

## Biological activities of essential oils from six genotypes of four *Ocotea* species

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Essential oils from four *Ocotea* species collected in southern Brazil were evaluated for chemical composition using gas chromatography coupled with mass spectrometry. The primary compound identified in *O. acutifolia* essential oil was an unsaturated tetracyclic diterpene, phyllocladene (67.7%), followed by a sesquiterpene hydrocarbon,  $\beta$ -selinene (18.0%). The sesquiterpene fraction was predominant in oils from two collections of *O. puberula*;  $\beta$ -caryophyllene (25.2%) and globulol (22.6%) were the major compounds identified in collections 1 and 2, respectively. *O. silvestris* essential oil contained predominantly germacrene D and bicyclogermacrene. These compounds were also predominant in essential oil from *O. indecora* leaves collected from shady habitats. By contrast, essential oil extracted from *O. indecora* grown under direct sunlight contained mainly oxygenated sesquiterpenes, such as guaiol (30.2%),  $\alpha$ -eudesmol (27.6%), and  $\beta$ -eudesmol (12.7%). Chemotaxis assays showed that *Ocotea* essential oils had no significant inhibitory activity on leukocyte migration compared with a chemotactic stimulant (lipopolysaccharide from *Escherichia coli*). However, the oils exhibited antifungal activity against *Candida parapsilosis*, with a minimum inhibitory concentration of 500  $\mu$ g/mL. To our knowledge, this is the first study to investigate the *in vitro* antifungal and antichemotactic activities of essential oils from *Ocotea* species native to southern Brazil.

**Keywords:** Antifungal. Biological activity. Essential oil. Lauraceae. *Ocotea*.

### INTRODUCTION

Plants are rich natural sources of compounds capable of exerting the most diverse pharmacological actions. This property, combined with an outstanding chemical diversity, makes products of plant origin excellent sources of new drugs. Perhaps the most promising and widely researched plant secondary metabolites are essential oils, one of the largest groups of natural products and potential sources of biologically active substances. Knowledge of plant composition and secondary metabolite profiles

may also assist in botanical classification, particularly for species that exhibit morphological changes as an adaptation to different environments or climates (Freire *et al.*, 2011).

In recent years, studies have reported increasing resistance to antifungal agents, and numerous efforts have been undertaken to develop alternative treatment and prophylactic strategies, such as investigations on the pharmacological activity of compounds isolated from plants (Bajpai *et al.*, 2009a). Terpenoids are an example of such compounds. These volatile constituents of essential oils are associated with important biological activities, including antibacterial and antifungal properties (Reddy *et al.*, 2017; Blažeković *et al.*, 2018; Danielli *et al.*, 2018).

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In addition to antimicrobial activity, several essential oils exhibit anti-inflammatory and antioxidant effects. A product possessing these properties may aid in wound healing, as infection commonly triggers an inflammatory process (Oliveira *et al.*, 2011). By combining antifungal and anti-inflammatory actions, it is possible to limit symptoms such as itching, burning, and erythema, given that antioxidant compounds eliminate free radicals that cause tissue damage during inflammation (Havlickova, Friedrich, 2008; Mastelic *et al.*, 2008).

The family Lauraceae comprises about 50 genera, including *Ocotea*, about 2500–3000 species, and an extensive fossil record. This family is primarily found in tropical and subtropical regions and is well represented in the Americas, Asia, Australia, Madagascar, and rarely, in Africa (Van Der Werff, Ritcher, 1996; Bannister, Conran, Lee, 2012). The genus *Ocotea* Aubl. is the largest in the neotropical region, consisting of circa 350 species. It is highly prevalent in the Brazilian Atlantic Forest, with 120–160 species (Baitello, 2001; Baitello *et al.*, 2003).

