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Antioxidant and Cytotoxic Properties of Essential Oils from Native Brazilian Lauraceae Species

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Received: 15 December 2018 Accepted: 1 January 2019 Published: 15 January 2019

Abstract

Analysis of the leaf and stem essential oils from three Brazilian Lauraceae species showed that the major constituent of O. odorifera was safrole (57.1 and 88.5

Index terms— Ocotea odorifera, Ocotea indecora, Persea venosa, essential oil, biological activities.

1 Introduction

ssential oils from herbal sources are used as food flavours, perfumes and pharmaceuticals purposes (Burt, 2004). Leaves and barks of some Lauraceae species are popular spice ingredients and flavoring agents, such as cinnamon and laurel (Joshi et al., 2010). Additionally, the essential oils from some species within the genera Aniba Aubl., Cinnamomun Spreng., Nectandra Rottb. and Ocotea Aubl. have been largely used in the industry (Marques, 2001). Lauraceae comprises about 55 genera and over 2000 species mostly found in tropical, subtropical and mild temperate regions (Takaku et al., 2007). Due to their commercial importance, some Lauraceae species have already been studied regarding their essential oil contents and biological activities, however there are still many neglected species regarding their chemical composition.

Ocotea is one of the largest genera in the Neotropics, containing ca. 350 species from which 170 are found in Brazil (Brotto et al., 2013). Among the native Brazilian Ocotea species, O. odorifera (Vell.) Rohwer is a tree found in the Atlantic Rainforest, and it is popularly known as 'canela-sassafrás, producing a highly valued essential oil by the cosmetic and pharmaceutical industries due to the high concentration of safrole. The commercial importance had led this species to near extinction and currently it is federally protected **??**IBAMA, 1992). On the other hand, there are also some species that have not yet been chemically or biologically studied, such as O. indecora (Schott) Mez., 'canela-cheirosa', whose barks are commonly used in traditional medicine as sudorific, antirheumatic and anti-syphilic (Marques, 2001). Although it is a native species widely distributed along the Southeastern and Southern Atlantic Rainforest (Brotto et al., 2013), there is only one previous report on the leaf essential oil composition of O. indecora (Gonçalves et al., 2018). To the best of our knowledge, there is no previous study about the biological properties of this species.

As a part of a research on aromatic species of the Brazilian Atlantic Rainforest aiming to aggregate value to them, in order to increase the interest in their sustainable use. The present study deals with the chemical analysis of the essential oils obtained from leaves and stems of Ocotea odorifera, O. indecora, and Persea Mill. is known as the oldest Lauraceae genus (Scora and Bergh, 1992). It is typically represented by the avocado (P. americana Mill.), the most important edible species within the genus. The Neotropical Persea species are distributed from Brazil and Chile in South America to Central America and Mexico (Moraes et al., 2014). In Brazil, about 30 Persea species are found dispersed among the biomes Amazon, Cerrado and Atlantic Rainforest (Flora do Brasil 2020, 2017). P. venosa Nees & Mart. is a native Brazilian species, popularly known as 'pau-de-andrade' and 'canela-sebo', it is found in Minas Gerais, São Paulo, Paraná and Santa Catarina (Flora do Brasil 2020, 2017). This species is also used in the traditional medicine for treating wounds and skin ulcers (Mazza, 2000). Although it is a rare species, the tree was extensively harvested for its wood which has put it in a high threat of extinction (Biodiversitas, 2019). P. venosa. The oils' biological activity was assessed by evaluating their antioxidant capacity and cytotoxic activity.

2 II.

3 Materials and Methods

4 a) Chemicals and cell lines

The linear alkane mixture (C 6 -C 40), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethylsulfoxide (DMSO) and methanol were obtained from Merck (Darmstadt, Germany).

Human colon (HCT-116) and breast (MCF-7) adenocarcinoma cell lines were cultivated in DMEM/F12 (SK-Mel-19) or DMEM Glutamax (RPE) medium with 10% fetal bovine serum (v/v), 2 mmol/L glutamine, 100 U/mL penicillin and 100 ?g/mL streptomycin at 37 °C under 5% CO 2 atmosphere.

5 b) Plant Material

Leaves and stems of three species were collected at different areas from Minas Gerais, Brazil along Atlantic Rainforest areas, the specific collection sites are presented in Table 1. The plant materials were (Instituto de Botânica, São Paulo, Brazil). Voucher specimens were deposited in the Herbarium of the same institution. The leaves and stems were separated and dried at room temperature. The dried stems were pulverized in a hammer mill. The extractions were carried for 4 h and the oils were dried over anhydrous sodium sulfate and stored in a freezer (-20 °C) until further use. The essential oil yields were calculated based on the dry weight of each sample (Table **??**).

