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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 (Omoruyi 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 (Inan 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 Pérola, Paraná, Brazil (23º 50' 56.6" S 53º 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

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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 4ºC 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 and Salari, 2012). Mass spectra were obtained with a scan range of 40 to 500 m/z and a solvent delay time of 3 min, and compounds were identified based on comparison of their retention indices (RI), 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 sabinene (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%). Dadalioglu 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 sabinene (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.*

Peak	Constituent*	RI**	% Area	Identification methods
1	α-Thujene	931	0.25	a,b
2	α-Pinene	937	2.53	a,b
3	Camphene	952	0.05	a,b
4	Sabinene	978	5.71	a,b
5	β-Pinene	981	2.36	a,b
6	β-Myrcene	997	0.32	a,b
7	δ-2-Carene	1010	0.03	a,b
8	α-Phellandrene	1015	0.14	a,b
9	α-Terpinene	1021	0.23	a,b
10	o-Cymene	1029	0.37	a,b
11	Limonene	1033	1.57	a,b
12	1,8-Cineole	1035	40.14	a,b
13	(E)-β-Ocimene	1053	0.03	a,b
14	γ-Terpinene	1063	0.61	a,b
15	cis-Sabinene hydrate	1071	0.36	a,b
16	Terpinolene	1094	0.17	a,b
17	n.i.	1104	0.34	a,b
18	Linalool	1106	15.69	a,b
19	cis-ρ-Menth-2-en-1-ol	1126	0.08	a,b
20	δ-Terpineol	1172	0.36	a,b
21	Terpinen-4-ol	1182	3.17	a,b
22	α-Terpineol	1195	6.22	a,b
23	n.i.	1234	0.13	a,b
24	Linalool acetate	1263	0.08	a,b
25	δ-Terpinyl acetate	1323	0.42	a,b
26	α-Terpinyl acetate	1355	11.70	a,b
27	Eugenol	1363	0.20	a,b
28	β-Elemene	1397	0.21	a,b
29	Methyl eugenol	1411	5.84	a,b
30	(E)-Caryophyllene	1424	0.44	a,b
31	γ-Cadinene	1529	0.04	a,b
32	Caryophyllene oxide	1587	0.21	a,b
	Compound groups (%)			
Monoterpene hydrocarbons			14.44	
Oxygenated monoterpenes			78.15	
Sesquiterpene hydrocarbons			0.69	
Oxygenated sesquiterpenes			0.21	
Phenylpropanoids			6.04	
Total of identified compounds			99.54	

*Compounds listed in order of elution from HP-5MS column; **RI = Retention index; aIdentification based on RI; bIdentification 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).

FR3 moderately inhibited *C. krusei* (MIC 1.25 mg/mL) and *C. neoformans* (MIC 0.62 mg/mL), and strongly

Table 2. Minimum inhibitory concentration (MIC) of essential oil and fractions of *L. nobilis* (mg/mL).

Microorganisms	Essential oil of laurel	FR 1	FR 2	FR 3	FR 4	FR 5
Candida albicans	5.00[b]	>10.00[a]	>10.00[a]	>10.00[a]	1.25[c]	1.25[c]
Candida krusei	10.00[b]	>10.00[a]	>10.00[a]	1.25[d]	2.50[c]	2.50[c]
Candida parapsilosis	5.00[b]	>10.00[a]	>10.00[a]	>10.00[a]	5.00[b]	2.50[c]
Candida tropicalis	10.00[b]	>10.00[a]	>10.00[a]	>10.00[a]	1.25[c]	1.25[c]
Cryptococcus gattii	0.31[d]	1.25[b]	5.00[a]	0.31[d]	1.25[b]	0.62[c]
Cryptococcus neoformans	0.62[d]	>10.00[a]	5.00[b]	0.62[d]	1.25[c]	0.62[d]

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.

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 in FR 4 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 be1.23 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

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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REFERENCES

Abu-Dahab R, Kasabri V, Afifi FU (2014). Evaluation of the volatile oil composition and antiproliferative activity of Laurus nobilis L.(Lauraceae) on breast cancer cell line models. Rec. Nat. Prod. 8(2):136-147.

Adams RP (2007). Identification of essential oil components by gas chromatography/mass spectroscopy. J. Am. Soc. Mass Spectrom. 6(8):671-672.

Adegoke GO, Iwahashi H, KomatsuY, Obuchi K, Iwahashi Y (2000). Inhibition of food spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice Aframomum danielli. Flavour Fragr. J. 15:147-150.

Alangaden GJ (2011). Nosocomial Fungal Infections: Epidemiology, Infection Control, and Prevention. Infect. Dis. Clin. N. Am. 25:201-225.

