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Chemical composition of essential oil from leaves of *Ocotea limae* Vattimo Gil. and *Ocotea gardneri* (Meisn.) Mez. growing wild in Atlantic forest of North-Eastern Brazil

[Composición química del aceite esencial de las hojas de *Ocotea limae* Vattimo Gil. y *Ocotea gardneri* (Meisn.) Mez. nativas de la floresta Atlántica del Nordeste de Brasil]

Marcilio M de Moraes, Claudio AG da Camara & Carolina A de Araujo

Department of Chemistry, University Federal Rural of Pernambuco, Recife, PE, Brazil
Contactos | Contacts: **Claudio AG da CAMARA - *E-mail address:*** **claudio_agc@hotmail.com**

Abstract: Essential oils from leaves of *Ocotea limae* and *Ocotea gardneri* species were analyzed by GC-FID and GC-MS. The analysis revealed the presence of 48 components accounting for 98.1 ± 1.3% e 98.0 ± 0.5% of the essential oils from the *O. limae* and *O. gardneri*, respectively. The oils were characterized by high percentage of sesquiterpenes. The mains compounds identified in the essential oils were spathulenol (13.3 ± 0.5%), β-caryophyllene (12.4 ± 0.5%), bicyclogermacrene (11.3 ± 0.4%) and germacrene D (10.9 ± 0.3%) from the *O. limae* and germacrene D (26.9 ± 0.9%) e bicyclogermacrene (21.7 ± 0.7%) from the O. glomerata.

Keywords: Essential oil, GC-MS, sesquiterpenes, *Ocotea limae* and *Ocotea gardneri*.

Resumen: Los aceites esenciales de hojas de *Ocotea limae* y *Ocotea gardneri* fueron analizados por GC-FID y GC-MS. El análisis reveló la presencia de 48 componentes que representan 98,1 ± 1,3% y 98,0 ± 0,5% de los aceites esenciales de *O. limae* y *O. gardneri*, respectivamente. Los aceites se caracterizaron por un alto porcentaje de sesquiterpenos. Los principales compuestos identificados en los aceites esenciales fueron espatulenol (13,3 ± 0,5%), β-cariofileno (12,4 ± 0,5%), biciclogermacreno (11,3 ± 0,4%) y germacreno D (10,9 ± 0,3%) de *O. limae* y germacreno D (26,9 ± 0,9%) y bicyclogermacreno (21,7 ± 0,7%) de la O. glomerata.

Palabras clave: Aceites esenciales, GC-MS, sesquiterpenos, *Ocotea limae* y *Ocotea gardneri*.

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INTRODUCTION

Ocotea is one of the most representative genera of the family Lauraceae, with approximately 400 species. Three hundred species are found in the Americas, 150 of which occur in Brazil (Rohwer, 1993; Quinet *et al*., 2010), with 52 recorded for the northeastern region of the country and 11 for the state of Pernambuco (Barreto, 1990; Quinet *et al.*, 2010). Many plants in the genus have high commercial value due to the fact that their wood is used in furniture making and civil construction (Moraes, 2005). Predatory extractivism in Brazil has led some species of *Ocotea* to be listed as endangered, which could represent a loss of genetic heritage and chemical knowledge of native aromatic plants (IBAMA, 1992).

Species of this genus are characterized by the production of essential oils that are used in folk medicine in the form of tea or infusions of the leaves for the treatment of headache, fever, cough and diarrhea (Coutinho *et al*., 2006; Moraes, 2005). Studies on pharmacological activity have demonstrated the species of *Octotea* have chemical substances with antimicrobial (Leporatti *et al*., 2014), muscle relaxing (Ribeiro *et al*., 2003) and anti-inflammatory (Destryana *et al*., 2014) properties. Previous investigations on the characterization of the chemical constituents of species of *Ocotea* have revealed the occurrence of alkaloids (Dias *et al*., 2003), flavanoids (Garrett *et al*., 2012), lignans, neolignans (Barbosa-Filho *et al*., 1999) and essential oils comprised basically of monoterpenes (Olivero-Verbel *et al*., 2010), sesquiterpenes, diterpenes (Takaku *et al*., 2007; Yamacuchi *et al.*, 2013) and phenylpropanoids (Oltramari *et al.*, 2001; Mossi *et al.*, 2013; Leporatti *et al*., 2014;).

Ocotea limae and *Ocotea gardneri* are exclusive to Brazilian flora and are found in fragments of the Atlantic Forest in the state of Pernambuco in the northeastern region of the country, the biodiversity of which is at risk due to anthropogenic pressure. According to information obtained *in loco*, the leaves of these species are used in the form of tea to alleviate headache and stomach problems. However, a survey of the literature reveals that no chemical studies have yet been conducted for the species.

