ORIGINAL ARTICLE

Chemical composition of the Brazilian native *Cinnamomum stenophyllum* (Meisn.) Vattimo-Gil essential oil by GC-qMS and GC × GC-TOFMS, and its cytotoxic activity

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Abstract *Cinnamomum stenophyllum* (Meisn.) Vattimo-Gil (Lauraceae) is a native and vulnerable Brazilian species restricted to the Atlantic Forest. The leaf essential oil obtained by hydrodistillation was characterized for the first time by two-dimensional gas chromatography with time-of-flight mass spectrometry (GC × GC-TOFMS). This analysis resulted in the tentatively identification of 80 compounds, showing the superior performance of this method in comparison to the seven compounds identified by GC–MS. The identified compounds included 8 ketones, 7 monoterpene hydrocarbons, 30 oxygenated monoterpenes, 4 sesquiterpene hydrocarbons and 23 oxygenated sesquiterpenes, showing that the *C. stenophyllum* oil contained mostly oxygenated mono and sesquiterpenes. The oil cytotoxicity was tested against two human cancer cell lines, colon adenocarcinoma (HCT-116) and breast cancer carcinoma (MCF-7), and the non-tumor retinal pigment epithelial cells (RPE) using the colorimetric MTT assay. Both cancer cell lines were sensible to leaf

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essential oil, with $IC_{50} < 20$ μg/mL (HCT, $IC_{50} = 9.95$ μg/mL and MCF-7, $IC_{50} = 16.65$ μg/mL), while there was no cytotoxicity against the non-tumor cells at tested concentrations ($IC_{50} > 50$ μg/mL), suggesting selectivity to cancer cells. The results showed that the *C. stenophyllum* leaf essential oil has a cytotoxic potential, presenting several compounds already known as biologically active against tumor cells.

1. Introduction

The Atlantic Forest, an important biodiversity hotspot, has been experiencing heavy habitat loss over the centuries (Neves et al., 2017). Currently, with only about 11% of the original area remaining in small fragments (less than 100 ha), degradation and deforestation processes are critical, causing a rapid decline of several plant populations (Zwiener et al., 2017). For this reason, prospecting the Atlantic Forest for commercially valued biologically active compounds is very important economically, and it may also encourage the preservation of the species (Felipe et al., 2017).

Lauraceae is one of the five most expressive plant families in the Atlantic Forest, represented by about 55 species and 12 genera (Lima et al., 2012). One of the traits of this family is the production of essential oil, which can be highly valued, such as *Aniba rosiodora* Ducke, whose linalool-rich essential oil is used in fine perfumery (Maia et al., 2007; Cunha and Guedes, 2013), and *O. odorifera* (Vell.) Rohwer, highly valued by their safrole-rich essential oils used in fine chemistry (Costa, 2000).

Cinnamomum Schaeffer, another Lauraceae genus, presents interesting species, such as *C. zeylanicum* Blume, *C. loureiroi* Nees, *C. burmanni* (Nees & T. Nees) Blume and *C. cassia* (L.) J. Presl. These four species are marketed all over the world as the spice cinnamon, with a characteristic aroma given by the cinnamaldehyde found in the bark essential oils (Ranasinghe et al., 2013). The essential oils of few other *Cinnamomum* species have also been studied, presenting compositions varying mainly on the predominance of oxygenated monoterpenes or phenylpropanoids (Yuangzheng et al., 1986; Jantan et al., 2008). The biological properties of the *Cinnamomum* spp. essential oils have been reported, as antimicrobial, antifungal, analgesic, antispasmodic, aphrodisiac, homeostatic, insecticide, anti-inflammatory and antitumor activities (Chao et al., 2005; Jantan et al., 2008; Unlu et al., 2010; Barros et al, 2016). To our knowledge, there are no studies with any of the nine Brazilian *Cinnamomum* species from the Atlantic Rainforest (native and/or endemic).

In this way, *Cinnamomum stenophyllum* (Meisn.) Vattimo-Gil is a species of potential interest. It is a native Brazilian and endemic species, restricted to the Atlantic Forest. *C. stenophyllum* is a vulnerable species, with a small and declining population, due to wood exploitation (Lorenzi, 2002; Quinet et al., 2015). To date, no chemical or biological study has been reported for this species. The only mention was an unsuccessful attempt to extract essential oils from its fresh and dried leaves (Amaral et al., 2017).

Essential oils are complex mixtures that may contain hundreds of compounds, as terpenoids, benzenoids, phenylpropanoids, aliphatic aldehydes, alcohols and others (Bakkali et al., 2008). Because of their pleasant odor, biological activity and/or medicinal properties, the pharmaceutical and cosmetic industries have great interest in determining the composition of unknown essential oils and to evaluate their cosmeceutical and pharmacological activities (Barbieri and Borsotto, 2018).

Nowadays, the comprehensive two-dimension gas chromatography time-of-flight mass spectrometry (GC × GC-TOFMS) is known as one of the most accurate techniques for separation and characterization of essential oil components, especially for extremely complex samples (Lebanov et al., 2019). In contrast with the commonly used GC–MS technique, it employs two columns with independent separation mechanisms, conferring orthogonality to the separation. In addition, the reconcentration of the eluates leaving the first column, through the modulation process, provides several advantages that generates a much more efficient separation of compounds, such as superior chromatographic peak capacity, selectivity and low detection limit for analytical trace compounds (Tranchida et al., 2010; Lebanov et al., 2019).

