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

By Fabiana Lima Silva, Amanda Espírito Santo, Paola Cristina Branco, Letícia Veras Costa-Lotufo, Maria Claúdia Marx Young, Cynthia Murakami, Inês Cordeiro, Sueli A. Nicolau, Leticia Megumi Ishibaru & Paulo Roberto H. Moreno

Universidade Paulista

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Fabiana Lima Silva [α], Amanda Espírito Santo [σ], Paola Cristina Branco [ρ], Letícia Veras Costa-Lotufo [ω], Maria Claúdia Marx Young [¥], Cynthia Murakami [§], Inês Cordeiro [χ], Sueli A. Nicolau [ν], Leticia Megumi Ishibaru [θ] & Paulo Roberto H. Moreno [ζ]

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% for leaves and stems, respectively) while for *O. indecora* leaves the major ones were the α-pinene (12.8%) and β-pinene (12.4%) and β-bisabolol (12.2%) in the stems. For the third species, *Persea venosa*, the main components of leaf and stem oils were spathulenol (27.8 and 14.7%, respectively) and humulene epoxide II (11.3 and 5.1%, respectively). The bioactivity results indicated that the leaf essential oil from *O. indecora* was the most active for both radical scavenging activity (IC_{50}=0.142 mg/mL) and cytotoxicity against human tumor cells, with growth inhibitions for the colon tumoral line HCT-116 (100%) and breast cancer cell line MCF-7 (99.2%), at 5 µg/mL concentration. This activity might be related to the presence of α-pinene and β-pinene in the leaf essential oil of *O. indecora*. However, further studies with the isolated compounds are necessary to fully understand these bioactivites.

Keywords: Ocotea odorifera, Ocotea indecora, Persea venosa, essential oil, biological activities.

I. Introduction

Essential 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.

Author α σ: Instituto de Ciências da Saúde, Universidade Paulista, 06542-001, Santana do Parnaíba, SP, Brazil.
e-mail: falimasilva@hotmail.com
Author ρ ω : Instituto de Ciências Biomédicas, Universidade de São Paulo, 05508-900, São Paulo, SP, Brazil.
Author ¥ § χ ν: Instituto de Botânica do Estado de São Paulo, 04301-902, São Paulo, SP, Brazil.
Author θ ζ: Instituto de Química, Universidade de São Paulo, 05508-000, São Paulo, SP, Brazil. e-mail: prmoreno@iq.usp.br

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.

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).

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

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P. venosa. The oils' biological activity was assessed by evaluating their antioxidant capacity and cytotoxic activity.

II. Materials and Methods

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). Dimethyl-sulfoxide (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 µg/mL streptomycin at 37 °C under 5% CO_2 atmosphere.

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 identified by Dr. Inês Cordeiro and Dr. Sueli Nicolau (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.

Table 1: Collection sites of the Lauraceae species and Voucher numbers

Species	Voucher number	Collection site	GPS Localization
Ocotea odorifera (Vell.) Rohwer	S. Nicolau 3885	Morro Grande, Caldas	21.92° S and 46.39° W
Ocotea indecora (Schott) Mez.	Cordeiro 3113	Serra do Selado, Poços de Caldas	21.79° S and 46.56° W
Persea venosa Nees & Mart.	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. 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 2).

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 µm 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).

d) Biological Assays

i. DPPH Radical Scavenging Assay

The antioxidant assay by the DPPH method was performed as described by Machado et al. (2017) with some modifications. Essential oils were tested at final concentrations ranging 33.75-10,000 µg/mL in methanol. Briefly, in a 96-well microplate was added 160 µL of DPPH methanol solution (0.08 mg/mL) and 40 µL of sample solution of different concentrations. Methanol was used as blank solution and the control consisted of 160 µL of DPPH solution plus 40 µL 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.

ii. Cytotoxicity Assay

The cytotoxic activity of the essential oils was measured by reduction of soluble MTT to water-insoluble 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 µg/mL of each essential oil for 72 h. DMSO was used as vehicle control and diluent of the essential oils. Following the incubation, 150 µL 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,

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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). The highest yields were obtained for the leaves (2.790 %) and stems (1.870 %) of *O. odorifera* and the lowest for the stems of *P. venosa* (0.003%).

Table 2: Essential oil yields for leaves and stems of *Ocotea odorifera*, *O. indecora* and *Persea venosa.*

Plant species	Part used	Yield* (w/w %)
Ocotea odorifera	Leaf	2.790
	Stem	1.870
Ocotea indecora	Leaf	0.090
	Stem	0.200
Persea venosa	Leaf	0.280
	Stem	0.003

* On a dry weight basis.

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.

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%).