Chemical composition of *O. gardneri* essential oil was previously reported by our research group at two congresses (Botelho *et al.*, 2009; Moraes *et al.*, 2010) and a sample collected in Paraíba-Brazil was also communicated by Dias *et al.* (2006). Right now, new triplicate analyzes were performed and a more complete chemical composition of *O. gardneri* oil is presented in the present work. As part of a systematic investigation of the chemical composition of aromatic flora in northeastern Brazil, this paper offers the first report of the chemical composition of essential oils from the leaves of *O. limae* that occur in a fragment of the Atlantic Forest in the state of Pernambuco, Brazil.

MATERIAL AND METHODS

Collection of plant material

The fresh leaves of *Ocotea limae* and *Ocotea gardneri* were collected in the Mata de Cruzinha, in Igarassú, Pernambuco state, in March 2008. The geographical coordinates of the collection point were: S 07º49'25.5" W 034º 58'46.6" and S 08º20'05.0" W 035º 47'09.7", respectively. The plant was idenfied by botanist Dr. Maria RCS de Melo (University Federal Rural of Pernambuco). Voucher of also samples were mounted and deposited in the Vasconcelos Sobrinho Herbarium of the UFRPE, under numbers: 45859 *Ocotea limae* Vattimo Gil. and 45790 *Ocotea gardneri* (Meisn.) Mez.

Isolation of essential oils

The essential oils from fresh leaves (100 g) were separately isolated using a modified Clevenger-type apparatus and hydrodistillation for 4h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, at a low temperature (-5º C) until analysis. Total oil yields were expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate.

Optical rotation

Optical rotation of the essential oils was performed with a digital polarimeter (A. Krüss model Px800, Germany) at 589 nm and 25° C in a dichloromethane solution.

Chemicals

All monoterpenes (α-pinene and β-pinene) and sesquiterpenes (β-caryophyllene, aromadendrene, α-humulene e valencene, germacrene D, bicyclogermarene spathulenol and caryophyllene oxide) used in the identifications of volatile components were purchased from Sigma-Aldrich - Brazil.

Gas chromatography analysis

Quantitative GC analysis were carried out using a Hewlett-Packard 5890 Series II GC apparatus equipped with a flame ionization detector (FID) and a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μm) (J & W Scientific). The oven temperature was programmed from 60 to 240° C at a rate 3° C min^{-1}. Injector and detector temperatures were 260° C. Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1} in split mode (1:30). The injection volume was 0.5 μL of diluted solution (1/100) of oil in *n*-hexane. The amount of each compound was calculated from GC peak areas in the order of DB-5 column elution and expressed as a relative percentage of the area of the chromatograms. Analysis were carried out in triplicate.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the essential oils was carried out using a Varian 220-MS IT GC system with a mass selective detector, mass spectrometer in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 D fitted with the same column and temperature program as that for the GC experiments, with the following parameters: carrier gas = helium; flow rate = 1 mL min^{-1}; split mode (1:30); injected volume = 1 μL of diluted solution (1/100) of oil in *n*-hexane.

Identification of components

Identification of the components was based on GC retention indices with reference to a homologous series of C_8-C_{40} n-alkanes calculated using the Van den Dool and Kratz equation (Van den Dool & Kratz, 1963) and by computer matching against the mass spectral library of the GC-MS data system (NIST version 14 and WILEY version 11) and co-injection with authentic standards as well as other published mass spectra (Adams, 2007). Area percentages were obtained from the GC-FID response without the use of an internal standard or correction factors.

RESULTS

The essential oils from the leaves of *O. limae* and *O. gardineri* obtained through hydrodistillation had a yellowish coloration and citric aroma, with respective yields of 1.0 ± 0.0% and 0.9 ± 0.1%. The oils were levorotatory, with a specific rotation of $[\alpha]\ ^{D}_{25}$ = -25.2° (c.=1, CH_2Cl_2) for the oil from *O. limae* and $[\alpha]\ ^{D}_{25}$ = -29.1° (c.=1, CH_2Cl_2) for the oil from *O. gardneri.* The chemical analysis using GC-MS enabled the identification of 48 compounds, representing 98.1 ± 1.3% and 98.0 ± 0.5% of the composition of the oils from *O. limae* and *O. gardneri*, respectively. The oils exhibited a terpene chemical profile, with a predominance of sesquiterpenes (96.8 ± 0.8% and 98.0 ± 0.5% for the oils from *O. limae* and *O. gardneri*, respectively) (Table 1).