Aligiannis N, Kalpotzakis E, Mitaku S, Chinou IB (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species. J. Agric. Food Chem. 40:4168-4170.

Angelini P, Pagiotti R, Menghini A, Vianello B (2006). Antimicrobial activities of various essential oils against foodborne pathogenic or spoilage moulds. Ann. Microbiol. 56(1):65-69.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils – A review. Food Chem. Toxicol. 46:446-475.

Belletti N, Ndagihimana M, Sisto C, Guerzoni M, Lanciotti R, Gardini F (2004). Evaluation of the Antimicrobial Activity of Citrus Essences on *Saccharomyces cerevisae*.Agric. Food Chem. 52:6932-6938.

Clinical and Laboratory Standards Institute (CLSI) (2008). Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. 3ed. 940 West Valley Road, Suite 1400, Wayne, Pensylvania 19087-1898 USA. (CLSI document M27-A3).

Dadalioglu I, Evrendilek GA (2004). Chemical Compositions and Antibacterial Effects of Essential Oils of Turkish Oregano (*Origanum minutiflorum*), Bay Laurel (*Laurus nobilis*), Spanish Lavender (*Lavandula stoechas* L.) and Fennel (*Foeniculum vulgare*) on Commom Foodborne Pathogens. J. Agric. Food Chem. 52:8255-8260.

Derwich E, Benziane Z, Boukir A (2009). Chemical Composition and Antibacterial Activity of Leaves Essential Oil of *Laurus nobilis* from Morocco. Aust. J. Basic Appl. Sci. 3(4):3818-3824.

Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C (2005). Anti-candida activity of Brazilian medicinal plants. J. Ethnopharmacol. 97:305-311.

Duggan S, Leonhardt I, Hunniger K, Kurzai O (2015). Host response to *Candida albicans* bloodstream infection and sepsis. Virulence 6(4):316-326.

Edris AE (2007). Pharmaceutical and Therapeutic Potencials of Essential Oils and Their Individual Volatile Constituents: A Review. Phytother. Res. 21:308-323.

Erturk O, Ozbucak TB, Bayrak A (2006). Antimicrobial activities of some medicinal essential oils. Herba Pol. 52(1):58-66.

Fiorini C, Fourasteâ I, David B, Bessieáre JM (1997). Composition of the Flower, Leaf and Stem Essential Oils from *Laurus nobilis* L. Flavour Frag. J. 12:91-93.

Gullo FP, Rossi AS, Sardi JCO, Teodoro VLI, Mendes-Giannini MJS, Fusco-Almeida AM (2013). Cryptococcosis: epidemiology, fungal resistance, and new alternatives for treatment. Eur. J. Clin. Microbiol. Infect. Dis. 32:1377-1391.

Gumus T, Demirci AS, Sagdic O, Arici M (2010). Inhibition of Heat Resistant Molds: *Aspergillus fumigates* and *Paecilomyces variotti* by Some Plant Essential Oils. Food Sci. Biotechnol. 19:1241-1244.

Hammer KA, Carson CF, Riley TV (2003). Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J. Appl. Microbiol. 95:853-860.

Hsu CC, Lai WL, Chuang KC, Lee MH, Tsai YC (2013). The inhibitory activity of linalool against the filamentous growth and biofilm formation in *Candida albicans*. Med. Mycol. 51:473-482.

Inan O, Özcan MM, Juhaimi FYA (2012). Antioxidant effect of mint, laurel and myrtle leaves essential oils on pomegranate kernel, poppy, grape and linseed oils. J. Clean. Prod. 27:151-154.

Joly AB (1993). Botânica: Introdução à taxonomia vegetal. São Paulo: Editora Nacional.

Lemke A, Kiderlen AF, Kayser O (2005). Amphotericin B. Appl. Microbiol. Biotechnol. 68:151-162.

Lira PDL, Retta D, Tkacik E, Ringuelet J, Coussio JD, Van Baren C, Bandoni AL (2009). Essential oil and by-products of distillation of bay leaves (*Laurus nobilis* L.) from Argentina. Ind. Crop Prod. 30:259-264.

Lorenzi H, Matos FJA (2008). Plantas Medicinais no Brasil Nativas e Exóticas, 2 ed. São Paulo: Instituto Plantarum.

Marcos-Arias C, Eraso E, Madariaga L, Quindós G (2011). *In vitro* activities of natural products against oral *Candida* isolates from denture wearers. BMC Complement. Altern. Med. 11(119):1-7.