Considering the importance of Lauraceae for the Atlantic Forest and the lack of studies with these species, the specific interest of this work was to obtain the chemical characterization of the essential oil from *C. stenophyllum* leaves using two chromatographic techniques GC-qMS and GC × GC-TOFMS. In addition, its potential antitumor activity was evaluated against two human cancer cell lines, colon adenocarcinoma (HCT-116) and breast cancer carcinoma (MCF-7), and the non-tumor retinal pigment epithelial cells (RPE).

2. Experimental

2.1. Materials

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Leaves and stems from *Cinnamomum stenophyllum* (Meisn.) Vattimo-Gil were collected at Arujá (23°23′47″S and 46°19′17″W), São Paulo, Brazil, in the Atlantic Rainforest area. The plant material was identified by Dr. Sueli Nicolau (Instituto Botânico, São Paulo, Brazil). Voucher specimen (*Cinammomum stenophyllum* area 615 Arujá) were deposited in the Herbarium of the same institution. The leaves were separated and dried at room temperature until constant weight.

2.2. Oil extraction

Leaf essential oil was obtained by hydrodistillation using a Clevenger-type apparatus. The extraction was carried for 4 h and the oil was dried over anhydrous sodium sulfate and

stored in a freezer (−20 °C) until further use (Machado et al., 2017). The essential oil yield (0.144%, w/v) was calculated based on the dry weight.

2.3. GC-qMS analysis

The leaf essential oil from *C. stenophyllum* was diluted with dichloromethane (1:10 v/v) and a single aliquot of 1 μL was analyzed using an Agilent 6890 Series GC apparatus (Agilent Technologies, Santa Clara, CA, USA) with a fused silica capillary column (DB-5, 5%-phenyl-95%-methylsiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) hyphenated with an electron ionization system 5973 quadrupole MS detector (Agilent Technologies, Santa Clara, CA, USA) operating at 70 eV. The detector temperature was 250 °C, scan time of 0.1 scan/sec and acquisition mass range of *m/z* 35–500. Carrier gas helium (99.999% purity) was used at a flow rate of 1 mL/min. The sample was injected using split less mode. The injector temperature was set at 250 °C and the oven temperature was programmed from 40 °C (1 min) to 240 °C at 3 °C/min. The essential oil components were identified by comparing their retention index (RI), calculated about in a series of *n*-alkanes (C_6-C_{40}), and by comparison of their mass spectra with three commercial libraries: HPCH2205 (Adams, 2007), NIST 08/Wiley (2014). For peak assignment, only peaks with similar mass spectra higher than 80% and RI difference (RI calculated – RI literature) of ±10 unit were tentatively identified (Tranchida et al., 2013).

2.4. GC × GC-TOFMS analysis

For the GC × GC analysis of the leaf essential oil (1 μL) from *C. stenophyllum* a GC × GC-TOFMS system (Model Pegasus 4D, Leco, St. Joseph, MI, USA) was used. This equipment is composed of a gas chromatograph (Model 7890, Agilent Technologies, Palo Alto, CA, USA) equipped with a secondary oven, a non-moving quad-jet and dual-stage cryogenic modulator and a time-of-flight mass spectrometer (TOFMS) (Model Pegasus 4D, Leco, St. Joseph, MI, USA). The primary (1D) and secondary (2D) dimension columns were a non-polar (DB-5) and a short mid-polar (BPX 50, 50%-phenyl-50%-methylsiloxane), respectively. The 2D column and the TOFMS were connected by a 0.5 m × 0.25 mm i.d. uncoated deactivated fused silica capillary via SGE-mini-unions and SilTite[TM] metal ferrules (0.1–0.25 mm i.d.) (SGE, Ringwood, VIC, Australia). A single sample was injected into GC × GC inlet using split mode (1:10). Carrier gas helium (99.9999% purity) was used at a flow rate of 1 mL/min. Modulation was carried out every 6 s, with the duration of the hot pulse of 2.5 s. For the chromatographic run, the primary oven was programmed as 40 °C (1 min), at 3 °C/min to 150 °C, then at 2 °C/min to 270 °C. The secondary oven was maintained at 10 °C above primary oven temperature. Mass spectrometer source and transfer line temperature were kept at 230 and 280 °C, respectively. The electron ionization was at 70 eV and the spectral acquisition rate was of 100 spectrum/s with full scan mode using a mass range of 40–450 Dalton.

GC × GC-TOFMS data acquisition and processing were performed using ChromaTOF® software (4.51.6.0, Leco, St. Joseph, MI, USA). After the data acquisition, the peaks that showed a signal-to-noise ratio above 1000: 1 were selected for processing. The areas and the mass spectra of the individual peaks were obtained. The compound identification was performed using the Mass Spectral Library NIST (NIST MS Search Program version 2.0) and Adams (2007), and the comparison of calculated 1D linear retention indices (related to the same C_6-C_{40} series of n-alkanes used in GC–MS) with literature values. After the analysis, only peaks with similar mass spectra higher than 80% and RI difference (RI calculated – RI literature) of ± 10 unit were tentatively identified (Tranchida et al., 2013).