Several studies assessed the biological activities of *Ocotea* essential oils. Important properties were identified, such as anti-inflammatory (Ballabeni *et al.*, 2010; Leporatti *et al.*, 2014), antithrombotic (Ballabeni *et al.*, 2007), cardiovascular (Barbosa-Filho *et al.*, 2008), antimicrobial (Leporatti *et al.*, 2014, Da Silva *et al.*, 2017), and cytotoxic (Da Silva *et al.*, 2017). Other studies investigated associations between groups of

compounds and cytotoxicity, such as Garcez *et al.* (2011), who reported that the alkaloid aporphine, isolated from *O. acutifolia* leaves, was cytotoxic to human cancer cell lines. Another study reported the mutagenic effects of five aporphinoid alkaloids isolated from *O. acutifolia* on *Drosophila melanogaster* wing cells (Guterres *et al.*, 2013). It was also found that the chloroform fraction of *O. puberula* fruits containing the alkaloid dicentrine demonstrated antinociceptive effects (Montrucchio *et al.*, 2012).

This study aimed to determine the chemical composition and assess the *in vitro* antifungal and anti-inflammatory activities of essential oils from *Ocotea* species occurring in southern Brazil. To our knowledge, no study has yet reported the chemical or pharmacological properties of these oils.

## MATERIAL AND METHODS

### Plant material

Leaves of *Ocotea* species were collected from native populations in southern Brazil (Table I). The plant material was identified by the botanist Dr. Sérgio L. Bordignon, and a voucher specimen of each species was deposited in the Herbarium of the Federal University of Rio Grande do Sul (ICN-UFRGS) under the following numbers: *O. acutifolia*, 176762; *O. indecora*, 192541; *O. puberula*, 176761; and *O. silvestris*, 176763.

**TABLE I** - Collection period, locality, and essential oil yield of different *Ocotea* species

Species	Code	Collection period	Locality	Yield (%)
<i>O. acutifolia</i>	OA	September 2012	Caçapava do Sul (30°30'44"S 53°29'29"W)	0.2
<i>O. indecora</i>	OI1	July 2013	Santo Antônio da Patrulha (29°49'0.3"S 50°31'11"W)	0.3
	OI2	July 2013		0.2
<i>O. puberula</i>	OP1	July 2012	Nova Petrópolis (29°22'33"S 51°06'43"W)	0.2
	OP2	April 2013		0.2
<i>O. silvestris</i>	OS1	July 2012	Nova Petrópolis (29°22'33"S 51°06'43"W)	0.2
	OS2	April 2013		0.2

## Essential oil extraction

Essential oils from *Ocotea* species were obtained from fresh material by hydrodistillation using a Clevenger-type apparatus for 4 h, according to the procedures described in the Brazilian Pharmacopoeia (2010). Yield was determined as the ratio of weight to volume (w/v). Samples were stored in glass vials in a refrigerator at 4–5 °C until analysis to minimize chemical degradation.

## Chemical analysis

For chemical analysis, the essential oils were diluted in ethyl ether at a ratio of 2:100 (v/v) and analyzed on a gas chromatograph coupled to a mass spectrometer (GC-MS) (Shimadzu QP5000) equipped with a Durabond DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 µm). The injector and detector temperatures were set at 220 and 250 °C, respectively, using a column temperature program of 60–300 °C at 3 °C/min, with helium as carrier gas at a flow rate of 1 mL/min. Quantitative GC analysis was carried out using a GC-PerkinElmer Autosystem 158 XL chromatograph equipped with a Durabond DB-5 column and TotalChrom™ Workstation software. Injector, detector, and oven temperatures were the same as described for the GC-MS analysis. Nitrogen was used as carrier gas (1.0 mL/min) with a constant makeup flow of 40 mL/min hydrogen and 400 mL/min analytical grade air. Percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID) without taking into account relative response factors.

Compound identification was based on the comparison of retention indices calculated by linear interpolation relative to retention times of a series of *n*-alkenes and comparison of mass spectra with those of standard samples, literature data (Adams, 2009), and National Institute of Standards and Technology (NIST) 12 and 62 spectral databases. Relative compositions were calculated by normalization of GC peak areas.

