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ARTICLE

Chemical composition and antifungal activity of essential oil and fractions extracted from the leaves of *Laurus nobilis* L. cultivated in Southern Brazil

Full Length Research Paper

Chemical composition and antifungal activity of essential oil and fractions extracted from the leaves of Laurus nobilis L. cultivated in Southern Brazil

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Laurus nobilis L., popularly known as laurel, is a tree belonging to the Lauraceae family, native to Asia. It has long been used in traditional medicine to treat rheumatic disorders, and as a gastric stimulant. The aim of this study was to characterize the chemical composition of essential oils (EO) and fractions from laurel by column chromatography, and to evaluate their antifungal activity. The EO of L. nobilis leaves was obtained by hydrodistillation, and separated by column chromatography. Thirty-two EO constituents were identified, with 1,8-cineole and linalool comprising 40.14 and 15.69% of the total yield, respectively. The major constituents of the fractions (FR) were: α-terpinyl acetate (FR1: 52.65%), 1,8-cineole (FR2: 76.88%), 1,8-cineole (FR3: 84.24%), linalool (FR4: 67.26%), and linalool (FR5: 90.64%). Antifungal activity of EO and fractions were tested by a broth microdilution method, whereby minimum inhibitory concentration (MIC) was determined against several fungal organisms (Candida albicans, Candida krusei, Candida parapsilosis, Candida tropicalis, Cryptococcus gattii, and Cryptococcus neoformans). EO showed moderate inhibition of C. neoformans (MIC 0.62 mg/mL), and strongly inhibited of C. gattii (MIC 0.31 mg/mL). FR3 moderately inhibited C. neoformans (0.62 mg/mL), and strongly inhibited C. gattii (MIC 0.31 mg/mL). FR5 moderately inhibited strains of C. gattii and C. neoformans (MIC 0.62 mg/mL). Laurel’s EO and the fractions analyzed in this study were confirmed to have antifungal properties. However, further studies on toxicity of these substances and in vivo experiments are necessary to confirm the results presented herein.

Key words: Laurus nobilis, antifungal, linalool, 1,8-cineole.

INTRODUCTION

Infectious diseases caused by fungi are responsible for morbidity and mortality in thousands of hospitalized and immune compromised individuals annually (Lemke et al., 2005; Alangaden, 2011). Therefore, the development of
novel antifungal drugs is of vital importance. Patients with human immunodeficiency virus infection (HIV infection/AIDS) comprise a highly susceptible group, and the number of opportunistic infections reported for this group has increased dramatically (Omoriy et al., 2014). Cryptococcosis, a systemic mycosis caused by yeasts of the Cryptococcus genus, most commonly Cryptococcus gattii and Cryptococcus neoformans, is the third most prevalent disease in people with HIV infection/AIDS (Gullo et al., 2013; Maziarz and Perfect, 2016). These agents are responsible for cryptococcal meningitis, a disease most commonly diagnosed in sub-Saharan Africa, where it may kill more people each year than tuberculosis. Globally, one million new cases of cryptococcosis are estimated to occur in HIV-positive individuals annually, resulting in nearly 624,700 deaths, most due to meningitis (Park et al., 2009).

Additionally, the Candida yeasts are of clinical importance, causing opportunistic infections. Candidemia (disseminated hematogenous infection) or deep-seated infection in normally sterile body sites of immunosuppressed patients cause high morbidity and mortality, and also increase medical costs by increasing the duration of hospitalization (Patterson, 2005; Alangaden, 2011; Menezes et al., 2012). C. albicans is one of the major causes of infection of skin and mucosal surfaces, it can infect any organ and in cases of infections in the bloodstream can lead to death, if left untreated (Noble and Johnson, 2007; Duggan et al., 2015). Another species of great importance is C. parapsilosis, which has recently emerged as the second most commonly isolated species in candidemia, infects groups such as neonates, transplant patients, and individuals receiving parenteral nutrition. C. parapsilosis has the ability to form biofilms with high affinity for intravascular and parenteral nutrition devices, being more prevalent than C. albicans in patients using such devices (Trofa et al., 2008; Menezes et al., 2012). C. tropicalis is increasingly isolated from patients with hematologic malignancies, and its presence is predictive for infection causing mucositis and neutropenia. C. krusei is the fifth most common species in immunocompromised patients, with high mortality rates because of resistance to commonly used antifungal drugs such as fluconazole (Pfaller et al., 2008; Sipsas et al., 2009; Alangaden, 2011).

