germ tubes with good hyphal elongation (more than 2 cm). All isolates were maintained on Sabouraud Dextrose Agar (SDA) (BBL, Becton Dickinson, Cockeysville, USA) slants at 4°C, until performing the experiments. *C. parapsilosis* ATCC 22019 was used as quality control strain for the antifungal susceptibility test, in accordance with the NCCLS method [7].

**Antifungal susceptibility testing**

Antifungal susceptibility testing of *C. albicans* isolates was performed according to the NCCLS reference microdilution method [7]. Serial two-fold dilutions of the test substances were prepared in RPMI 1640 medium buffered to pH 7.0 with 0.165 mol/l MOPS buffer (3-N-morpholinos propane sulfonic acid, Sigma-Aldrich) and were distributed in 96-well microtitre plates in 0.1 ml volumes.

The final concentrations of the substances were: 0.0078–4% for lavender oil, linalool and linalyl acetate; 0.031–16 µg/ml for miconazole and ketoconazole; and 0.031–64 µg/ml for fluconazole. The final concentration of the solvent did not exceed 2% for ethanol or 1% for DMSO, and it did not influence the growth of yeasts. Drug-free control wells, both with and without solvent, and sterility controls, were always included. The inoculum was obtained as described in the NCCLS method [7]. Each well was inoculated with 0.1 ml of the inoculum suspension (0.5 × 10⁵–2.5 × 10⁶ cells/ml). After 48 h incubation at 35°C, yeast growth was evaluated visually and the minimum inhibitory concentration (MIC) was determined. MIC was defined as the lowest concentration at which total inhibition of growth (optically clear) was observed. MIC<sub>50</sub> and MIC<sub>90</sub> were defined as the concentrations of each substance necessary to inhibit 50 and 90% of the isolates tested. The *in vitro* minimum fungicidal concentration (MFC the lowest concentration that killed ≥99.9% of the initial inoculum) was also evaluated. To determine MFC, 50 µl from each well showing no growth was spread on Sabouraud agar plates and incubated at 35°C for 48 h. The colony forming units (c.f.u.) were counted and compared to control plates. MFC<sub>50</sub> and MFC<sub>90</sub> were defined as the drug concentrations necessary to kill 50 and 90% of the isolates, respectively.

**Cytocidal assay**

The cytocidal assay was performed on living, yet not actively growing, cells of *C. albicans* ATCC 3153. *Candida albicans* ATCC 3153 cells (0.5 ml of a 2 × 10⁶ cells/ml suspension in normal saline) were added to a buffer solution (4.5 ml of 0.165 mol/l MOPS, pH 7.0) containing lavender oil concentrations ranging from 0.125 to 2% and incubated at room temperature for pre-established times (range 30 s–30 min). Following incubation, samples were diluted 1:1000 in sterile saline; they were then added to melted
SDA and overlaid on an SDA plate; finally, a third layer was poured on top [8]. The plates were incubated for 48 h at 35°C under aerobic conditions. The c.f.u. were counted to assess viability. Each experiment was performed in triplicate.

Germ tube inhibition test

The germ tube inhibition test was performed in N-acetylg glucosamine (NAG) medium [9] (Composition for 1 l: 1 g N-acetylg glucosamine [Sigma]; 100 mg L-alanine [Merck, Darmstadt, Germany]; 0.1 mg L-biotin [Merck]; 100 mg L-proline [Merck]; 50 ml 0.2 mol/l imidazole-HCl buffer, pH 6.6; and distilled water up to 1 l). The inoculum was prepared by picking three to five colonies (about 1 mm diameter) from 24 h old cultures. The colonies were suspended in 5 ml NAG medium and the cell density was adjusted to an OD of 0.08—0.1 at 625 nm. This procedure yielded a working suspension of 1/5 x 10^6 cells/ml. The working suspension (0.1 ml) was added to 0.9 ml NAG containing scalar dilutions of the tested substances (concentration range 0.0018—4%). Substance-free controls, containing the solvent, were always included. After 3 h incubation at 37°C with shaking, the percentage of germ tube forming cells was calculated by observing about 250 cells from each sample with an optical microscope (magnification x 320). The MIC was defined as the lowest concentration that completely inhibited germ tube formation; MIC50 and MIC90 were the lowest concentrations that inhibited germ tube formation in 50 and 90% of the isolates, respectively. From 80 to 90% of the substance-free control cells showed germ tube formation. The effect of lavender oil on hyphal elongation was evaluated on C. albicans ATCC 3153. The hyphal length was measured with an ocular micrometer and expressed as the percentage of the hyphal length observed in the control. Each test was performed in triplicate.