## Antifungal activity

The antifungal activity of essential oils was evaluated against clinical isolates of *Candida albicans*

(CA15), *C. glabrata* (CG09), *C. krusei* (CK03), *C. parapsilosis* (CP52), *C. tropicalis* (CT750), *Cryptococcus neoformans* (CN16), *Microsporium canis* (MC38), *M. gypseum* (MG01), *Trichophyton mentagrophytes* (TM32), and *T. rubrum* (TR51). All isolates are deposited in the Mycology Collection of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Prior to the antifungal activity assays, yeasts were cultured in Sabouraud agar containing chloramphenicol for 24 h at 35 °C and filamentous fungi were incubated at 32 °C for 5 days. A screening test was conducted with all essential oils at a fixed concentration of 500 µg/mL. The minimum inhibitory concentration (MIC) was determined only in cases where antifungal activity was detected in the screening test. MIC values were assessed by the broth microdilution method, according to the Clinical Laboratory Standards Institute protocol (CLSI, 2008). Essential oil samples were tested at concentrations from 1.95 to 500 µg/mL. The MIC was defined as the lowest concentration of essential oil at which the tested microorganism did not show visible growth. Experiments were performed in quadruplicate.

## Chemotaxis assay

Antichemotactic activity was evaluated according to the modified Boyden chamber method as described by Suyenaga *et al.* (2011). Before the assay, neutrophils were treated with essential oils dissolved in Hank's balanced salt solution (pH 7.4) at concentrations of 0.3125–5 µg/mL at 37 °C for 30 min. Samples were diluted in 1% polysorbate 80. A neutrophil suspension without antichemotactic agent and a solution of 1% polysorbate 80 were used as negative controls. Indomethacin (10 µg/mL) was used as positive control.