Marques CA (2001). Importância da Família Lauraceae. Floresta Ambient. 8(1):195-206.

Maubon D, Garnaud C, Calandra T, Sanglard D, Cornet M (2014). Resistance of *Candida* spp. to antifungal drugs in the ICU: where are we now? Intensive Care Med. 40:1241-1255.

Maziarz EK, Perfect JR (2016). Cryptococcosis. Infect Dis. Clin. N. Am. 30:179-206.

Menezes EA, Júnior AAV, Cunha FA, Cunha MCSO, Braz BHL, Capelo LG, Silva CLF (2012). Molecular identification and antifungal susceptibility of *Candida parapsilosis* isolates in Ceará, Brazil. J. Bras. Patol. Med. Lab. 48(6):415-420.

Moghtader M, Salari H (2012). Comparative survey on the essential oil composition from the leaves and flowers of *Laurus nobilis* L. from Kerman province. J. Ecol. Nat. Environ. 4(6):150-153.

Noble SM, Johnson AD (2007). Genetics of *Candida albicans*, a Diploid Human Fungal Pathogen. Annu. Rev. Genet. 41:193-211.

Okoh OO, Sadimenko AP, Afolayan AJ (2010). Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem. 120:308-312.

Omoruyi EB, Afolayan AJ, Bradley G (2014). The inhibitory effect of *Mesembryanthemum edule* (L.) bolus essential oil on some pathogenic fungal isolates. BMC Complement. Altern. Med. 14(168):1-7.

Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM (2009). Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23:525-530.

Patterson TF (2005). Advances in the management of invasive mycoses. Lancet 336(9490):1013-1025.

Pattnaik S, Subramanyam VR, Bapaji M, Kole CR (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 89:39-46.

Peixoto LR, Rosalen PL, Ferreira GL, Freires IA, de Carvalho FG, Castellano LR, de Castro RD (2017). Antifungal activity, mode of action and anti-biofilm effects of *Laurus nobilis* Linnaeus essential oil against *Candida* spp. Arch Oral Biol. 73:179-185.

Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Nagy E, Dobiasova S,

Rinaldi M, Barton R, Veselov A, Global Antifungal Surveillance Group (2008). Candida krusei, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J. clin. Microbial. 46(2):515-521.

Pfaller MA (2012). Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. Am. J. Med. 125:S3-S13.

Pina ES, Coppede JS, Sartoratto A, Fachin AL, Bertoni BW, França SC, Pereira MAS (2012). Antimicrobial activity and chemical composition of essential oils from *Aloysia polystachya* (Griseb.) Moldenke grown in Brazil. J. Med. Plants Res. 6(41):5412-5416.

Politeo O, Jukic M, Milós M (2006). Chemical Composition and Antioxidant Activity of Essential Oils of Twelve Spice Plants. Croat. Chem. Acta 79:545-552.

Santos FS, Novales MGM (2012). Essential oils from aromatic herbs as antimicrobial agents. Curr. Opin. Biotechnol. 23:136-141.

Sayyah M, Saroukhani G, Peirovi A, Kamalinejad M (2003). Analgesic and Anti-inflammatory Activity of the Leaf Essential oil of *Laurus nobilis* Linn. Phytother. Res. 17:733-736.

Sellami IH, Wannes WA, Bettaieb I, Berrima S, Chahed T, Marzouk B, Limam F (2011). Qualitative and quantitative changes in the essential oil of *Laurus nobilis* L. leaves as affected by different drying methods. Food Chem. 126:691-697.

Sertkaya E, Kaya K, Soylu S (2010). Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (Acarina: Tetranychidae). Ind. Crop. Prod. 31:107-112.

Silveira SM, Júnior AC, Scheuermann GN, Secchi FL, Vieira CRW (2012). Chemical composition and antimicrobial activity of essential oils from selected herbs cultivated in the South of Brazil against food spoilage and foodborne pathogens. Cienc. Rural 42:1300-1306.

Simões CMO, Spitzer V (2007). Óleos voláteis. In: Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR. Farmacognosia: da planta ao medicamento. 6 ed. Florianópolis: UFSC, Porto Alegre: UFRGS.

Sipsas NV, Lewis RE, Tarrand J, Hachem R, Rolston KV, Raad II, Kontoyiannis DP (2009). Candidemia in patients with hematologic malignancies in the era of new antifungal agents (2001–2007): stable incidence but changing epidemiology of a still frequently lethal infection. Cancer 115(20):4745-4752.

Trofa D, Gácser A, Nosanchuk JD (2008). *Candida parapsilosis*, an Emerging Fungal Pathogen. Clin. Microbiol. Rev. 21(4):606-625.