2.5. Cytotoxic assay

The evaluation of the cytotoxic activity from *C. stenophyllum* essential oil was obtained through the colorimetric MTT assay (Mosmann, 1983; Costa-Lotufo et al., 2010). Three cell lines were used in this study: Colon adenocarcinoma (HCT-116), Breast cancer carcinoma (MCF-7) and the non-tumor retinal pigment epithelial cells (RPE). They were maintained in RPMI 1640 (HCT-116 and RPE) or DMEM Glutamax (MCF-7) medium supplemented with 10% fetal bovine serum (v/v), 2 mmol/L glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were cultured at 37 °C under a 5% CO_2 atmosphere. After incubation, they were seeded into a 96-well microplate at a density of 5×10^4 cells/mL and cultured for 24 h in the proper culture medium. Cells were then treated with different concentrations of each sample and incubated for 72 h. Three hours before the end of treatment, 150 μL of MTT (5 mg/mL) was added into each well and then incubated for an additional 3 h. The absorbance intensity was measured at 595 nm using a multi-well scanning spectrophotometer (Multiskan FC, Fisher Scientific, USA).

The IC_{50} experiments included triplicates for each 5-fold dilution and two independent assays. Final concentrations ranged from 0.002 to 50 μg/mL. Doxorubicin (0.001–5.43 μg/mL) was used as positive control. DMSO served as a negative control. The cell viability was calculated based on the control cells.

3. Results and discussion

3.1. Chemical analysis

Initially, the essential oil was subjected to GC-*q*MS analysis. The GC–MS chromatogram showed a chromatogram with many coelutions resulting in a very low number of compounds identified. Of the 41 detected peaks, only 7 analytes could be identified with the described method, belonging to the following chemical groups: ketone (4-hydroxy-4-methyl-pentanone, **1**), oxygenated monoterpenes (nopinone, **2**; *p*-mentha-1, 5-dien-8-ol, **5** and verbenone, **7**), phenylpropanoid (methyl eugenol, **9**) and oxygenated sesquiterpenes (spathulenol, **17**; mustakone, **33**) (Fig. 1, Table 1). In previous works with the leaf essential oils from Asian *Cinnamomum* species the main constituents identified were phenylpropanoids (eugenol, safrol and cinnamoyl derivatives), benzenoids (benzyl benzoate) and oxygenated monoterpenes (1,8-cineole and camphor) (Simic et al., 2004; Jantan et al., 2004, 2008). Although most of the detected compounds could not be identified in the Brazilian species, no cinnamoyl derivatives were observed. Despite this difference, results obtained with the GC–MS technique pre-
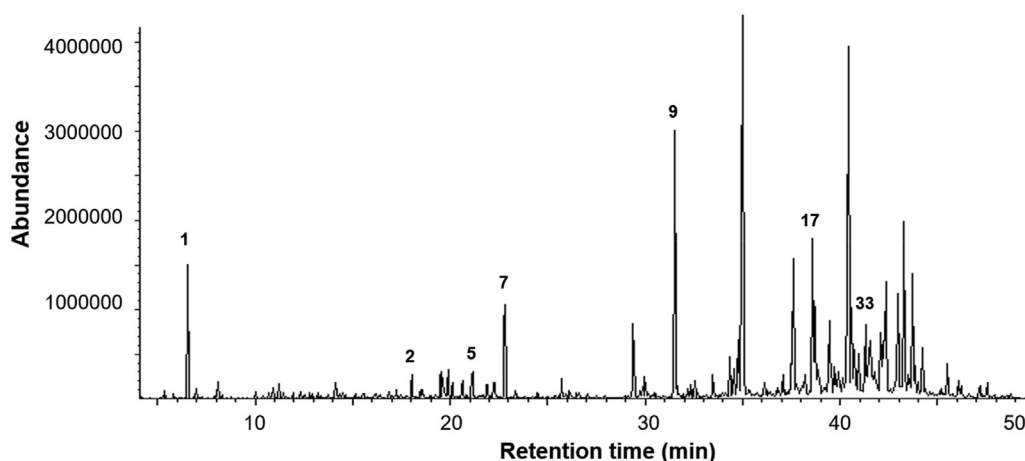
Fig. 1 Chromatographic profile of the *C. stenophyllum* leaf essential oil by GC-*q*MS (see Table 1 for peak assignment).

cluded further consideration, since most of the compounds remained unassigned probably due to the high co-elution rate.

Although separation of essential oil components is mainly accomplished by GC–MS, it is not always easy to obtain a reliable mass spectrum, without mutual interference of other compounds (Han et al., 2018), due to the large number of unresolved peaks, mainly when using quadrupole analyzer and with low resolution mass spectrometer. This fact explains the low rate of identification in the GC–MS analysis (Fig. 1). For this reason, GC × GC-TOFMS technique was used to obtain a more detailed characterization of the *C. stenophyllum* leaf essential oil. The use of two columns, on the basis of volatility (first dimension, DB-5) and polarity (second dimension, BPX 50) increased the chromatographic resolution, allowing a better separation of the analytes. In addition, the modulation used in the GC × GC system provided an increased sensibility and peak capacity, due to the accumulation and rapid release of the compounds (Lebanov et al., 2019).

The structured bidimensional GC × GC chromatogram allowed a better visualization of the compound classes present in the *C. stenophyllum* oil (Fig. 2A), indicating an abundance in oxygenated mono- and sesquiterpenes. Finally, the 3D chromatogram obtained allowed to observe the relative concentrations of the compounds and their better separation (Fig. 2B).