Previous investigation of the leaf oil from *O. odorifera* revealed safrole contents between 36.3 – 42% (Cansian et al, 2010; Mossi et al., 2014; Alcoba 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 phenylpropanoids, as safrole or other eugenol 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 α-pinene (12.8%), β-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.

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

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 from *Ocotea spp.* (α-pinene, β-pinene, β-elemene, β-caryophyllene, α-humulene, germacrene D, γ-cadinene, δ-cadinene and α-cadinene). In our case, only two of these compounds (α-pinene and β-pinene) were found in the leaf oil of *O. indecora*.

The essential oil of some *Persea* species have been chemically investigated (Bergh et al., 1973; Scora 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 twenty-two 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* 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

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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 α- and β-pinene, and sesquiterpene hydrocarbons, represented by β-caryophyllene, while in other taxa phenylpropanoids, such as estragole (methyl chavicol) and (E)-anethole, were the main components (Bergh et al., 1973; Scora and Scora, 2000).

Table 3: Chemical composition of the essential oils from three Lauraceae species collected in Atlantic Rainforest areas.

Compounds	RI lit[a]	RI[b]	%					
			1	2	3	4	5	6
4-hydroxy-4-methyl-2-pentanone	831	832	1.6	1.8	1.2	-	-	-
α-thujene	924	923	-	-	1.2	-	-	-
α-pinene	932	929	0.9	-	12.8	1.9	-	-
camphene	946	945	-	-	0.6	-	-	-
sabinene	962	971	-	-	11.0	-	-	-
β-pinene	974	972	0.6	-	12.4	0.7	1.3	-
myrcene	988	988	-	-	5.4	-	-	-
α-phellandrene	1002	1003	-	-	1.0	-	-	-
δ-3-carene	1008	1005	-	-	-	3.4	-	-
α-terpinene	1014	1015	-	-	1.3	-	-	-
p-cymene	1020	1016	-	-	-	1.1	-	-
o-cymene	1022	1021	-	-	3.3	1.8	1.6	-
limonene	1024	1026	-	-	2.2	-	-	-
1,8-cineole	1026	1028	1.1	4.0	3.2	1.7	-	-
γ-terpinene	1054	1054	-	-	2.0	-	1.6	-
cis-sabinene hydrate	1065	1066	-	-	0.9	-	-	-
terpinolene	1086	1081	-	-	0.5	-	-	-
linalool	1095	1097	-	0.9	2.6	2.2	3.8	-
terpinen-4-ol	1174	1176	-	-	5.5	1.6	4.9	-
α-terpineol	1186	1190	0.5	-	0.9	0.7	-	-
(Z)-safrole	1285	1292	57.1	88.5	-	-	-	-
bicycloelemene	1336	1324	-	-	0.6	-	-	-
α-cubebene	1345	1341	-	-	-	0.7	-	-
eugenol	1356	1346	1.5	0.5	-	-	-	-
α-copaene	1374	1371	-	-	-	2.3	5.5	1.5
7-epi-sesquithujene	1390	1383	-	-	-	1.9	-	-
(Z)-caryophyllene	1408	1413	-	-	-	0.9	1.9	-
α-cis-bergamotene	1411	1428	-	-	-	3.2	-	-
β-funebrene	1413	1435	-	-	-	1.4	-	-
α-humulene	1452	1448	-	-	-	-	1.6	-
β-trans-farnesene	1454	1448	-	-	-	3.1	-	-
α-acoradiene	1464	1473	-	-	-	3.4	-	-
dauca-5,8-diene	1471	1474	-	-	0.6	-	-	-
ar-curcumene	1479	1476	-	-	-	1.9	-	-
amorpha-4,7(11)-diene	1479	1478	-	-	-	0.7	-	-
γ-himachalene	1481	1483	-	-	-	-	4.7	1.7
γ-curcumene	1481	1488	1.7	-	-	-	-	-
aristolochene	1487	1488	-	-	-	0.7	-	-
β-selinene	1489	1489	-	-	-	-	2.0	-
N.I.: M^+ 121 (100%), 73 (93%), 107 (52%), 91 (40%)		1489	-	-	3.5	-	-	-
2-tridecanone	1495	1491	-	-	-	1.6	-	-
γ-patchoulene	1502	1497	0.6	-	-	-	-	-
α-cuprenene	1505	1505	-	-	-	5.2	-	-
δ-amorphene	1511	1511	-	-	-	1.4	1.8	2.0
trans-calamenene	1521	1515	-	-	-	1.6	-	-
α-dehydro-ar-himachalene	1516	1534	-	-	-	-	1.8	1.7
(E)-iso-γ-bisabolene	1528	1538	-	-	-	2.4	-	-
hedycaryol	1546	1542	-	-	1.7	1.8	-	-