Monoterpenes were only identified in *O. limae* at a proportion of less than 1%: *α*-pinene (0.5 ± 0.0%) and *β*-pinene (0.8 ± 0.0%).

The major constituents in the oil from *O. gardneri* were germacrene D (26.9 ± 0.9%) and bicyclogermacrene (21.7 ± 0.7%). Other compounds found in expressive quantities were *β*-caryophyllene (6.1 ± 0.0%), germacrene B (4.9 ± 0.1%), caryophyllene oxide (4.0 ± 0.0%), viridiflorol (4.5 ± 0.0%) and *α*-cadinol (3.3 ± 0.0%). The major constituents in the oil from *O. limae* were spathulenol (13.3 ± 0.5%), *β*-caryophyllene (12.4 ± 0.5%), bicyclogermacrene (11.3 ± 0.4%) and germacrene D (10.9 ± 0.3%). Other compounds found in expressive quantities were aromadendrene (5.7 ± 0.0%), viridiflorol (5.3 ± 0.4%), *epi*-*α*-muurolol (4.4 ± 0.1%) and *α*-cadinol (3.8 ± 0.0%).

DISCUSSION

No significant differences were found in the yields of oils from the fresh leaves of the two *Ocotea* species that grow wild in fragments of the Atlantic Forest in the state of Pernambuco, northeastern Brazil. The yields from both *O. limae* and *O. gardneri* are within the range reported in the literature for leaf oils from other species that occur in Brazil, such as 0.23% for *O. nigrescens*, 0.35% for *O. splendens* (Yamaguchi *et al*., 2013), 0.70% for *O. duckei* (Barbosa-Filho *et al*., 2008) and 1.5% for *O. puchury-major* (Leporatti *et al*., 2014). Differences in the yields between the plants examined herein and congeners may be explained by the influence of abiotic factors, such as temperature, luminosity, seasonality, nutrition and water availability. With regard to specific rotation, the oils of the species analyzed herein were levorotatory, with similar rotation angles (Table 1). No measures of specific rotation were found in the literature for essential oils from species of *Ocotea*.

Among the major constituents identified in the oil from *O. limae*, only spathulenol was not found in the oil from *O. gardneri*. β-caryophyllene was the second major component of the *O. limae* oil, but was found in the *O. gardneri* oil at a proportion of less

than 10%. The overall similarity in the constituents identified in the two species was 33.3%, indicating that the chemical profiles of these oils differ both qualitatively and quantitatively.

Table 1
Percentage composition, yield and optical rotation of essential oils from *Ocotea limae* and *Ocotea gardneri*