The GC × GC-TOFMS analysis allowed an increase in the number of peaks detected, 213 in total. However, despite the high chromatographic resolution power of the comprehensive two-dimensional gas chromatography, only 80 compounds could be identified (Table 1). There were five main chemical classes identified by GC × GC-TOFMS system in the essential oil: ketones (8 compounds), monoterpene hydrocarbons (7 compounds), oxygenated monoterpenes (30 compounds), sesquiterpene hydrocarbons (4 compounds) and oxygenated sesquiterpenes (23 compounds) (Table 1).

It is interesting to note that, due to the increase of the GC × GC chromatographic resolution, α-terpineol (**43**) was identified with 93% of similarity while in the one-dimensional GC analysis it was assigned with 58% of similarity. Background interferences makes GC–MS analysis less reliable. Furthermore, some compounds, myrtenol (**44**, ^1t_R 1680 s, ^2t_R 2.63 s) and myrtenal (**45**, ^1t_R 1680 s, ^2t_R 2.91 s) for example, were resolved only in the second dimension (Fig. 3).

Despite the better resolution and many compounds detected and identified, there was still some compound coelution, where many of them could be resolved using the mass spectra deconvolution. In the present case, a particularly problematic coelution was observed in the elution region for oxygenated sesquiterpenes (Fig. 4). These compounds are known to have identical or nearly identical mass spectra (Shellie et al., 2004; Zoccali et al., 2015). As displayed in Fig. 4, GC × GC-TOFMS analysis showed that three compounds overlapped in the first dimension and even after modulation and the second-dimension elution these peaks were not resolved. With the mass spectra deconvolution, the identification of the oxygenated sesquiterpenes was still not clear due to the similarities in the MS spectra of **146** and **148**. Therefore, the individual tentatively identification of 10-epi-γ-eudesmol (**146**), selin-6 -en-4-ol (**147**) and γ-eudesmol (**148**) was possible only by comparing their RI with libraries reference data.

The composition of the essential oils from Asian *Cinnamomum* species has been widely studied, but to our knowledge this is the first analysis of a native Brazilian one. For those species [*C. zeylanicum*, *C. cassia*, *C. tamala* (Buch.-Ham.) T.Nees & Eberm., *C. burmannii*, *C. pauciflorum* Nees, *C. rhynchophyllum* Miq., *C. cordatum* Kosterm., *C. microphyllum* Ridl., *C. scortechinii* Gamble, *C. pubescens* Kochummen, *C. impressicostatum* Kosterm., *C. mollissimum* Hook.f., *C. camphora* (L.) J.Presl], the leaf and bark oils contained mainly cinnamoyl derivatives, benzyl benzoates, oxygenated monoterpenes and other phenylpropranoids (Jantan et al., 2008; Wang et al., 2009; Unlu et al., 2010; Jiang et al., 2016; Barros et al, 2016), while in *C. stenophyllum* oil the majority were oxygenated mono and sesquiterpenes.

Considering first the cinnamoyl derivatives, it is interesting to note that *trans*-cinnamaldehyde, cinnamyl alcohol or methyl-cinnamate are found in almost all the leaf oils of the above-mentioned Asian species, with the exception of *C. microphyllum*, *C. mollissimum* and *C. camphora*, in which benzyl-benzoate and camphor were the major compounds (Jantan et al., 2008; Jiang et al., 2016). However, it is important to notice that none of these compounds was found in the *C. stenophyllum* leaf oil. The only neotropical *Cinnamomum* species studied [*C. amoenum* (Nees & Mart.) Kosterm.] also did not present the cinnamoyl derivatives, benzyl-benzoate and camphor in leaf oil (Maciel et al., 2019).

Table 1 Compounds identified in the *C. stenophyllum* leaf essential oil by GC-qMS and GC × GC-TOFMS.