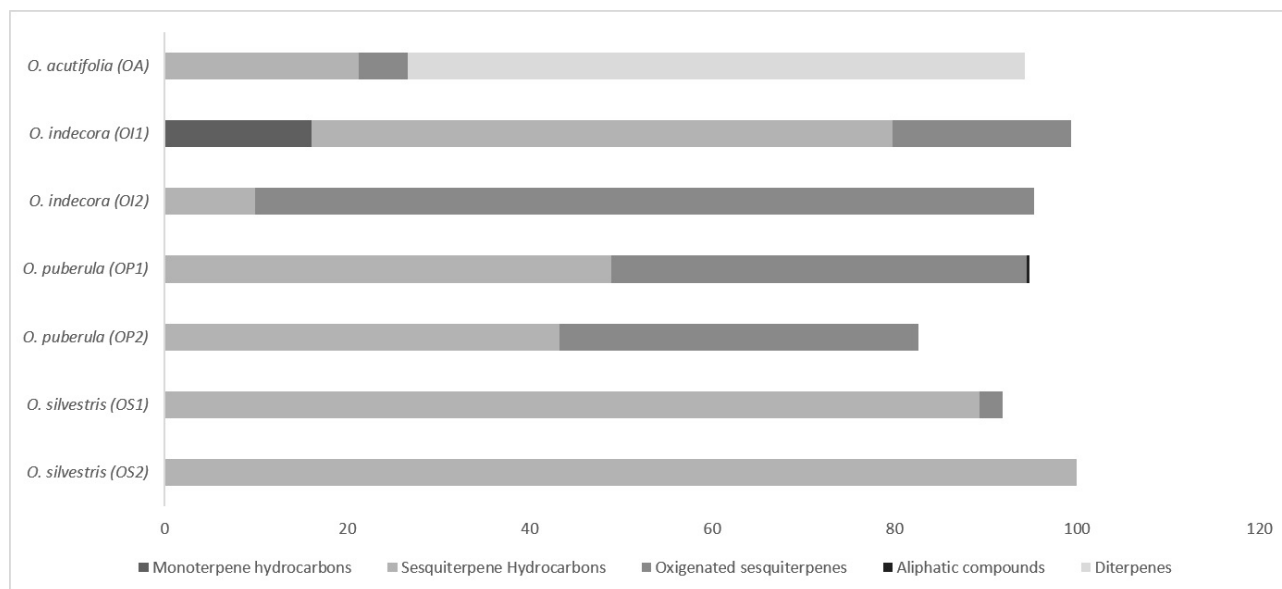
## RESULTS AND DISCUSSION

### Chemical composition of essential oils

The essential oils of fresh leaves of four *Ocotea* species were analyzed in this study. Two specimens of *O. indecora*, *O. puberula*, and *O. silvestris* and one specimen of *O. acutifolia* were collected. Details on

collection periods and localities are shown in Table I. No significant differences in essential oil yields were observed; however, there were important quantitative and qualitative variations in chemical composition (more specifically, terpene content) among species. Most samples were characterized by the presence of

sesquiterpenes. Monoterpenes were not detected in the investigated species, except in the essential oil of one *O. indecora* specimen, which contained 16% monoterpene hydrocarbons, represented by  $\alpha$ - and  $\beta$ -pinene (Figure 1). The chemical composition of essential oils obtained from leaves of *Ocotea* species is described in Table II.



**FIGURE I** - Terpenoid content in essential oils from four *Ocotea* species.

**TABLE II** - Percentage composition of essential oils extracted by hydrodistillation of fresh leaves of four *Ocotea* species

RI <sub>lit</sub>	RI <sub>calc</sub>	Component	<i>O. acutifolia</i>		<i>O. indecora</i>		<i>O. puberula</i>		<i>O. silvestris</i>	
			OA		OI1	OI2	OP1	OP2	OS1	OS2
<i>Monoterpene hydrocarbons</i>										
939	926	$\alpha$ -Pinene	-		4.2	-	-	-	-	-
979	968	$\beta$ -Pinene	-		8.9	-	-	-	-	-
991	985	Myrcene	-		0.7	-	-	-	-	-
1029	1021	Limonene	-		2.3	-	-	-	-	-
<i>Sesquiterpene hydrocarbons</i>										
1377	1365	$\alpha$ -Copaene	-		3.2	-	1.1	-	-	-
1391	1383	$\beta$ -Elemene	-		1.3	-	-	-	0.3	-
-	1394	Iso-caryophyllene	-		-	-	9.5	-	-	-
1419	1407	$\beta$ -Caryophyllene	-		1.8	3.0	25.2	7.7	1.9	-
1441	1426	Aromadendrene	-		-	-	0.7	11.5	-	-
1439	1436	$\beta$ -Humulene	-		0.9	-	2.7	tr	-	-