Microbial resistance develops through naturally occurring mutations in fungal cells during prolonged antifungal treatment, resulting in selection of the most resistant strain (Pfaller, 2012). Resistance to drugs is a major concern worldwide because of the limited number of antifungal drug classes, and because the number of patients requiring antifungal treatment is increasing (Maubon et al., 2014). Because of the pressing need for novel therapies to treat the fungal infections, researchers have directed their studies toward the discovery of natural substances with greater efficacy and lower toxicity (Pina et al., 2012; Santos and Novales, 2012).

Secondary metabolism in plants produces many compounds that have complex chemical structures, many of these substances have been reported to have antimicrobial properties as essential oils (EOs) (Edris, 2007). EOs are important natural products, being multifunctional, well accepted by consumers, and safer than synthetic additives. Thus, they have been targeted for research on natural food preservation, crop protection, pharmaceutical applications, and cosmetic production (Bakkali et al., 2008; Okoh et al., 2010).

Laurus nobilis L. is a tree belonging to the Lauraceae family, native to Asia. The plant is popularly known as laurel, and is cultivated in south and southeast Brazil (Marques, 2001; Lorenzi and Matos, 2008). Laurel is an aromatic spice, commonly used to season recipes owing to its aroma. Laurel leaf is also used in folk medicine as infusions or decoctions, being considered a gastric stimulant as well as a treatment for rheumatic disorders. It is also used externally for rheumatism, and as an antiseptic for dandruff and lice (Joly, 1993; Marques, 2001; Lorenzi and Matos, 2008).

Laurel leaves are widely used in the food, cosmetic, and perfumery industries, and their essential oil (EO) is highly valued. Large amounts of phytoactive agents are found in EO among which is terpenes. The EO are widely studied, and their antibacterial (Angelini et al., 2006), antifungal (Gumus et al., 2010), antioxidant (Iran et al., 2012), insecticidal (Sertkaya et al., 2010), antiproliferative (Abu-Dahab et al., 2014), analgesic, and anti-inflammatory properties (Sayyah et al., 2003) reported.

This present study was designed to evaluate the antifungal activity of EO and fractions extracted from the leaves of L. nobilis cultivated in Southern Brazil.

MATERIALS AND METHODS

Plant material

Fresh leaves of L. nobilis L. were collected in January 2014, in the city of Pêrola, Paraná, Brazil (23° 50’ 56.6” S 53° 41’ 06.2” W, 20 m), identified by Msc. Mayara Lautert and Camila Vanessa Buturi, as sample number 1615, and were deposited at the Herbarium of the State University of Western Paraná.

Essential oil extraction

Fresh leaves of L. nobilis L. were subjected to hydrodistillation in a...
apparatus for 2 h (Fiorini et al., 1997). EO was collected, dried over sodium sulfate, filtrated, and stored in amber-colored vials at 4°C. After total evaporation of the solvent, the EO was weighed to calculate oil yield (%).

Obtaining L. nobilis EO fractions

EO (4.0 g) was submitted to silica gel column chromatography and eluted sequentially with n-hexane, dichloromethane, ethyl acetate, methanol, and hexane: dichloromethane (9:1; 8:2; 7:3, and 5:5 v/v), dichloromethane: ethyl acetate (9:1, 8:2, 7:3, and 5:5 v/v), and ethyl acetate: methanol (9:1, 8:2, 7:3, and 5:5 v/v) mixtures. The fractions were then concentrated under reduced pressure using a rotary evaporator (Tecnal TE-211) to reduce the volume to about 2.0 mL, transferred to amber vials, dried, and stored at 4°C for the duration of the experiment.