6 ii. GC-MS analysis

Essential oil samples were dissolved in acetone (0.1% v/v) and injected(1.0 µL) in a gas chromatograph Agilent 6890 Series GC apparatus (Agilent, Santa Clara, CA, USA) with a fused silica capillary column (DB-5, 30 m x 0.25 mm i.d. x 0.25 ?m film thickness) hyphenated in an electron ionization system 5973 quadrupole MS detector (Agilent, Santa Clara, CA, USA) operating at 70 eV, with a detector temperature of 250 °C, scan time of 0.1 scans/s, acquisition mass range of m/z 35 -500 and using helium as carrier gas (1 mL/min). For the chromatographic run, the injector temperature was set at 250 °C and the oven temperature was programmed to run from 40 °C (1 min) to 240 °C at 3 °C/min. The essential oil components were identified by comparing their retention indices (RI), calculated in relation to a series of n-alkanes (C 6 -C 40) and by comparison of their mass spectra with those reported in the literature (Adams, 2007; NIST).

7 d) Biological Assays i. DPPH Radical Scavenging Assay

The antioxidant assay by the DPPH method was performed as described by **??**achado et al. (2017) with some modifications. Essential oils were tested at final methanol. Briefly, in a 96-well microplate was added 160 ?L of DPPH methanol solution (0.08 mg/mL) and 40 ?L of sample solution of different concentrations. Methanol was used as blank solution and the control consisted of 160 ?L of DPPH solution plus 40 ?L of methanol. After 30 min of incubation in the dark at room temperature, the decrease in the absorbance was measured at 517 nm using a multi-well scanning spectrophotometer (Synergy HT Biotek, Winooski, VT, USA). The radical scavenging activity was calculated using the equation [(Abs control -Abs sample)/(Abs control -Abs blank)] x 100. The IC 50 value was calculated by non-linear regression (GraphPad Prism 5.01). The experiment included triplicates for each concentration. Quercetin was used as a positive control. The results of the antioxidant activity were presented as mean±SD.

8 ii. Cytotoxicity Assay

The cytotoxic activity of the essential oils was measured by reduction of soluble MTT to waterinsoluble formazan, as described by Costa-Lotufo et al. **??**2010). Prior the assay, cells of colon (HCT-116) and breast (MCF-7) tumor lines were seeded into a 96-well microplate at a density of 5 x 10 4 cells/mL per well, separately, and expected to grow for 24h at 37°C under 5% CO 2 atmosphere. The cells were then treated with a final concentration of 50 and 5 ?g/mL of each essential oil for 72 h. DMSO was used as vehicle control and diluent of the essential oils. Following the incubation, 150 ?L of MTT (5 mg/mL) were added to each well and the cells were incubated for additional period of 3h at 37°C. Differences in the cell viability were measured at 595 nm by using a microplate reader (Multiskan FC, The GC/MS analyses of the leaf and stem essential oils from the three species allowed the identification of 82 compounds, accounting for 91.0-100% of the total components (Table 3). Number of components in the oils ranged from 6 in O. odorifera stems to 42 in O. indecora stems.

The key chemical characteristic for leaf and stem essential oils from O. odorifera was the high amounts of safrole, a phenylpropanoid, reaching 57.1 and 88.5%, respectively. In addition to phenylpropanoids, the leaf oil contained still oxygenated sesquiterpenoids (22.7%), sesquiterpene hydrocarbons (3.1%), oxygenated monoterpenoids (1.6%) and monoterpene hydrocarbons (1.6%), where the oxygenated sesquiterpene spathulenol (13.8%) was the second major constituent.

Previous investigation of the leaf oil from O. odorifera revealed safrole contents between 36.3 -42% (Cansian et al, 2010;Mossi et al., 2014; **??**lcoba et al., 2018). The marked differences in the % of safrole could be attributed to many factors as growing stage or extrinsic factors (Sari et al., 2006).