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RI <sub>lit</sub>	RI <sub>calc</sub>	Component	<i>O. acutifolia</i>		<i>O. indecora</i>		<i>O. puberula</i>		<i>O. silvestris</i>	
			OA	OI1	OI2	OP1	OP2	OS1	OS2	
1460	1447	Allo-aromadendrene	-	-	-	0.8	tr	-	-	
1466	1461	$\alpha$ -Acoradiene	-	-	-	1.4	3.7	-	-	
1471	1463	$\beta$ -Acoradiene	-	-	-	5.4	12.5	-	-	
1480	1466	$\gamma$ -Muurokene	-	-	-	-	-	0.6	-	
1485	1467	Germacrene D	-	10.5	1.7	0.8	-	54.1	73.3	
1490	1470	$\beta$ -Selinene	18.0	-	-	-	-	-	-	
1498	1480	$\alpha$ -Selinene	3.2	-	-	-	-	-	-	
1500	1483	Bicyclogermacrene	-	40.7	3.1	1.0	5.9	29.8	26.7	
1500	1488	$\alpha$ -Muurokene	-	-	-	-	-	0.3	-	
1509	1488	Germacrene A	-	0.8	-	-	-	-	-	
1523	1509	$\delta$ -Cadinene	-	3.3	2.2	0.5	1.9	2.3	-	
1561	1539	Germacrene B	-	1.1	-	-	-	-	-	
<i>Oxygenated sesquiterpenes</i>										
1563	1554	( <i>E</i> )-Nerolidol	-	-	-	13.0	5.9	-	-	
1578	1567	Spathulenol	-	4.6	2.0	11.8	6.3	1.1	-	
1583	1570	Caryophyllene oxide	3.0	tr	4.4	7.9	-	-	-	
1585	1575	Globulol	-	2.5	1.9	6.6	22.6	1.3	-	
-	1581	Epi-Globulol	-	0.6	-	4.7	4.4	-	-	
1601	1588	Guaiol	-	-	30.2	-	-	-	-	
1627 <sup>#</sup>	1615	1- <i>epi</i> -Cubenol	-	-	1.5	-	-	-	-	
1624	1625	Iso-spathulenol	-	1.6	-	-	-	-	-	
	1631	$\tau$ -Cadinol	-	-	4.9	-	-	-	-	
1646	1645	$\alpha$ -Muurokol	-	-	-	1.4	-	0.2	-	
1651	1639	$\beta$ -Eudesmol	-	-	12.7	-	-	-	-	
1654	1647	$\alpha$ -Eudesmol	2.3	-	27.6	-	-	-	-	
1652	1642	$\alpha$ -Cadinol	-	10.2	-	-	-	-	-	
<i>Diterpenes</i>										
2017	2019	Phyllocladene	67.7	-	-	-	-	-	-	
<i>Aliphatic compounds</i>										
-	2099	Nonadecanal	-	-	-	0.4	-	-	-	
-	1826	Unidentified*	-	-	-	-	10.7	-	-	
		Monoterpene hydrocarbons	-	16.1	-	-	-	-	-	
		Sesquiterpene hydrocarbons	21.2	63.6	10.0	49.1	43.2	89.3	100.0	

(continues on the next page...)



RI <sub>lit</sub>	RI <sub>calc</sub>	Component	<i>O. acutifolia</i>		<i>O. indecora</i>		<i>O. puberula</i>		<i>O. silvestris</i>	
			OA	OI1	OI2	OP1	OP2	OS1	OS2	
		Oxygenated sesquiterpenes	5.3	19.5	85.2	45.4	39.2	2.6	-	
		Diterpenes	67.7	-	-	-	-	-	-	
		Aliphatic compounds	-	-	-	0.4	-	-	-	
		Total compounds	94.2	99.2	95.2	94.9	82.4	91.9	100.0	

Compounds are listed in order of elution on a DB-5 column. RI<sub>lit</sub>, literature retention index (Adams, 2001 and 2009); RI<sub>calc</sub>, calculated retention index; OA, *O. acutifolia*; OI1, *O. indecora* collection 1; OI2, *O. indecora* collection 2; OP1, *O. puberula* collection 1; OP2, *O. puberula* collection 2; OS1, *O. silvestris* collection 1; OS2, *O. silvestris* collection 2; tr, traces. \*m/z (rel. int.): 41 (100), 55 (28), 69 (33), 91 (32), 105 (41), 119 (19), 131 (26), 145 (32), 159 (42), 173 (9), 187 (8), 202 (41), 220 (3).

*O. acutifolia* essential oil, obtained in 0.2% yield, was orange in color and had a viscous consistency. Chemical analysis revealed the presence of five major compounds, accounting for about 94% of the total oil content. The oil was characterized by a predominance of an unsaturated tetracyclic diterpene, phyllocladene (67.7%), followed by a sesquiterpene hydrocarbon,  $\beta$ -selinene (18.0%), formed in the biosynthetic pathway of germacrene. Monoterpenes were not identified in this essential oil.