GC-MS analysis

Analysis of EO was carried out in a gas chromatograph (Agilent 7890 B) coupled to a mass spectrometer (Agilent 5977 A) equipped with an Agilent HP-5MS capillary column (30 m × 0.250 mm × 0.25 μm), using the following conditions: injector temperature of 250°C, injection volume 1 μL at a ratio of 1:30 (split mode), initial column temperature of 50°C, heated gradually to 260°C at 3°C/min rate. The carrier gas (helium) flow was set at 1 mL/min. The temperatures of the transfer line, ion source, and quadrupole were 250, 230 and 150°C, respectively (Derwich et al., 2009; Moghtader Salari, 2012). Mass spectra were obtained using various n-alkanes (C8-C25). In addition, their electron ionization (EI) mass spectra were compared with the NIST 11.0 library spectra according to Adams (2007).

Determination of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) of EO were determined against C. albicans ATCC 18804, C. krusei ATCC 20298, C. parapsilosis ATCC 20019, C. tropicalis ATCC 750, C. gattii L21/01, and C. neoformans H99. An 80 mg/mL of the EO solution was prepared, diluted with 2% polysorbate 80 (tween 80) in Muller Hinton Broth with the addition of 2% glucose for yeasts. The culture medium (100 μL) was distributed into the wells of a microdilution plate, and then 200 μL EO solution was added to the second well. Following homogenization, this was transferred to the third well, and so on until the tenth. Thus, the final concentrations obtained were 40.00, 20.00, 10.00, 5.00, 2.50, 1.25, 0.62 and 0.31 mg/mL. A microbial suspension was prepared in saline with turbidity equivalent to 0.5 on the McFarland scale (1 × 108 UFC/mL). Next, the 1:50 yeast suspension was diluted to 1:20 in Mueller Hinton Broth modified for fungi to yield 1 × 105 UFC/mL inoculums. Hundred microliters of the suspensions was inoculated in triplicate into each well containing the various EO concentrations. Well 1 was used as sterility control. The toxicity control was well 11 with 2% polysorbate 80 in culture medium. Well 12 was used as the growth control, where microbial suspension was added to the culture medium. Microplates were incubated at 35°C for 24 h in aerobic conditions. MICs were determined by examining the plates. The lowest concentration of EO causing complete inhibition of growth (CLSI, 2008) was reported. The same procedure was performed with fractions of the essential oil, using an initial solution of 20 mg/mL and the fluconazole was used as positive control.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and comparisons of means by Tukey’s test at a 5% significance level.

RESULTS AND DISCUSSION

Hydrodistillation of laurel leaves resulted in a 0.66% yield of EO. The yield obtained is in accordance with that reported by Lira et al. (2009), who obtained a yield between 0.3 and 1.2% during their 15-month study.

Thirty-two different constituents were identified (Table 1). The majority were terpenoids (93.50%), followed by phenylpropanoids (6.04%). The major terpenoid constituents obtained were monoterpene hydrocarbons (14.44%), oxygenated monoterpenes (78.15%), sesquiterpene hydrocarbons (0.69%) and oxygenated sesquiterpenes (0.21%). Oxygenated monoterpenes form the majority of the EO, 1,8-cineole being the predominant component (40.14%), followed by linalool (15.69%), and α-terpinyl acetate (11.70%). Sellami et al. (2011) also reported 1,8-cineole (61.17%) to be the major compound in samples of fresh laurel leaf EO, and Moghtader and Salari (2012) showed that the EO of dried laurel leaves contained 25.7% 1,8-cineole.

Silveira et al. (2012) analyzed the EO of laurel cultivated in Concordia (Santa Catarina - Brazil) by GC-MS. The authors observed that the oil contained 1,8-cineole (35.50%) as its major constituent, followed by linalool (14.10%), α-terpinyl acetate (9.65%), and sabine (9.45%). The results of the present work are in line with those reported by these authors. In Croatia, Politeo et al. (2006) reported the major compounds in Laurel EO to be 1,8-cineole (34.9%), linalool (13.5%), methyl eugenol (13.5%), and α-terpinyl acetate (12.2%). Dadaligolu and Evrendilek (2004) analyzed the EO of fresh L. nobilis leaves collected in Hatay, Turkey, and found the major constituents to be 1,8-cineole (60.72%), α-terpinyl acetate (12.53%), and sabine (12.12%). The differences in the chemical composition of EO of laurel can be attributed to plant origin, time of harvesting, drying processes, and other factors such as climate, soil, vegetative stage, and processing (extraction) (Simões and Spitzer, 2007).