The oils from O. indecora did not contain derivatives, showing that the biosynthetic pathways in this species mainly favored the formation of terpenoids. In the leaf essential oil from O. indecora were identified twenty-five compounds, constituting 93.8% of the sample. Monoterpenoids had a clear predominance in the volatile profile, presenting eighteen compounds that contribute with 53.8% (monoterpene hydrocarbons) and 13.2% (oxygenated monoterpenoids) of the oil, from which ?-pinene (12.8%), ?-pinene (12.4%) and sabinene (11.02%) were the major components. The third most important class in this oil was oxygenated sesquiterpenes, with spathulenol (9.06%) as the most important.

Unlike our results, a recent study on the chemical composition of the leaf essential oil from O. indecora showed that the main component was the sesquiterpene hydrocarbon bicyclogermacrene (29.8%) (Gonçalves et al., 2018), a compound that was not found in our specimen. In the oil of other Ocotea species from Costa Rica, the presence of bicyclogermacrene and other germacrene derivatives were also detected, among them germacrene D, considered apparently common to the Ocotea species from Costa Rica (Takaku et al., 2007). Still, nine compounds could be apparently common in the leaf oil caryophyllene, ?-humulene, germacrene D, ?-cadinene, ?-cadinene and ?-cadinene). In our case, only two of these compounds (?-pinene and ?-pinene) were found in the leaf oil of O. indecora.

showed qualitative similarity in oxygenated sesquiterpenes (leaf, 57.6% and stem, 45.6%) content, that were also the major compound class. The major compounds for both leaf and stem oils were spathulenol (27.8 and 14.7%), humulene epoxide II (11.3 and 5.1%) and caryophyllene oxide (7.6 and 4.8%), respectively. Besides these compounds, the leaf oil still contained sesquiterpene hydrocarbons (26.4%) and oxygenated monoterpenoids (10.3%) and does not contain phenylpropanoids. For the stem oil, besides %) and stems (1.870 %) of O. odorifera and the lowest for the stems of P. venosa (0.003%).

9 Ocotea odorifera Ocotea indecora

10 Persea venosa

Table **??**: Essential oil yields for leaves and stems of Ocotea odorifera, O. indecora and Persea venosa.

The stem oil from O. odorifera did not present mono-and sesquiterpene hydrocarbons, but their oxygenated counterparts were found in lower amounts. The most abundant compounds from those classes were spathulenol (4.2%) and 1, 8-cineole (4.0%).

11 phenylpropanoids, as safrole or other eugenol

Compared to leaf oil, the O. Indecora stem oil high amounts of oxygenated sesquiterpenoids (41.8%), followed by sesquiterpene hydrocarbons (30.9%), with ?-bisabolol (12.2%), ?-cuprenene (5.2%) and ?-eudesmol (4.0%) as the main constituents. from Ocotea spp. (?-pinene, ?-pinene, ?-elemene, ?-

The essential oil of some Persea species have been chemically investigated (Bergh et al., 1973; **??**cora and Scora, 2000), however, this is the first study conducted with the essential oil from P. venosa. The present analysis resulted in the identification of twentytwo and nineteen compounds from the leaf and stem oils, respectively, representing of them 98.8% and 91.0% of the total constituents. Leaf and stem oils of P. venosa

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Antioxidant and Cytotoxic Properties of Essential Oils from Native Brazilian Lauraceae Species phenylpropanoids, no monoterpenoids were found, however this oil presented a high amount of a fatty acid ester, methyl octadecanoate (23.7%). Leaf essential oils from Persea spp. can vary a lot, some species presented a higher concentration of monoterpene hydrocarbons, mostly ?-and ?-pinene, and sesquiterpene hydrocarbons, represented by ?-caryophyllene, while in other taxa phenylpropanoids, such as estragole (methyl chavicol) and (E)-anethole, were the main components (Bergh et al., 1973; **??**cora and Scora, 2000). The free radical scavenging activity for DPPH radical expressed as IC 50 ranged from 0.142 to 10 and stem (0.180 mg/mL) oils from O. indecora were the most active as compared to the other plant essential oilhere studied.

In previous studies evaluating the antioxidant activity of essential oils, using DPPH assay, it was observed that more expressive activities may be related to the presence of compounds containing phenolic groups (Miguel, 2010). Among the studied oils, O. odorifera (leaf and stem) presented eugenol (1.5 and 0.5%, respectively), as the only representative phenolic compound and the phenylpropanoid safrole (57.1 and 88.5%, respectively), that can also form stable radicals. However, the most active oil was O. indecora leaf oil, which was composed mostly by monoterpene hydrocarbons. Comparing the results between the cytotoxic and antioxidant activities, the most active essential oil (O. indecora leaf oil) against the two cell lines also presented the highest DPPH free radical scavenging capacity. Many studies have reported different biological activities for essential oils and their isolated compounds, including cytotoxic activity against tumor cell lines. This activity might be associated with their antioxidant capacity. (Bayala et al., 2014).