Compounds	RI [a]	RI [b]	*O.limae*	*O. gardneri*	Method of Identification
Yield (%) ± SD			1.0 ± 0.0	0.9 ± 0.1	
$[\alpha]_D^{25}$(c.=1,CH_2Cl_2)			-25.2°	-29.1°	
α-pinene	935	932	0.5 ± 0.0	-	RI, MS,CI
β-pinene	980	974	0.8 ± 0.0	-	RI, MS,CI
δ-elemene	1335	1335	1.0 ± 0.0	1.8 ± 0.0	RI, MS
α-cubebene	1345	1345	0.4 ± 0.1	0.1 ± 0.0	RI, MS
α-elangene	1374	1373	1.6 ± 0.3	0.3 ± 0.0	RI, MS
α-copaene	1379	1374	-	0.3 ± 0.0	RI, MS
β-elemene	1389	1389	2.3 ± 0.5	2.2 ± 0.0	RI, MS
β-longipinene	1402	1400	-	0.1 ± 0.0	RI, MS
***β*-caryophyllene**	**1414**	**1417**	**12.4 ± 0.5**	**6.1 ± 0.0**	RI, MS,CI
β-copaene	1434	1430	1.0 ± 0.0	0.4 ± 0.0	RI, MS
β-gurjunene	1435	1431	0.6 ± 0.1	-	RI, MS
α-*trans*-bergamotene	1436	1432	0.4 ± 0.3	-	RI, MS
γ-elemene	1438	1434	-	0.9 ± 0.0	RI, MS
Aromadendrene	1442	1439	5.7 ± 0.0	0.8 ± 0.0	RI, MS,CI
6,9-guaiadiene	1446	1442	-	0.7 ± 0.0	RI, MS
Spirolepechinene	1449	1449	-	0.6 ± 0.0	RI, MS
α-humulene	1452	1452	2.1 ± 0.0	2.6 ± 0.0	RI, MS,CI
9-*epi*-(*E*)-caryophyllene	1469	1464	1.3 ± 0.1	-	RI, MS
germacrene D	**1488**	**1484**	**10.9 ± 0.3**	**26.9 ± 0.9**	RI, MS,CI
β-selinene	1492	1489	0.4 ± 0.0	-	RI, MS
γ-amorphene	1499	1495	0.5 ± 0.0	0.1 ± 0.0	RI, MS
Bicyclogermacrene	**1505**	**1500**	**11.3 ± 0.4**	**21.7 ± 0.7**	RI, MS,CI
γ-cadinene	1515	1513	-	1.4 ± 0.2	RI, MS
δ-cadinene	1526	1522	2.0 ± 0.1	2.9 ± 0.1	RI, MS
Zonarene	1532	1528	2.5 ± 0.0	-	RI, MS
α-cadinene	1536	1537	0.7 ± 0.1	1.3 ± 0.2	RI, MS
italicene epoxide	1550	1547	1.3 ± 0.0	-	RI, MS
germacrene B	1558	1559	-	4.9 ± 0.1	RI, MS
Maaiol	1567	1566	-	1.2 ± 0.1	RI, MS
Spathulenol	**1581**	**1577**	**13.3 ± 0.5**	-	RI, MS,CI
Caryophyllene oxide	1583	1582	-	4.0 ± 0.0	RI, MS,CI
Viridiflorol	1589	1592	5.3 ± 0.4	4.5 ± 0.0	RI, MS
Carotol	1599	1594	3.8 ± 0.2	-	RI, MS
Widdrol	1601	1599	0.8 ± 0.2	-	RI, MS
Rosifoliol	1604	1600	-	1.5 ± 0.0	RI, MS
Cedrol	1605	1600	1.3 ± 0.1	-	RI, MS
Junenol	1619	1618	0.6 ± 0.1	2.1 ± 0.1	RI, MS
α-acorenol	1635	1632	0.7 ± 0.3	-	RI, MS
cis-caden-4-em-7-ol	1636	1635	-	0.8 ± 0.0	RI, MS
epi-*α*-muurolol	1643	1640	4.4 ± 0.1	-	RI, MS

α-muurolol	1651	1644	0.8 ± 0.1	-	RI, MS
β-eudesmol	1654	1649	0.6 ± 0.1	-	RI, MS
α-cadinol	1658	1652	3.8 ± 0.2	3.3 ± 0.0	RI, MS
α-selin-11-em-4- ol	1660	1658	-	3.5 ± 0.1	RI, MS
trans-calamenen-10-ol	1672	1668	3.3 ± 0.2	-	RI, MS
eudesm-7 (11)-em-4-ol	1696	1700	-	0.3 ± 0.0	RI, MS
6*R*,7*R*-bisabolone	1735	1740	-	0.3 ± 0.0	RI, MS
β-costol	1764	1765	-	0.3 ± 0.2	RI, MS
Monoterpenes			1.3 ± 0.1	-	
Sesquiterpenes			96.8 ± 0.8	98.0 ± 0.5	
Total			98.1 ± 1.3	98.0 ± 0.5	

[a]Retention indices calculated from retention times in relation to those of a series of n-alkanes on a 30m DB-5 capillary column. [b]Linear retention indices from the literature. RI = retention index, SD = standard deviation, MS = mass spectrum, CI = co-injection with authentic standards.

The chemical composition of *O. gardneri* leaves oil found in the present study differed from the composition presented at a congress by Dias et al. (2006) for a sample collected in Paraíba-Brazil, where β-caryophyllene (29.1%) was identified As a major component, whereas germacrene D, the main constituents found in the sample collected in Pernambuco-Brazil (26.9 ± 0.9) was found in a percentage lower than 10% in the oil collected in Paraíba. Bicyclogermacrene, characterized as the second main constituent of *O. gardneri* oil collected in Pernambuco (21.7 ± 0.7), was not identified in the sample investigated by Dias et al (2006).