No. (1D/2D)	Compound	RI lit	Leaf			
			1D GC		GC × GC	
			t_R (s)	RI calc	$^1t_R/^2t_R$ (s)	RI calc
1/1	4-hydroxy-4-methyl-2-pentanone	831	394	828	648/2.24	660
-/2	3-(2-propenyl)-cyclohexene	876	–	–	756/1.59	770
-/3	2,5-dimethyl-3-hexanone	–	–	–	762/1.82	776
-/4	2-heptanone	889	–	–	774/2.09	788
-/5	cumene	924	–	–	870/2.04	886
-/6	α-pinene	932	–	–	900/1.71	898
-/7	camphene	946	–	–	942/1.82	915
-/8	thuja-2,4(10)-diene	953	–	–	954/1.89	918
-/9	6-methyl-2-heptanone	954	–	–	954/2.09	918
-/10	benzaldehyde	961	–	–	978/3.1	927
-/11	2-methyl-1-hepten-6-one	966	–	–	996/2.28	934
-/12	β-pinene	974	–	–	1026/1.88	945
-/13	6-methyl-5-hepten-2-one	981	–	–	1050/2.36	954
-/14	3-p-menthene	984	–	–	1080/2.34	966
-/15	p-cymene	1020	–	–	1164/2.19	998
-/16	1-acetyl-cyclohexene	1023	–	–	1176/2.66	1002
-/17	1,8-cineole	1026	–	–	1188/2.09	1007
-/18	2,2,6-trimethyl-cyclohexanone	1036	–	–	1194/2.35	1009
-/23	camphenilone	1078	–	–	1344/2.84	1066
-/24	nonanal	1100	–	–	1404/2.21	1089
-/25	α-fenchocamphorone	1104	–	–	1410/2.99	1090
-/27	dehydro-sabina ketone	1117	–	–	1452/3.12	1107
2/28	nopinone	1135	1171	1133	1506/3.11	1128
-/30	trans-pinocarveol	1135	–	–	1512/2.58	1129
-/32	trans-sabinol	1137	–	–	1524/2.54	1134
-/34	isomenthone	1158	–	–	1554/2.46	1145
-/35	trans-pinocamphone	1158	–	–	1572/2.71	1152
-/36	cis-dihydro-α-terpineol	1160	–	–	1578/2.39	1154
-/38	pinocarvone	1160	–	–	1578/2.85	1154
-/39	borneol	1165	–	–	1590/2.60	1159
5/40	p-mentha-1,5-dien-8-ol	1166	1269	1167	1590/2.67	1159
-/41	p-cymen-8-ol	1179	–	–	1644/2.89	1179
-/42	p-methyl-acetophenone	1179	–	–	1644/3.25	1179
-/43	α-terpineol	1186	–	–	1662/2.54	1186
-/44	myrtenol	1194	–	–	1680/2.63	1193
-/45	myrtenal	1195	–	–	1680/2.91	1193
-/46	decanal	1203	–	–	1698/2.22	1200
7/47	verbenone	1204	1368	1201	1716/3.16	1207
-/48	trans-carveol	1215	–	–	1740/2.66	1216
-/50	carvone	1239	–	–	1812/2.91	1243
-/51	nonanoic acid	1272	–	–	1878/2.30	1268
-/52	dihydro-linalool acetate	1272	–	–	1878/2.58	1268
-/57	carvacrol	1298	–	–	1968/2.85	1302
-/59	neoiso-dihydrocarveol acetate	1356	–	–	2136/2.34	1366
-/61	eugenol	1356	–	–	2154/3.21	1373
-/63	(E)-β-damascenone	1383	–	–	2190/2.74	1386
9/64	methyl eugenol	1403	1892	1396	2238/3.37	1404
-/65	dodecanal	1408	–	–	2250/2.23	1409
-/74	(E)-α-ionone	1428	–	–	2304/2.63	1430
-/76	geranyl acetone	1453	–	–	2358/2.56	1451
-/85	α-amorphene	1483	–	–	2448/2.47	1486
-/87	β-ionone	1487	–	–	2454/2.91	1488
-/88	β-ionone-5,6-epoxide	n	–	–	2454/3.01	1488
-/94	δ-selinene	1492	–	–	2478/2.52	1498
-/100	cis-calamenene	1528	–	–	2562/2.81	1529
-/104	furopelargone A	1538	–	–	2598/2.88	1542
-/107	α-calacorene	1546	–	–	2616/3.02	1549
-/109	elemol	1548	–	–	2628/2.80	1553
-/111	elemicin	1555	–	–	2634/3.73	1555
-/115	longicamphenylone	1562	–	–	2646/3.28	1560

Table 1 (*continued*)

No. (1D/2D)	Compound	RI lit	Leaf			
			1D GC		GC × GC	
			t_R (s)	RI calc	$^1t_R/^2t_R$ (s)	RI calc
17/123	spathulenol	1577	2315	1569	2718/3.16	1587
-/125	caryophyllene oxide	1582	–	–	2730/3.11	1591
-/133	salvial-4(14)-en-1-one	1594	–	–	2760/3.12	1602
-/134	ledol	1602	–	–	2766/2.94	1604
-/142	humulene epoxide III	1608	–	–	2802/3.22	1617
-/146	10-epi-γ-eudesmol	1622	–	–	2826/3.35	1625
-/147	selina-6-en-4-ol	1624	–	–	2832/3.40	1628
-/148	γ-eudesmol	1630	–	–	2832/3.47	1628
-/150	*cis*-cadin-4-en-7-ol	1635	–	–	2850/3.02	1634
-/151	*allo*-aromadendrene epoxide	1639	–	–	2850/3.21	1634
-/154	hinesol	1640	–	–	2862/3.15	1638
-/159	ledene oxide III	1646	–	–	2880/3.24	1645
-/163	β-eudesmol	1649	–	–	2916/3.36	1657
-/165	α-cadinol	1652	–	–	2922/3.25	1660
-/178	8-oxo-neoisolongifolene	n	–	–	2952/3.50	1679
-/179	cadalene	1675	–	–	2982/3.68	1681
33/182	mustakone	1676	2535	1665	2994/3.70	1685
-/191	podocephalol	n	–	–	3054/3.69	1706
-/208	*epi*-cyclo-colarenone	1774	–	–	3288/3.96	1789
-/210	eudesm-11-en-4α,6α-diol	1808	–	–	3402/4.17	1830

t_R (s): retention time for GC–MS (in seconds); ^1t_R (s): first dimension retention time (in seconds); ^2t_R (s): second dimension retention time (in seconds); **RI lit**: retention index of the literature; **RI calc**: retention index calculated; **n**: compounds without retention index of the literature; (–): compounds not found in GC–MS.

In contrast, the number of oxygenated monoterpenes identified for the *C. stenophyllum* oil (30 compounds) by the GC × GC technique was higher than that reported for those species, previously studied only by GC–MS, with only 3–13 compounds within this class (Jantan et al., 2008; Wang et al., 2009; Jiang et al., 2016). Among these, 1,8-cineole, also detected in *C. stenophyllum* oil, was also found in the leaf oils from *C. rhyncophyllum, C. mollissimum, C. camphora* and *C. glanduliferum* (Wall.) Meisn., even as the major component for the latter (Jantan et al., 2008; Singh et al., 2014).