Silva *et al.* (2013) analyzed essential oil of *O. acutifolia* leaves collected in Rio Grande do Sul and reported a total of 24 compounds, the major of which were oxygenated sesquiterpenes, such as caryophyllene oxide (56.9%), calarene epoxide (11.7%), and  $\gamma$ -elemene (8.2%). However, different from that observed in the current study, the authors identified the monoterpenes  $\alpha$ -thujene,  $\alpha$ -pinene, limonene, and linalool.

Herein, we report for the first time the presence of phyllocladene in *Ocotea* species. This compound was previously identified in samples of *Cryptomeria japonica* D. Don (Cupressaceae) from Portugal (Moiteiro *et al.*, 2013) and in the leaves, branches, and roots of *Eryngium aquifolium* Cav. (Apiaceae) from Spain (Palá-Paúl *et al.*, 2010).

Samples of *O. puberula* (OP) were collected from two close individuals under direct sunlight. Essential oil yield was 0.2% for both samples, and the color was light yellow. A total of 18 compounds were identified, representing about 94% and 82% of the total oil content of samples from collections 1 (OP1) and 2 (OP2), respectively. The

sesquiterpene fraction was predominant in both samples.  $\beta$ -Caryophyllene (25.2%), (*E*)-nerolidol (13%), and spathulenol (11.8%) were the major compounds in OP1 essential oil, whereas globulol (22.6%),  $\beta$ -acoradiene (12.5%), and aromadendrene (11.5%) predominated in OP2 essential oil. No monoterpenes were identified in OP1 or OP2 essential oils. It is important to emphasize that all compounds identified in OP2 were also detected in OP1, indicating some level of similarity. Chemical differences between samples might have been due to seasonality and/or ontogenetic factors, such as plant age. The proximity of OP1 and OP2 might also have contributed to the slight variation in oil composition and the high sesquiterpene content of both samples. Quantitative variation in volatile oil components may be attributed to plant development stage, level of sunlight incidence, or presence of herbivores and pathogens, as terpenoids are related to plant defense.

In a previous study, De Araújo *et al.* (2001) reported the presence of 3 monoterpenes and 10 sesquiterpenes in the essential oil of *O. puberula* leaves, with  $\beta$ -caryophyllene and bicyclogermacrene as the most abundant compounds. Later, Raggi (2008) examined the chemical composition of the leaf essential oil of three *O. puberula* individuals and reported a predominance of the sesquiterpene hydrocarbons  $\beta$ -caryophyllene,  $\beta$ -elemene, bicyclogermacrene,  $\alpha$ -copaene, and  $\alpha$ -humulene. The results of our study were similar to those of De Araújo *et al.* (2001).

*O. silvestris* essential oil was obtained in 0.2% yield from both specimens (OS1 and OS2). The oils

were transparent and light yellow in color. Samples were collected from close individuals under direct sunlight. Bicyclogermacrene and germacrene D were the major compounds, accounting for 83.9% of the total oil content in OS1 and 100% in OS2. OS1 essential oil was found to contain 10 terpenes, which together accounted for about 92% of the total oil content. In this sample, germacrene D (54.1%) and bicyclogermacrene (29.8%) were the predominant compounds, and other oxygenated sesquiterpenes and sesquiterpene hydrocarbons were identified at smaller concentrations. OS2 oil contained only germacrene D and bicyclogermacrene, representing 100% of the total oil content.

*O. indecora* samples were collected at the same locality but from different individuals, one located under the shade of large trees (OI1) and the other under direct sunlight (OI2). The yields of leaf essential oils were 0.3 and 0.2% for OI1 and OI2, respectively. Oils exhibited a limpid and clear yellow aspect. A total of 24 components were identified in OI1 and OI2 oils, accounting for 99.2 and 95.2% of the total oil content, respectively. The sesquiterpene fraction was the most predominant in both samples. In OI1, bicyclogermacrene (40.7%), germacrene D (10.5%), and  $\alpha$ -cadinol (10.2%) were the major components; only OI1 contained monoterpene compounds. The oil of OI2 leaves, collected under direct sunlight, had the following oxygenated sesquiterpenes as the major compounds: guaiol (30.2%),  $\alpha$ -eudesmol (27.6%), and  $\beta$ -eudesmol (12.7%).