Gas chromatography and gas chromatography—mass spectrometry

Gas chromatography was performed using a Perkin Elmer AutoSystem with two fused-silica SPB five columns (60 m x 0.25 mm i.d.; film thickness 0.25 μm), mounted in parallel in the same oven, with two detectors: FID (GC-FID) and TurboMass Mass Spectrometer (GC-MS) (electron ionization 70 eV electron energy, transfer line 220°C). The carrier gases were oxygen and moisture-free helium, obtained with a SUPELCO™ High Capacity Heated Carrier Gas Purifier, equipped with an OMI-2 indicating tube, at an average flow rate of 1 ml/min. The oven temperature program was as follows: 60°C for 4 min, followed increasing by 2°C/min to 180°C then by 3°C/min to 250°C. The detector and the injector temperatures were 280°C. The injected volume of essential oil or pure substance was 0.1 μl, and the split ratio was 1:50. Two distinct data systems were connected to the GC-FID or GC-MS: Turbochrom and TurboMass Analytical Workstation Software with NIST/EPA/MSDC Mass Spectral database, respectively.

Chemical identification and quantitative estimation

Chemical components were identified by co-gas chromatography of the oil, adding pure substances and comparing their spectra with those of the NIST/EPA/MSDC Mass Spectral database, and considering their retention indices [10]. Quantitative data were based on peak area normalization without use of correction factors.

Results

The chemical composition of lavender oil is reported in Table 1. As expected, linalool and linalyl acetate were the main components, constituting 32.75 and 43.13% of the essential oil, respectively.

<table>
<thead>
<tr>
<th>Component</th>
<th>Kovats Index</th>
<th>Content % (vol./vol.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Octanone/myrcene</td>
<td>0988</td>
<td>2.41</td>
</tr>
<tr>
<td>Limonene</td>
<td>1031</td>
<td>0.27</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1033</td>
<td>0.60</td>
</tr>
<tr>
<td>trans-Ocimene</td>
<td>1050</td>
<td>1.50</td>
</tr>
<tr>
<td>Linalool</td>
<td>1098</td>
<td>32.75</td>
</tr>
<tr>
<td>Camphor</td>
<td>1143</td>
<td>0.52</td>
</tr>
<tr>
<td>Borneol</td>
<td>1165</td>
<td>0.76</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1177</td>
<td>3.10</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>1189</td>
<td>1.04</td>
</tr>
<tr>
<td>Linalyl acetate</td>
<td>1257</td>
<td>43.13</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>1404</td>
<td>4.95</td>
</tr>
<tr>
<td>β-Farnesene</td>
<td>1433</td>
<td>0.82</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>1440</td>
<td>0.41</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>1591</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Values represent the mean of four determinations.

Table 1 Main constituents of the essential oil of Lavandula angustifolia
1.8% for oropharyngeal strains (Table 2). Of the two major constituents, linalool was the most effective: MIC of 0.09% for vaginal strains and 0.29% for oropharyngeal strains; MFC of 0.1% for vaginal strains and 0.3% for oropharyngeal strains. The strains showed a low susceptibility to linalyl acetate (Table 2). The strains showed a low susceptibility to linalyl acetate (Table 2). The MIC and MFC of lavender oil and the pure substances for \textit{C. albicans} ATCC 3153 were in the range of values observed for the clinical isolates (Table 2) and were the same as those for the quality control strain \textit{C. parapsilosis} ATCC 22019.