Previous studies on *Cinnamomum* spp. essential oils indicated a smaller variety of oxygenated sesquiterpenes, with only 1–14 compounds for the Asian species (Jantan et al., 2008; Wang et al., 2009; Jiang et al., 2016), compared to the current study where 23 of these compounds were found after the GC × GC-TOFMS. The predominance of oxygenated terpenes in *C. stenophyllum* essential oil suggests cytotoxic effects since these compounds are known for their pro-oxidant effects at the cellular level (Sharma et al., 2013).

3.2. Cytotoxic assay

Cytotoxicity studies have shown that essential oils can be active, generally without causing mutagenicity in the treated organisms (Bakkali et al., 2008). Considering that selective cytotoxicity for tumor cells is a characteristic of interest for potential antitumor agents, the cytotoxic activity of *C. stenophyllum* oil was evaluated for two cancer cells, MCF-7 (breast) and HCT-116 (colon), and compared to that observed using the non-tumor retinal pigment epithelial cells (RPE). The two tumor cell lines are part of our initial panel for investigation of cytotoxic activity because those cells have some characteristics that make them useful as experimental models, little aggressive tumors, noninvasive and low metastatic potential in MCF-7 and the invasive ability and high sensitivity of HCT-116 (Rajput et al., 2008; Comşa et al., 2015).

As shown in Table 2, the leaf essential oil was toxic against the two tumor cell lines, with IC_{50} values lower than 20 μg/mL, threshold to be considered as active (Bézivin et al., 2003). On the other hand, towards non-tumor cells (RPE), the oil presented $IC_{50} > 50$ μg/mL, which demonstrates a selectivity for the cancer cells, i.e. some of its components might be acting in specific metabolic pathways from these cells (Unlu et al., 2010).