Of note, OI2 oil had a higher content of oxygenated compounds than OI1 oil, suggesting that sunlight can directly influence essential oil composition. Germacrene D and bicyclogermacrene, detected in OI1 oil, may undergo rearrangements and diverse or nonenzymatic oxidation, affording different sesquiterpenoids, such as cadinene and guaiane or those with a eudesmane skeleton. These reactions can be stimulated by elevation of temperature (Bulow, Konig, 2000). We highlight that specimens were stored in a freezer until extraction and oils were stored at low temperatures until chemical analysis.

Another difference between the two samples was the presence of monoterpenes in OI1, collected in a shady habitat. Monoterpenes are derived from geranyl pyrophosphate, the initial precursor of monoterpenes,

which, after some cyclization reactions, gives rise to structural compounds such as pinenes ( $\alpha$ - and  $\beta$ -pinene), *p*-menthanes (limonene), myrcene, and acyclic compounds (Poulose, Croteau, 1978). No terpenes of this class were identified in OI2 essential oil.

Quantitative and qualitative chemical variability may be attributed to several factors. Biochemical and physiological changes occurring throughout plant development can modify the production of biologically active substances, thereby influencing the content and quality of essential oils. For this reason, oils do not have constant quality or quantity and are directly influenced by environmental factors, such as soil, light, temperature, radiation, water deficiency, and place and time of collection. Different combinations of soil (acidity or alkalinity), climate, topography, moisture, temperature, and sunlight conditions can result in differences in the chemical and physical characteristics of oils. This is supported by the fact that plants collected from areas under different light, heat, and temperature conditions may differ in development (Almeida *et al.*, 2016). Species that grow under light may suffer more from the action of herbivores and pathogens, as they are more exposed than plants grown under shade (Herms, Mattson, 1992). Thus, we can infer that the qualitative difference in essential oil composition between OI1 and OI2 might be related to the adaptation of plants to their environment. Possibly, the high percentage of oxygenated compounds is associated with direct sunlight, suggesting that these compounds do not have such a high deterrent property.

Chemical characterization of essential oil from *Dalbergia frutescens* (Vell.) Britton (Fabaceae) and analysis of the influence of environmental conditions revealed that essential oil yield was strongly affected by environmental conditions, being directly proportional to temperature and inversely proportional to cloudiness and rainfall (Mendes *et al.*, 2012). The concentrations of linalool,  $\alpha$ -ionone, and  $\beta$ -ionone, the major compounds of essential oil extracted in the spring, were directly influenced by temperature and sunlight. It was also observed that autumn and winter specimens were characterized by the presence of  $\beta$ -damascenone and geranyl acetone, compounds that, at first, were not related to environmental conditions (Mendes *et al.*, 2012).

## Antifungal activity

Antifungal activity was assessed against yeasts and filamentous fungi by the broth microdilution method. For this assay, oils obtained from *O. acutifolia*, *O. indecora* (OI1 and OI2), *O. puberula* (OP2), and *O. silvestris* (OS1) were tested at 500 µg/mL. In the screening assay, samples were tested against four species of filamentous fungi (*M. canis*, *M. gypseum*, *T. mentagrophytes*, and *T. rubrum*) and six species of yeasts (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. neoformans*). Oils did not inhibit the growth of yeasts or fungi, except that of *C. parapsilosis*, whose growth was inhibited by all samples.

Given this result, the MIC values of essential oils were determined against *C. parapsilosis* isolates. Filamentous fungal strains were not analyzed because the oils did not show inhibitory activity at the tested concentration. MIC was determined by the broth microdilution method against five strains of *C. parapsilosis* (RL07, RL11, RL13, RL27, and RL52). All tested essential oils showed a MIC of 500 µg/mL.