The fractions tested were identified by GC-MS. The fractions (FR) were characterized as follows: FR1; dichloromethane: hexane (7:3) fraction composed of α-terpinyl acetate (52.65%), 1,8-cineole (29.70%), eugenol (4.28%), and methyl eugenol (9.52%); FR2; dichloromethane: hexane (8:2) fraction composed of 1,8-cineole (76.88%), methyl eugenol (21.07%), α-terpinyl acetate (1.28%), and eugenol (0.77%); FR3; dichloromethane: hexane (9:1) fraction composed of 1,8-cineole (84.24%) and linalool (6.78%); FR4; dichloromethane fraction composed of 67.26% linalool and 1,8-cineole (19.68%); and FR5; dichloromethane: ethyl acetate (9:1) fraction composed of 90.64% linalool.
Table 1. Chemical composition of essential oil and fractions obtained from the leaves of *Laurus nobilis*.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Constituent†</th>
<th>RI*</th>
<th>% Area</th>
<th>Identification methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Thujene</td>
<td>931</td>
<td>0.25</td>
<td>a,b</td>
</tr>
<tr>
<td>2</td>
<td>α-Pinene</td>
<td>937</td>
<td>2.53</td>
<td>a,b</td>
</tr>
<tr>
<td>3</td>
<td>Camphene</td>
<td>952</td>
<td>0.05</td>
<td>a,b</td>
</tr>
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<td>4</td>
<td>Sabinene</td>
<td>978</td>
<td>5.71</td>
<td>a,b</td>
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<tr>
<td>5</td>
<td>β-Pinene</td>
<td>981</td>
<td>2.36</td>
<td>a,b</td>
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<td>6</td>
<td>β-Myrcene</td>
<td>997</td>
<td>0.32</td>
<td>a,b</td>
</tr>
<tr>
<td>7</td>
<td>δ-2-Carene</td>
<td>1010</td>
<td>0.03</td>
<td>a,b</td>
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<tr>
<td>8</td>
<td>α-Phellandrene</td>
<td>1015</td>
<td>0.14</td>
<td>a,b</td>
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<td>9</td>
<td>α-Terpine</td>
<td>1021</td>
<td>0.23</td>
<td>a,b</td>
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<tr>
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<td>α-Cymene</td>
<td>1029</td>
<td>0.37</td>
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<td>11</td>
<td>Limonene</td>
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<td>1.57</td>
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<td>12</td>
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<td>1035</td>
<td>40.14</td>
<td>a,b</td>
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<tr>
<td>13</td>
<td>(E)-β-Ocimene</td>
<td>1053</td>
<td>0.03</td>
<td>a,b</td>
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<tr>
<td>14</td>
<td>γ-Terpine</td>
<td>1063</td>
<td>0.61</td>
<td>a,b</td>
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<tr>
<td>15</td>
<td>cis-Sabinene hydrate</td>
<td>1071</td>
<td>0.36</td>
<td>a,b</td>
</tr>
<tr>
<td>16</td>
<td>Terpinolene</td>
<td>1094</td>
<td>0.17</td>
<td>a,b</td>
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<td>n.i.</td>
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<td>a,b</td>
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<td>Linalool</td>
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<td>19</td>
<td>cis-p-Menth-2-en-1-ol</td>
<td>1126</td>
<td>0.08</td>
<td>a,b</td>
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<td>a,b</td>
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<td>Terpinen-4-ol</td>
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<td>a,b</td>
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<td>α-Terpineol</td>
<td>1195</td>
<td>6.22</td>
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<td>23</td>
<td>n.i.</td>
<td>1234</td>
<td>0.13</td>
<td>a,b</td>
</tr>
<tr>
<td>24</td>
<td>Linalool acetate</td>
<td>1263</td>
<td>0.08</td>
<td>a,b</td>
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<tr>
<td>25</td>
<td>δ-Terpinyl acetate</td>
<td>1323</td>
<td>0.42</td>
<td>a,b</td>
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<tr>
<td>26</td>
<td>α-Terpinyl acetate</td>
<td>1355</td>
<td>11.70</td>
<td>a,b</td>
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<tr>
<td>27</td>
<td>Eugenol</td>
<td>1363</td>
<td>0.20</td>
<td>a,b</td>
</tr>
<tr>
<td>28</td>
<td>β-Elemene</td>
<td>1397</td>
<td>0.21</td>
<td>a,b</td>
</tr>
<tr>
<td>29</td>
<td>Methyl eugenol</td>
<td>1411</td>
<td>5.84</td>
<td>a,b</td>
</tr>
<tr>
<td>30</td>
<td>(E)-Caryophyllene</td>
<td>1424</td>
<td>0.44</td>
<td>a,b</td>
</tr>
<tr>
<td>31</td>
<td>γ-Cadinene</td>
<td>1529</td>
<td>0.04</td>
<td>a,b</td>
</tr>
<tr>
<td>32</td>
<td>Caryophyllene oxide</td>
<td>1587</td>
<td>0.21</td>
<td>a,b</td>
</tr>
</tbody>
</table>