C. cassia and *C. zeylanicum* bark oils have been previously described to be cytotoxic against leukemia, colon, prostate, liver cancer among others. However, their mechanism of action is not fully elucidated (Dutta and Chakraborty, 2018). There is strong evidence that *C. zeylanicum* bark essential oil promotes apoptosis in cancer cell lines through different mechanisms. Especially in skin cancer models, the downregulation of EGFR tyrosinase kinase, an important signaling way for the growth and differentiation of the cells, was reported after treatment with this oil (Han and Parker, 2017). The bark extract from *C. cassia,* containing *trans*-cinnamic acid and cinnamaldehyde, decreased levels of tumor-associated growth factors such as EGF, FGF and VEGF, in a mouse melanoma model system (Kwon et al., 2009). Additionally, it promoted
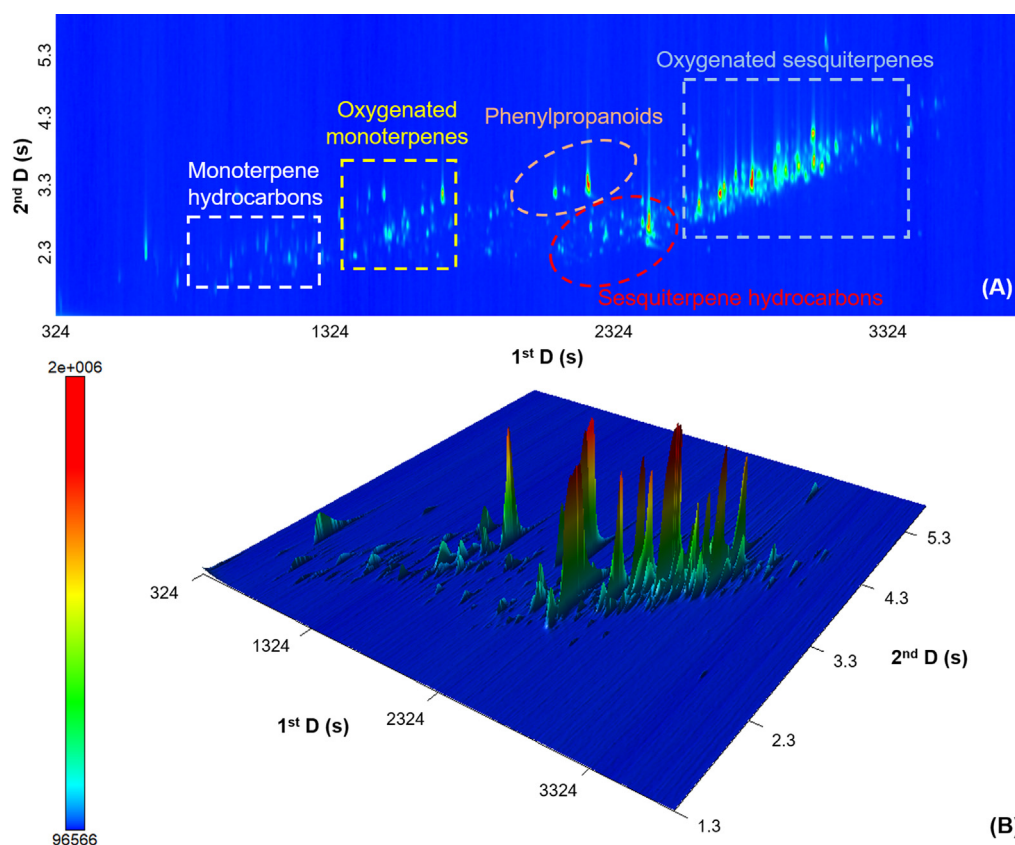
Fig 2 GC × GC-TOFMS total ion chromatogram (TIC) of the *C. stenophyllum* leaf essential oil represented as a topographic map, highlighting the main classes identified (A) and a tridimensional view (B).
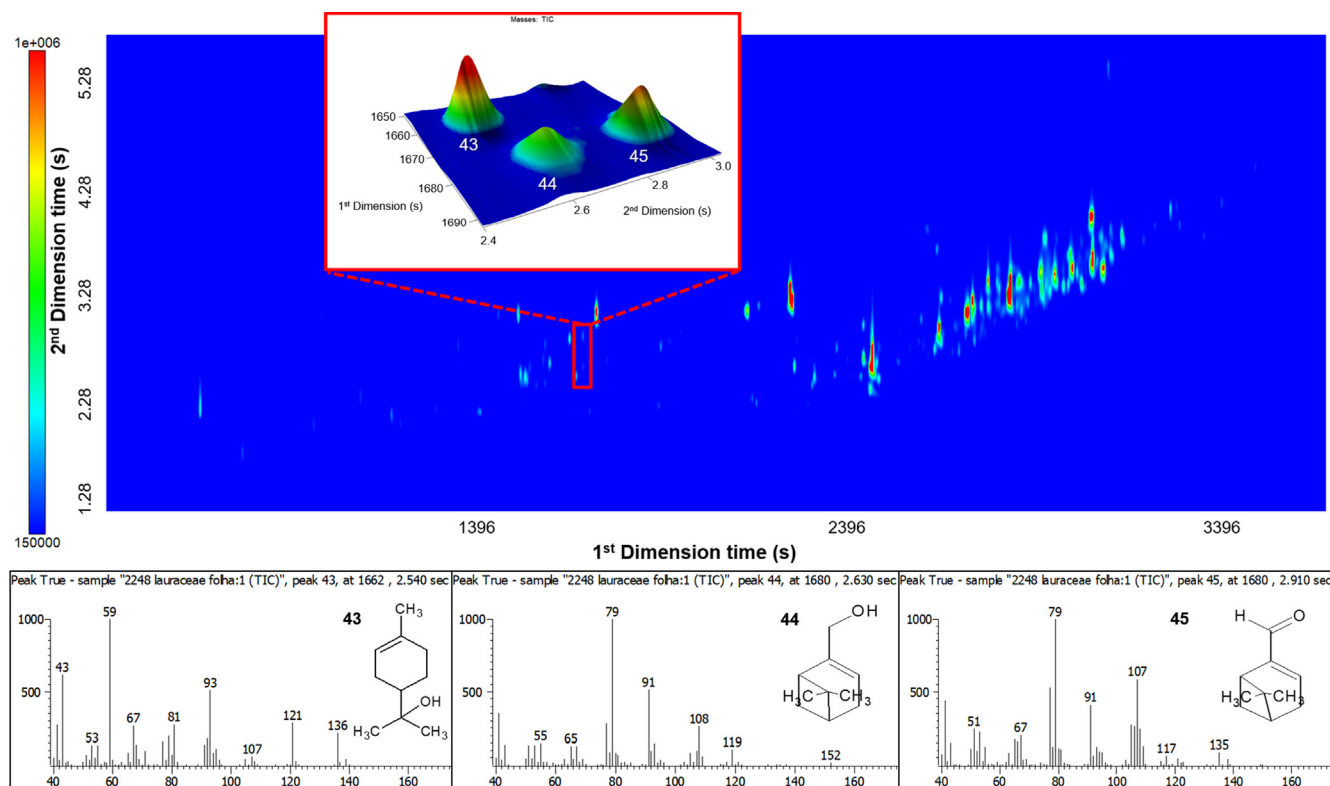
Fig 3 Oxygenated monoterpenes identified in the total ion chromatogram and their deconvoluted mass spectra: α-terpineol (**43**), myrtenol (**44**), and myrtenal (**45**).
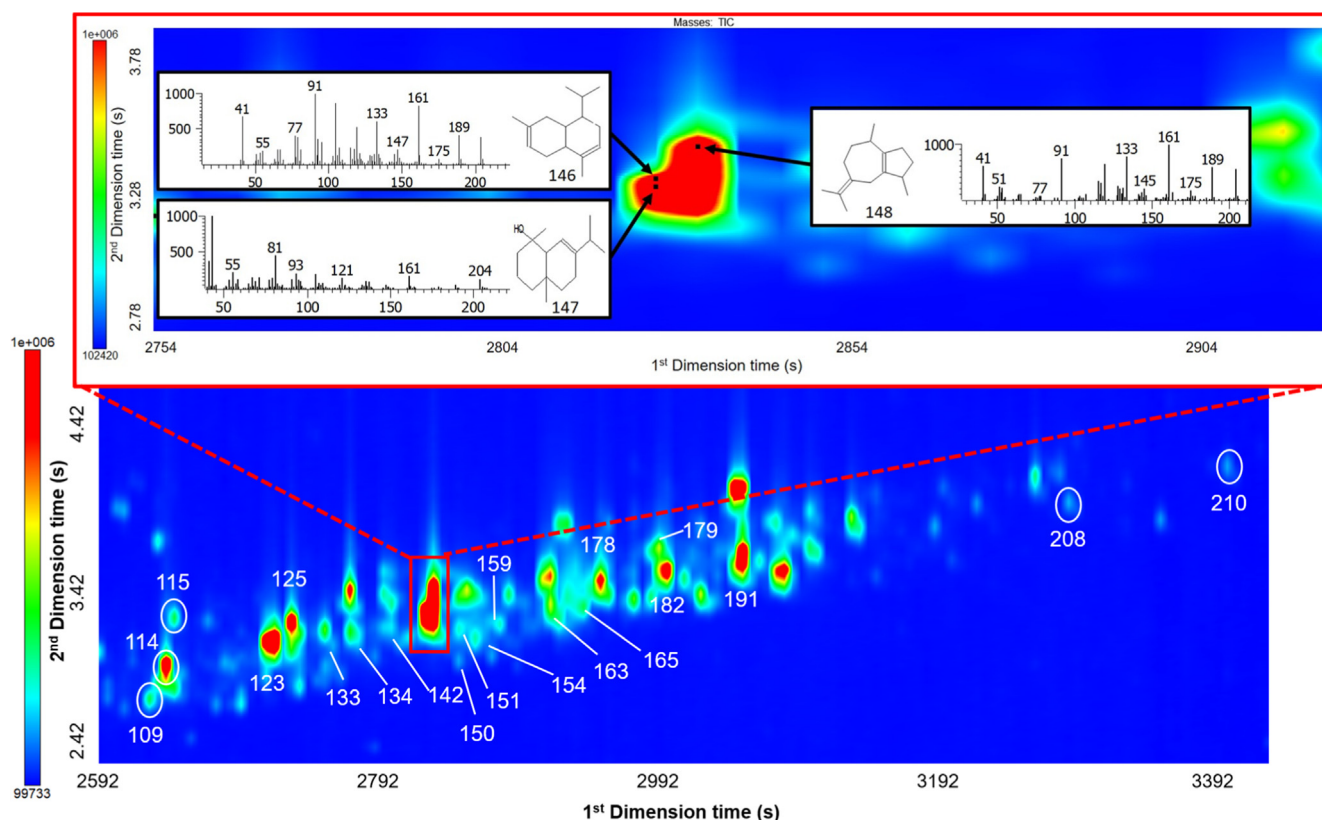
Fig 4 Separation of the oxygenated sesquiterpene region of leaf essential oil from *C. stenophyllum* using GC × GC technique. The amplified region still shows co-elution of compounds for first and second dimensions.