The literature offers reports of the chemical composition of essential oils from different parts of plants of the genus *Ocotea* that occur in different regions of Brazil and are rich in monoterpenes, sesquiterpenes and/or phenylpropanoids. In contrast to the oils from *O. limae* and *O. gardneri* analyzed herein from the state of Pernambuco, which were rich in sesquiterpenes, phenylpropanoids were to be the main group of compounds in oil from the fruit of *O. odorifiera* occurring in the state of Rio Grande do Sul in southern Brazil (Oltramari *et al*., 2001) and oil from the leaves of *O. pucbury-major* collected in the state of Amazonas in the northern region of the country (Leporatti *et al*., 2014). Monoterpenes are reported to be the major constituents in oil from the fruit of *O. duckei* in the state of Paraíaba in northeastern Brazil (Barbosa-Filho *et al.*, 2008) as well as leaf oil from *O. odorifera* collected in the state of Rio Grande do Sul in the southern region of the country (Mossi *et al.*, 2013). However, studies with other species of *Ocotea* report sesquiterpenes to be the most abundant class, such as *O. bracteosa* and *O. duckei* collected in the state of Paraíba in the northeastern region (Coutinho *et al*., 2007; Barbosa-Filho *et al*., 2008), *O. quixos*, *O. nigrescens* and *O. splendens* collected in the state of Amazonas in the northern region (Sacchetti *et al*., 2006; Yamaguchi *et al*., 2013), *O. notata* collected in the state of Rio de Janeiro in the southern region (Garrett *et al*., 2010) and *O. puberula* collected in the state of Paraná in the southern region of the country (Farago *et al*., 2005).

The predominance of sesquiterpenes in the chemical composition of the essential oils from species of *Ocotea* that occur in the state of Pernambuco is consistent with data reported in the literature for the majority of congeners distributed throughout different regions of Brazil. This sesquiterpene profile has also been observed in species of *Ocotea* that occur outside of Brazil, such as oils from the fruit and shoots of *O. quixos* in Ecuador (Bruni *et al*., 2004; Destryana *et al.*, 2014), oils from the leaves of *O. meziana*, *O. valeriana* and *O. whitei* in Costa Rica (Wright *et al*., 2007; Takaku *et al*., 2007) and oil from the leaves of *O. caudata* in Colombia (Gil *et al*., 2016).

The similarity in the chemical composition of the essential oils from the leaves of *O. limae* and *O. gardneri* with correlated data reported in the literature is not only related to the sesquiterpene chemical profile, but also the main component identified in the oils. Spathulenol (13.3 ± 0.5%), which was the main component in the oil from *O. limae* and absent from the oil from *O. gardneri*, is reported to be the a major constituent of the oil from leaves of *O. whitei* (15.3%) and the bark of stems from *O. puberula* (15.73%) collected in Costa Rica and Brazil, respectively (Takaku *et al*., 2007; Farago

et al., 2005). The major component in the oil from *O. gardneri* [Germacrene D (26.9 ± 0.9%)] was found at a significant proportion in the oil from *O. limae* (10.9 ± 0.3%) and is also reported to be a major constituent of oils from the leaves of *O. valeriana* (69.7%) (Takaku *et al*., 2007) and *O. mezina* (50.6%) collected in Costa Rica (Wright *et al*., 2007), *O. botrantha* (35.2%) collected in the United States (Scora & Scora, 2001) and *O. caudata* (55.8%) collected in Colombia (Gil *et al*., 2016).

The second major constituent in the oil from *O. gardneri* (21.7 ± 0.7%) and third major constituent in the oil from *O. limae* (11.3 ± 0.4%) was bicyclogermacrene, which is reported in quantities less than 10% in oils from the leaves of *O. morae* (8.8%) (Chaverri *et al*., 2011) and *O. caudata* (8.0%) (Gil *et al*., 2016) as well as oil from the bark of stems of *O. puberula* (9.38%). *β*-caryophyllene, which was the second major constituent of the oil from *O. limae* (12.4 ± 0.5%), is the most reported compound in the literature as a major component in other species of the genus *Ocotea*, such as oils from leaves of *O. meziana* (24.8%), *O. whitei* (15.3%) (Takaku *et al*., 2007), *O. duckei* (60.54%) (Barbosa-Filho *et al*., 2008), *O. notata* (22.9%) (Garrett *et al*., 2010), *O. nigrescens* (37.9%) and *O. splendens* (51.0%) (Yamaguchi *et al*., 2013) as well as oils from the shoots (28.2%), leaves (15.1%) and fruit (15.1%) of *O. quixos* (Destryana *et al*., 2014; Bruni *et al*., 2004; Sacchetti *et al*., 2006).

CONCLUSION

This is the first report of the chemical composition of essential oils from *O. limae*. The present study demonstrated that neither species has phenylpropanoids and both are basically comprised of serquiterpenes. The chemical profile of the oils of the two species differs both qualitatively and quantitatively. The major constituents in the oil from *O. limae* were spathulenol and *β*-caryophyllene, whereas the major constituents in the oil from *O. gardneri* were germacrene D and bicyclogermacrene and differed from those previously Communicated for a sample from Paraíba-Brazil by Dias et al (2006).

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