**Compound groups (%)**

<table>
<thead>
<tr>
<th>Compound groups</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoterpenes</td>
<td>14.44</td>
</tr>
<tr>
<td>Oxygenated monoterpenes</td>
<td>78.15</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>0.69</td>
</tr>
<tr>
<td>Oxygenated sesquiterpenes</td>
<td>0.21</td>
</tr>
<tr>
<td>Phenylpropanoids</td>
<td>6.04</td>
</tr>
<tr>
<td>Total of identified compounds</td>
<td>99.54</td>
</tr>
</tbody>
</table>

*Compounds listed in order of elution from HP-5MS column; **RI = Retention index; a=identification based on RI; b=identification based on comparison of mass spectra; n.i. = not identified.*

as the major constituents.

The results for MIC tests of EO and fractions are presented in Table 2. In order to compare the results, the values found in this study were compared with the classification values proposed by Aligiannis et al. (2001) and Duarte et al. (2005) for plant materials, based on MIC results. This classification system categorizes materials as strong inhibitors; MIC up to 0.5 mg/mL, moderate inhibitors; MIC between 0.6 and 1.5 mg/mL, and weak inhibitors; MIC above 1.6 mg/mL. In the present study, EO demonstrated low inhibition of Candida strains, moderate inhibition of *C. neoformans* (MIC 0.62 mg/mL), and high inhibition of *C. gattii* (MIC 0.31 mg/mL). FR1 moderately inhibited *C. gattii* (MIC 1.25 mg/mL) and *C. neoformans* (MIC 0.62 mg/mL), and strongly...
inhibited C. gattii (MIC 0.31 mg/mL). FR4 moderately inhibited C. albicans, C. tropicalis, C. gattii and C. neoformans (MIC 1.25 mg/mL). FR5 moderately inhibited C. albicans and C. tropicalis (MIC 1.25 mg/mL), C. gattii and C. neoformans (MIC 0.62 mg/mL). Both FR4 and FR5 slightly inhibited C. krusei and C. parapsilosis.

Studies carried out by Erturk et al. (2006) showed the antifungal activity of laurel EO against C. albicans, with an MIC of 2.4 mg/mL. Peixoto et al. (2017) evaluated the antifungal activity of EO of laurel collected in Brazil, and found isoeugenol (53.5%) and myrcene (16.6%) as major constituents. The EO showed activity against C. albicans strains (MIC 0.25 mg/mL), C. tropicalis (MIC 0.50 and 0.25 mg/mL), C. krusei and C. glabrata (MIC 0.5 mg/mL). The EO evaluated in the present study exhibited a higher MIC (10.00 and 5.00 mg/mL) for Candida strains; the differences in biological activities between these findings and the literature may be attributable to the differences in chemical composition of EO of laurel that directly influences its antimicrobial activity.