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N.I.: M^+ 79 (100%), 91 (85%), 106 (84%), 93 (78%)		1544	-	-	-	-	1.2	-
N.I.: M^+ 138 (100%), 96 (78%), 109 (73%), 95 (66%)	-	1548	-	-	-	0.6	-	-
β-vetivenene	1554	1554	0.8	-	-	-	-	-
(E)-nerolidol	1561	1561	-	-	-	-	1.3	-
spathulenol	1577	1569	13.8	4.2	9.1	1.9	27.8	14.7
trans-sesquisabinene hydrate	1577	1574	-	-	-	3.3	-	-
N.I.: M^+ 105 (100%), 43 (95%), 91 (91%), 93 (88%)		1577	-	-	-	0.6	-	-
N.I.1: M^+ 91 (100%), 43 (97%), 105, 159 (82%)		1578	-	-	1.6	-	-	-
caryophyllene oxide	1582	1580	-	-	-	-	7.6	4.8
globulol	1590	1596	3.8	-	-	1.6	-	-
viridiflorol	1592	1596	-	-	-	0.9	-	2.6
humulene epoxide II	1608	1601	-	-	-	1.9	11.3	5.1
β-atlantol	1608	1606	0.9	-	-	-	-	-
N.I.: M^+ 93 (100%), 91 (94%), 69 (91%), 119 (82%)		1608	-	-	-	1.0	-	-
tetradecanal	1611	1609	-	-	-	-	-	3.5
isolongifolan-7-α-ol	1618	1613	0.5	-	-	-	-	-
epi-cedrol	1618	1616	-	-	-	-	-	2.1
1,10-di-epi-cubenol	1618	1619	-	-	-	0.9	-	2.9
N.I.: M^+ 119 (100%), 91 (73%), 105 (72%), 161 (70%)		1620	0.7	-	-	-	-	-
N.I.: M^+ 161 (100%), 59 (59%), 119 (57%), 93 (51%)		1622	-	-	-	2.1	-	-
isospathulenol[c]	1627	1625	1.4	-	0.8	-	2.2	3.7
γ-eudesmol	1630	1625	-	-	-	1.5	-	-
muurola-4,10(14)-dien-1-β-ol	1630	1627	-	-	-	-	2.9	-
α-acorenol	1632	1630	-	-	-	3.8	-	-
camphoric acid	1634	1631	-	-	-	-	1.5	-
cis-cadin-4-en-7-ol	1635	1635	-	-	-	0.7	-	-
N.I.: M^+ 159 (100%),105 (79%), 91, 131		1635	0.5	-	-	-	-	-
α-epi-muurolol	1640	1636	1.1	-	1.1	0.6	-	-
hinesol	1640	1639	-	-	-	1.2	-	-
selina-3,11-dien-6- α-ol	1642	1639	0.6	-	-	-	3.5	1.8
α-eudesmol	1652	1647	-	-	-	4.0	-	-
α-cadinol	1652	1648	-	-	-	-	-	4.9
N.I.: M^+ 79 (100%),43 (75%), 80 (64%), 67 (50%)		1653	-	-	-	1.9	-	-
selin-11-en-4-α-ol	1658	1655	0.6	-	-	-	-	-
N.I.: M^+ 95 (100%),69 (97%), 109 (75%), 93 (67%)		1659	-	-	1.1	-	-	-
(E)-10,11-dihydroaltantone	1668	1660	-	-	-	3.8	-	-
β-bisabolol	1674	1666	-	-	-	12.2	-	-
cadalene	1675	1666	-	-	-	-	7.1	3.5
mustakone	1676	1668	-	-	-	-	-	2.9
α-bisabolol	1685	1681	-	-	-	1.5	-	-
N.I.: M^+ 58 (100%),43 (88%), 159 (55%), 71 (47%)		1692	-	-	-	-	-	1.6
2-α-hydroxy-amorpha-4,7(11)-diene	1775	1813	-	-	-	-	1.1	-
N.I.: M^+ 93 (100%),43 (48%), 121 (45%), 80 (31%)		1829	-	-	-	0.9	-	-
N.I.: M^+ 73 (100%),60 (75%), 43 (72%), 41 (67%)		1859	-	-	-	-	-	1.7
phthalic acid, isobutyl octyl ester*	-	2045	-	-	-	-	-	1.7
N.I.: M^+ 73 (100%),60 (78%), 43 (69%), 57 (60%)		2057	-	-	-	-	-	2.5
methyl octadecanoate	2124	2174	-	-	-	-	-	23.7
tricosane	2300	2307	-	-	-	-	-	1.9
N.I.1: M^+ 67 (100%),81 (93%), 55 (92%), 82 (86%)		2323	-	-	-	-	-	1.4
octacosane	2800	2732	-	-	-	-	-	5.8
nonacosane	2900	2936	-	-	-	3.4	-	-
triacontane	3000	2975	9.6	-	11.6	-	-	-
Total identified (%)			*98.8*	*100.0*	*93.8*	*92.8*	*98.8*	*91.1*
Monoterpene hydrocarbons			1.6	-	53.8	8.9	4.5	-
Oxygenated monoterpenes			1.6	5.0	13.2	6.2	10.3	-
Sesquiterpene hydrocarbons			3.1	-	1.3	30.9	26.4	10.4
Oxygenated sesquiterpenes			22.7	4.2	12.7	41.8	57.6	45.6
Phenylpropanoids			58.6	89.1	-	-	-	-
Fatty-acid-derived compounds			-	-	-	1.6	-	27.3