The clinical isolates, particularly the vaginal strains, were more susceptible to miconazole (MIC 2.1 μg/ml, MFC 8 μg/ml) and ketoconazole (MIC 3.7 μg/ml, MFC 10.9 μg/ml) than to fluconazole (MIC 8.9 μg/ml, MFC 64 μg/ml) (Table 2). For \textit{C. parapsilosis} ATCC 22019, the MIC was 2 μg/ml for fluconazole, 0.25 μg/ml for miconazole, and 0.25 μg/ml for ketoconazole.

The cytocidal effect against \textit{C. albicans} ATCC 3153 was investigated in relation to the time of exposure and concentration of the substance. At a concentration of 2%, the essential oil killed 99% of the cells within 5 min and 100% of the cells within 15 min. At a concentration of 0.5%, lavender oil killed 98% of the cells within 30 min. Linalool exhibited the highest cytocidal activity: at a concentration of 0.5% it killed 100% of the cells within 30 s. Linalyl acetate at a concentration of 2% killed 93% of the cells within 30 min (Fig. 1).

Lavender oil inhibited germ tube formation in clinical strains with a mean MIC of 0.062%, the mean MIC for linalool and linalyl acetate was 0.11% and 0.08%, respectively (Table 3). For \textit{C. albicans} ATCC 3153, both lavender oil and linalool completely inhibited germ tube formation at a concentration of 0.062%,...
whereas linalyl acetate completely inhibited formation at a concentration of 0.031% (Table 3). Lavender oil and the pure components also inhibited hyphal elongation in *C. albicans* ATCC 3153: at a concentration of 0.016%, the inhibition was about 50% for each substance (Fig. 2).

### Discussion

*Candida albicans* is an opportunistic pathogen responsible for about 60% of both superficial and systemic mycoses [11], and it is capable of reversible transition between the yeast and hyphal form. Factors involved in pathogenesis include adhesion, morphological conditions, the physiological state of the host and the ability of the fungus to penetrate the epithelial mucosa and other tissues [12,13]. Transition to the hyphal form, initiated by germ tube formation and the extension of its tip, can generate a strong pressure for tissue penetration. The hyphal tip is also the site of the secretion of proteases, lipases and other hystolitic enzymes that can facilitate the penetration in host tissues [14]. Furthermore, the hyphal form has an increased resistance to phagocytosis by macrophages and other leukocytes [15].

In the present study, lavender oil showed both fungistatic and fungicidal activity against *C. albicans* strains. It also inhibited germ tube formation at concentrations lower than those required for yeast inhibition. The finding that lavender oil exerts fungicidal activity against *C. albicans* is particularly important when considering that many patients with fungal diseases have a seriously impaired immune system. Given that lavender oil can inhibit germ tube formation and reduce hyphal elongation at concentrations lower than those required for inhibiting yeast, it can be considered as effective against *C. albicans* dimorphism, thus reducing fungal progression and the spread of the infection.

Gas-chromatography showed that lavender oil mainly consists of linalool and linalyl acetate (accounting for about 75% of the oil). According to our data, linalool is apparently responsible for the fungicidal activity, whereas linalyl acetate appears to be somewhat more active in inhibiting germ tube formation and hyphal elongation, even at concentrations lower than those necessary for inhibiting cell growth. The activity of lavender oil against *C. albicans* may depend on an additive effect of its major components; nonetheless, the potential contribution of minor constituents to the antimicrobial activity cannot be excluded.

Overall, the results of this study suggest that additional research is warranted for determining whether this essential oil can be used for treating superficial and mucosal *C. albicans* infections.

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References