Monoterpenes and sesquiterpenes with aromatic rings and phenol groups are capable of forming hydrogen bonds with the active sites of target enzymes, and this is the main mode of antimicrobial action of EO. Other compounds such as alcohols, aldehydes, and esters also contribute to antimicrobial activity (Belletti et al., 2004). The antifungal activity of the fractions can be attributed to the presence of terpenes. Linalool, the major constituent found in the present study for FR4 and FR5, was screened for activity against Candida isolates by Marcos-Arias et al. (2011), who reported findings against C. albicans (MIC, 0.30-2.50 mg/mL), C. tropicalis (0.60-2.50 mg/mL), C. parapsilosis (0.30 mg/mL), and C. krusei (0.60 mg/mL). The eugenol in FR1 and FR2, and terpinen-4-ol present in FR3–5 were also investigated by Marcos-Arias et al. (2011); against C. albicans eugenol had an MIC in the range of 0.60-2.50 mg/mL, and terpinen-4-ol in the range of 0.60-5.00 mg/mL; for C. tropicalis, the eugenol and terpinen-4-ol MIC range was 0.60-1.20 mg/mL; for C. parapsilosis, the MIC of both substances was 0.60 mg/mL; and for C. krusei, both had an MIC of 1.20 mg/mL. In general, the MIC values reported for the fractions in this study against Candida species are close to those found by Marcos-Arias et al. (2011), considering that they evaluated pure substances, while the fractions in this present study are a mixture of terpenes, which may or may not have synergistic effects. Hsu et al. (2013) determined the MIC against Candida species for linalool, and found it to be 1.23 to 4.93 mg/mL for C. albicans, and 2.47 mg/mL for C. tropicalis. These values are comparable to those found in the present study for FR4 and FR5, in which linalool is the major compound.

1,8-Cineole is present in all fractions, and is the major compound in FR2 and FR3 (76.88 and 84.24%, respectively); its antifungal activity was investigated by Adegoke et al. (2000) against Candida tropicalis yeast. It was found to have an MIC of 0.16 mg/mL against C. tropicalis yeast, with an activity superior to the that recorded for the fractions containing 1,8-cineole in the present study. However, these authors investigated the use of pure substances. Hammer et al. (2003) determined the MIC for 1,8-cineole against isolates of C. albicans (40.00 mg/mL) and C. parapsilosis (80.00 mg/mL). Pattnaik et al. (1997) analyzed the antifungal action of oxygenated linalool and 1,8-cineole monoterpenes, and found linalool to possess activity against C. albicans (MIC 0.20 mg/mL). C. albicans was resistant to 1,8-cineole at MIC up 5.00 mg/mL. This present study documented lower linalool activity in the fractions containing it as the major compound. Fractions containing 1,8-cineole had higher antifungal activity against C. albicans and C. parapsilosis in comparison with the results of the studies mentioned above; this may be explained by the fact that fractions are a mixture of antifungal substances acting in synergy.

To our knowledge, this is the first investigation documenting antifungal activity of EO and fractions extracted from fresh leaves of laurel against strains of C. parapsilosis, C. gattii, and C. neoformans. EO extracted and analyzed in this study, as well as its fractions, possess antifungal properties. The presence and proportion of the EO constituents are related to biological

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Essential oil of laurel</th>
<th>FR 1</th>
<th>FR 2</th>
<th>FR 3</th>
<th>FR 4</th>
<th>FR 5</th>
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<tbody>
<tr>
<td>Candida albicans</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>5.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Candida tropicalis</td>
<td>10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cryptococcus gattii</td>
<td>0.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>0.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;d&lt;/sup&gt;</td>
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FR1: Dichloromethane:hexane (7:3) was composed of α-terpinyl acetate (52.65%), 1,8-cineole (29.70%), eugenol (4.28%), and methyl eugenol (9.52%); FR2: Dichloromethane:hexane (8:2) of 1,8-cineole (76.88%), methyl eugenol (21.07%), α-terpinyl acetate (1.28%), and eugenol (0.77%); FR3: Dichloromethane:hexane (9:1) of 1,8-cineole (84.24%) and linalool (6.78%); FR4: Dichloromethane of linalool (67.26%) and 1,8-cineole (19.68%); FR5: Dichloromethane:ethyl acetate (9:1) of 90.64% of linalool as the major constituents. Values are the mean ± standard deviation of the experiment performed in triplicate. Different letters in the same line are different (p≤0.05) by Tukey's test.
properties of laurel. However, further studies on toxicity of these substances and in vivo experiments are necessary to confirm the results here presented.

Conflicts of Interests

The authors have not declared any conflict of interests.

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