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Hydrocarbons			9.6	-	11.6	3.4	-	7.8
Other compounds			1.6	1.8	1.2	-	-	-

1. Ocotea odorifera (leaf); 2. O. odorifera (stem); 3. O. indecora (leaf); 4. O. indecora (stem); 5. Persea venosa (leaf); 6. P. venosa (stem).
*solvent artefact.
RI = Retention indices on DB-5 column: [a]RI literature (Adams, 2007; NIST); [b] Calculated RI.
N.I.: not identified.

b) Biological Assays

i. *DPPH Radical Scavenging Assay*

The free radical scavenging activity for DPPH radical expressed as IC_{50} ranged from 0.142 to 10 mg/mL is shown in the Table 4.The leaf (0.142 mg/mL) 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.

Table 4: Antioxidant activities of the essential oils from Lauraceae species collected in Atlantic Rainforest areas

Species	Part used	IC_{50} (mg/mL) (M±SD)
Ocotea odorifera	Leaf	0.730±0.048
	Stem	1.670±0.110
Ocotea indecora	Leaf	0.142±0.002
	Stem	0.180±0.003
Persea venosa	Leaf	>10
	Stem	N.D.
Quercetin		0.010±0.009

M: average; SD: standard deviation; N.D.: not determined

ii. *Cytotoxicity Assay*

The essential oils' cytotoxic activity was assayed at two concentrations, where their respective growth inhibition percentages against two human tumor lines HCT-116 (colon) and MCF-7 (breast).The results obtained can be seen in Table 5. All essential oils showed activity, varying for HCT-116 from 42.4% (*O. indecora* stem oil) to 100% (*O. indecora* leaf oil) and for MCF-7 from 42.6% (*O. indecora*stem oil) to 99.2% (*O. indecora* leaf oil).Among the tested oils, *O. indecora* stem at the lowest concentration (5 μg/mL) showed low cytotoxic activity against the two cells tested.

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 α-pinene and β-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 5: Cytotoxic activity of essential oil from Lauraceae on human cell lines HCT-116 (colon adenocarcinoma), MCF-7 (breast cancer).

Species		Cell lines			
		HCT-116 (% mortality) (M±SD)		MCF-7 (% mortality) (M±SD)	
		At 50 μg/mL	*At 5 μg/mL*	*At 50 μg/mL*	*At 5 μg/mL*
Ocotea odorifera	Leaf	86.3±6.5	83.6±3.3	93.4±3.6	95.9±2.3
	Stem	95.7±1.9	88.1±1.0	86.1±1.4	89.8±1.3
Ocotea indecora	*Leaf*	*100*	*100*	*97.4±0.3*	*99.2±0.1*
	Stem	89.9±0.4	42.4±0.8	96.6±0.8	42.6±7.3
Persea venosa	Leaf	72.9±1.5	83.4±10.8	98.7±0.1	98.62±0.2
	Stem	86.5±1.1	95.3±0.4	63.2±36.2	98.1±0.1
Doxorubicin (IC_{50}, μg/mL) (C^+)		0.02 (0.02-0.03)		0.16 (0.09-0.29)	

HCT-166: colon tumor line; MCF-7: breast tumor line; M: average; SD: standard deviation; C^+: confidence interval

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IV. Conclusions

Essential oils and their components often exhibit interesting bioactivities useful in the fields of cosmetics, food and pharmaceuticals. For the species evaluated in this work, the leaf and stem essential oils from *Persea venosa* and stems of *O. indecora* were evaluated for the first time, opening the field for further studies of biological activity. In addition, *O. indecora* oil was promising for antioxidant and cytotoxic activities. Further evaluation studies of other biological activities and further cytotoxicity studies should be performed to determine the active compounds of this oil and their mechanisms of action.

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