Table 2 Cytotoxic activity of *C. stenophyllum* leaf essential oil on human cell lines HCT-116 (colon adenocarcinoma), MCF-7 (breast cancer) and RPE (retinal epithelium non-tumor cell).

	IC$_{50}$ (µg/mL)		
	HCT-116	MCF-7	RPE
EO	9.95 (N.D.)	16.65 (11.84–23.42)	> 50
Doxorubicin (C$^+$)	0.02 (0.02–0.03)	0.16 (0.09–0.29)	2.31 (0.85–6.29)

EO: leaf essential oil from *C. stenophyllum*. IC$_{50}$: inhibitory concentration of 50%, C$^+$: confidence interval, N.D.: not determined.

inhibition of the factors NFkappaB and AP1, key in tumorigenesis, and their target genes such as Bcl-2, BcL-xL and survivin, anti-apoptotic conductors (Kwon et al., 2010).

This cytotoxic activity of the bark essential oil from *C. zeylanicum* was associated with the presence of some components such as limonene, geraniol and 2'-benzyloxy-cinnamaldehyde (Unlu et al., 2010). Furthermore, cinnamaldehyde, cinnamic acid, 2-hydroxycinnamaldehyde and eugenol were also evaluated in different cancer models displaying cytotoxicity, and their mechanism of action involves apoptosis induction as evidenced by the increased levels of cleaved PARP and cleaved caspase-3. Other mechanisms such as induction Nrf2 activity, DNA damage and ROS production has also been reported for these compounds (Larasati and Meiyanto, 2018).

Although, the abovementioned compounds were not found in the *C. stenophyllum* oil and could not be accounted for the observed cytotoxicity. For instance, some of detected oxygenated monoterpenes have also been implicated with antitumor activities, such as linalyl acetate, α-terpineol, borneol, carvacrol, 1,8-cineole, carvone (Sobral et al., 2014). Additionally, two of the oxygenated sesquiterpenes, β-eudesmol and spathulenol, were also reported as having cytotoxic activity (Bomfim et al., 2016; Kotawong et al., 2018). Thus, the presence of this compounds could explain the observed cytotoxicity for *C. stenophyllum* leaf oil.

4. Conclusion

The present study demonstrated the utility of GC × GC coupled with rapid-scanning TOFMS for profiling complex mixtures of volatile constituents from essential oils. The combination of high chromatographic resolution and the possibility of first dimension coelutions to be resolved in the second dimension were the main improvements, allowing a more comprehensive analysis of the oil. The number of identified compounds increased from 8, by 1D-GC, to 80 using the 2D analysis. The *C. stenophyllum* essential oil was composed mostly of oxygenated monoterpenes and sesquiterpenes, is distinct from other *Cinnamomum* species, such as *C. zeylanicum* and *C. cassia*, in which the oils contained mainly cinnamoyl derivatives and other phenylpropanoids. Our results clearly show that this essential oil is selective against both tumor cell lines tested (HCT and MCF-7) when compared to the

non-tumor RPE cells. These cytotoxic properties could be explained, by the high amounts of oxygenated mono- and sesquiterpenes, which have been reported as cytotoxically active in previous studies. However, further studies with the isolated compounds are necessary to fully understand its bioactivity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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