According to Burt (2004), essential oils comprise a large number of chemical compounds, which is why their mechanism of action involves several targets in microbial cells. It is well accepted that the lipophilicity of essential oil constituents is the main factor responsible for antimicrobial properties, given that this characteristic can facilitate the interaction of compounds with cell membrane lipids and mitochondria, increasing membrane permeability and leading to leakage of cellular contents (Cowan, 1999). Nevertheless, as argued by Deuschle *et al.* (2007), this might not be the sole mechanism involved in the antimicrobial activity of essential oils. For example, germacrene D, a sesquiterpene hydrocarbon, exhibited pronounced lipophilic characteristics but did not inhibit microbial growth at concentrations lower than 500 µg/mL. This result corroborates the findings of Biavatti *et al.* (2001), who observed a lack of antimicrobial activity against different microorganisms.

Raggi (2008) determined the antifungal activity of *O. puberula* essential oil by bioautography on silica gel plate and observed low activity against *Cladosporium cladosporioides* and *C. sphaerospermum*. The author also assessed the antifungal activity of three *O. puberula*

specimens (collected at different times of the year) against strains of *Aspergillus niger* and *C. albicans*. The results showed no change in antifungal activity against *A. niger* between individuals or seasons, with a mean inhibition percentage of 67%. Essential oils did, however, differ in their antibacterial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The author suggested that this behavior was probably due to quantitative variations in oil composition. Against *S. aureus*, the essential oil of leaves collected in early autumn was the most active (94.4% inhibition), whereas the essential oil of leaves sampled in early spring was the least active. Plants collected in summer and early autumn produced essential oils that afforded a 70% inhibition of *P. aeruginosa*.

Essential oils containing germacrene B, the precursor of germacrene D (the major constituent in some samples of the current study), demonstrated fungitoxic activity (Fach *et al.*, 2002). Oils containing the sesquiterpene hydrocarbon bicyclogermacrene also demonstrated antimicrobial activity (Constantin *et al.*, 2001; Cysne *et al.*, 2005). The oxygenated sesquiterpene spathulenol was shown to exhibit important antibacterial properties and moderate cytotoxic activity (Santos *et al.*, 2012). It is important to highlight that each component contributes differently to the biological activity of essential oils (Daferera, Ziogas, Polissioi, 2003). Although the antimicrobial activity of an essential oil is attributed to its major compounds, the synergistic and antagonistic effects of those occurring at lower concentrations should be considered, possibly explaining a poorly pronounced biological effect.

## Antichemotactic activity

The ability of essential oils to inhibit leukocyte migration was determined using the Boyden chamber method. Essential oils were tested at 0.3125–5 µg/mL. Indomethacin, used as positive control, inhibited 62.9% of leukocyte migration at the tested concentration tested (10 µg/mL). None of the samples demonstrated antichemotactic activity compared with the negative control.

Previous research showed that *Ocotea* essential oils exert anti-inflammatory effects. *O. quixos* essential oil was found to significantly inhibit lipid polysaccharide-



induced cyclooxygenase-2 expression and carrageenan-induced edema in the rat paw (Ballabeni *et al.*, 2010). Of note, the oil was characterized by the predominance of *trans*-cinnamaldehyde and methyl cinnamate, whose anti-inflammatory action has not yet been elucidated.

## CONCLUSION

In this study, essential oils obtained from different *Ocotea* species showed important qualitative and quantitative variations in chemical composition, primarily in the sesquiterpene fraction. The main compounds identified were  $\beta$ -caryophyllene, germacrene D, bicyclgermacrene, globulol,  $\alpha$ -eudesmol, guaiol, (*E*)-nerolidol, and phyllocladene. The last compound was described for the first time in the genus *Ocotea*. All tested essential oils exhibited antifungal activity against *C. parapsilosis*. These findings contribute to the knowledge of the chemical composition and biological activities of *Ocotea* species.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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