For the O. indecora leaf oil, the observed cytotoxicity might be due to the presence of monoterpenes such as ?-pinene and ?-pinene, known for their synergistic association, regarding the cytotoxic effect (Zhang et al., 2015). Some other monoterpenes such as limonene, also present in small concentration in this oil, is pointed as capable to prevent the formation or progression of cancer cells, and it can also cause regression of existing malignant tumors (Crowell, 1999).

Table **??**: Cytotoxic activity of essential oil from Lauraceae on human cell lines HCT-116 (colon adenocarcinoma), MCF-7 (breast cancer).

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Species	Voucher number	Collection site	GPS Localization
	S. Nicolau 3885	Morro Grande, Caldas	21.92° S and 46.39° W
	Cordeiro 3113	Serra do Selado, Poços de Caldas	21.79° S and 46.56° W
	S. Nicolau 3876	Morro Grande, Caldas	21.92° S and 46.39° W

c) Chemical Evaluation

i. Essential oil extraction

Essential oils were obtained from leaves and stems by hydrodistillation using a Clevenger apparatus.

Figure 1: Table 1 :

Fisher Scientific, USA). The inhibition (%) of the cell proliferation was determined using the equation [(1 -Abs sample cells)/(Abs control cells)] x 100. The IC 50 value was calculated by non-linear regression (GraphPad Prism 5.01). The experiment included triplicate for each concentration and two independent assays.

III. Results and Discussion

a) Chemical Evaluation

i. Essential oil characterization

The essential oil yields (% w/w) for the target species varied from 0.003 to 2.790 % (w/w) (Table 2

Ocotea odorifera (Vell.) Rohwer Ocotea indecora (Schott) Mez. Persea venosa Nees & Mart. Plant

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*[Note: * On a dry weight basis.]*

Figure 2: B

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	Compounds	RI lit a	RI b
4-hydroxy-4-methyl-2-pentanone		831	832
Year	?-thujene ?-pinene	924 932 946 962	923
2019	camphene sabinene		929
			945
			971
4	?-pinene myrcene	974 988	972
			988
Volume	?-phellandrene ?-3-carene	1002 1003 1008 1005 1014 1015 1020 1016 1022 102	
XIX	?-terpinene p-cymene		
Issue III	o-cymene limonene		
Version	1,8-cineole ?-terpinene		
I	cis-sabinene hydrate		
	terpinolene linalool		
	terpinen-4-ol ?-terpineol		
	(Z)-safrole		
D D D D	bicycloelemene	1336 1324	
)			
(	?-cubebene	1345 1341	
Medical	eugenol ?-copaene 7-	1356 1346 1374 1371 1390 1383 1408 1413 1411 142	
Re-	epi-sesquithujene (Z)-		
search	caryophyllene ?-cis-		
	bergamotene ?-funebrene		
	?-humulene		
Global	?-trans-farnesene ?-	1454 1448 1464 1473 1471 1474 1479 1476 1479 147	
Journal	acoradiene dauca-5,8-		
of	diene ar-curcumene		
	amorpha-4,7(11)-diene		
	?-himachalene ?-curcumene		
	aristolochene		
	?-selinene	1489 1489	
N.I.: M + 121 (100%), 73 (93%), 107 (52%), 91 (40%)			1489
	2-tridecanone	1495 1491	
	?-patchoulene	1502 1497	
	?-cuprenene	1505 1505	
	?-amorphene	1511 1511	
	trans-calamenene	1521 1515	
	?-dehydro-ar-himachalene	1516 1534	
	(E)-iso-?-bisabolene	1528 1538	
	hedycaryol	1546 1542	

Figure 3: Table 3 :

4

Species	Part used	IC 50 (mg/mL) (M±SD)
	Leaf	0.730±0.048
	Stem	1.670±0.110
	Leaf	0.142±0.002
	Stem	0.180±0.003
	Leaf	>10
	Stem	N.D.
Quercetin		0.010±0.009

[Note: M: average; SD: standard deviation; N.D.: not determined ii.]

Figure 4: Table 4 :

.1 Acknowledgments

The authors are indebted to UNIP for the financial support and the grants offered to the undergraduate students. Authors are also thankful to USP for its support.

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