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BIOLOGICAL ACTIVITIES OF EXTRACTS AND SEASONAL VARIATION IN ESSENTIAL OIL COMPOSITION FROM *MYRCIARIA GLAZIOVIANA* (MYRTACEAE)

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ABSTRACT: Extracts from leaves, stems and flower buds of *Myrciaria glazioviana* were analyzed for their antioxidant and anticholinesterase activity. The methanolic extracts showed the best results in reducing power (623.52 - 806.15 mg AA/g of extract), DPPH radical scavenging (IC_{50} = 9.77 - 14.06 µg/mL) and lipid peroxidation inhibition activity (21.30 - 66.80%). These data were correlated to the total phenolic content (270.19 - 351.01 mg GA/g of extract). The dichloromethane extract of leaves exhibited a higher anti-cholinesterase potential (IC_{50} = 249.25 µg/mL). Furthermore, the chemical composition of the essential oils from leaves collected in all seasons of the year was analyzed by GC-MS and GC-FID. Only sesquiterpenes were identified in all essential oils, and non-functionalized sesquiterpenes comprised the major fractions (from 66.8 to 79.3%). An increase in non-functionalized compounds was observed in the warmer seasons. Germacrene B was the major compound in all seasons (from 26.8 to 30.1%). In winter and autumn, high contents of germacrene D (10.2 and 10.0%, respectively) and α-cadinol (12.1 and 12.0%, respectively) were identified; germacrene D (14.0%) was among the major compounds in summer and β-elemene (11.6%), in spring.

INTRODUCTION: The *Myrciaria* genus (Myrtaceae) is a uniquely American genus, occurring from Mexico to Uruguay [1]. In Brazil, it comprises about 30 species [2] and is spread across the Amazon Forest, Caatinga, Cerrado, Atlantic Forest and Pampa [3].

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Species of this genus are rich in phenolic compounds, such as anthocyanins, flavonoids, and tannins, as well as carotenoids, saponins and organic acids [3]. Furthermore, the essential oils (EOs) of *Myrciaria* species seem to be characterized by the presence of monoterpenes and sesquiterpenes. Several biological activities were also reported for this genus, such as antibacterial, antifungal, antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, anticholinesterase, cytotoxic and antigenotoxic [3].

Myrciaria glazioviana (Kiaerskou) G. Barroso ex Sobral is a tree native to Brazil and cultivated due

to its edible fruits [4]; it is popularly known as 'cabeludinha' [5]. Few studies about its chemical constitution and potential biological activities were performed [6], highlighting the isolation of a cytotoxic benzoquinone from its leaves, which was also detected in the fruits [7]. Reactive oxygen species (ROS) are highly reactive, which are generated normally in cells during metabolism and may cause severe oxidative damage to proteins, lipids, enzymes, and DNA, with subsequent tissue injury [8]. ROS have been implicated in the development of cancer, neurodegenerative disorders and inflammation [9]. Natural antioxidant agents, such as phenolic compounds, have attracted much interest because of their ability to scavenge free radicals and several medicinal plants are being investigated for their capacity to inhibit the oxidative processes [10].

Alzheimer's disease is the most common cause of dementia in the aged population. The main symptoms are cognitive decline and mental deterioration, which reflects the progressive loss of neurons from several different regions of the brain, especially in the cholinergic system [11]. Acetylcholine is involved in the signal transfer in the synapses and is usually hydrolyzed, giving choline and acetate in a reaction catalyzed by the enzyme acetylcholinesterase (AChE) [11, 12]. Thus, the main molecular basis of the drugs used up to now is their action as acetylcholinesterase inhibitors [11]. Essential oils (EOs) are complex mixtures of compounds characterized by a strong odor, produced by aromatic plants as secondary metabolites [13] and have several important medicinal properties [13, 14]. It is known that chemical composition and biological activity of the essential oils can be affected by environmental and geographic factors, as well as seasonal and circadian variations [15]. Therefore, the present study describes the antioxidant and anticholinesterase activity of the different extracts from aerial parts of *M. glazioviana* and the chemical composition of EOs from its fresh leaves collected in all seasons of the year.

MATERIALS AND METHODS:

Chemicals: All chemicals were of analytical grade and were acquired from Sigma-Aldrich® (Steinheim, Germany).

Plant Material: The aerial parts of *M. glazioviana* were collected in April 2013 in Florianopolis (Santa Catarina, Brazil) (27°36'09"S, 48°31'31"W) for the biological activities analysis. The leaves used for the oil analysis were collected in July and November 2013, March and May 2014 in the same local. Plant material was identified by Prof. Dr. Daniel Falkenberg (Federal University of Santa Catarina- UFSC). A voucher specimen was deposited at Herbarium FLOR (UFSC) under the number 24.265.

Preparation of the Extracts: The fresh leaves (690 g), flower buds (155 g) and stems (65 g) were extracted separately with dichloromethane (7.6 L, 0.4 L, and 0.3 L, respectively) at room temperature by maceration to 7 days and the extracts were concentrated under reduced pressure. The same procedure was carried out again using methanol (7.0 L, 0.4 L, and 0.3 L, respectively). The process afforded 11.0 g, 0.4 g and 0.14 g to leaves, flower buds and stemmed dichloromethane extracts, and 72.0 g, 2.7 g and 6.7 g to methanolic extracts, respectively.

Isolation of the Essential Oil: Essential oils (EOs) of fresh leaves from *M. glazioviana* were obtained by hydrodistillation for 4 hours in Clevenger-type apparatus. After extraction, the oils were dried with sodium sulfate and stored at low temperature.

GC-MS and GC–FID Analysis: The qualitative analysis was performed by gas chromatography coupled with mass spectrometry (GC-MS) in a Shimadzu® GCMS-QP2010 Plus chromatograph (nonpolar capillary column RTx®-5MS: 30 m x 0.25 mm x 0.25 μm film thickness), and the quantitative analysis by gas chromatography coupled with flame ionization detector (GC-FID) in a Shimadzu® GC-FID2010 chromatograph (non polar capillary column OV®-5:30 m × 0.25 mm × 0.25 μm film thickness). The quantitative analysis was performed in triplicate. The oven temperature program was at 60 °C for 5 min, with an increase of 3 °C per minute until 240 °C, remaining in this temperature for 5 min. Helium was used as carrier gas (flow rate of 1 mL/min). The injector temperature was at 250 °C (1:20 split) and the interface at 280 °C. Mass spectra were obtained at 70 eV.

Identification of the Essential Oil Compounds: Identification of the essential oil components was based upon their retention indexes (in comparison with a homologous series of alkanes from C_8 to C_{19}), and by comparison of these and their mass spectral patterns with those reported in the literature [16] and stored in the MS library NIST 2008 (National Institute for Standards and Technology) database.

The quantitative variation of the essential oils constituents in the seasons was analyzed using descriptive statistics, and the coefficient of variation (CV) was calculated for each compound. The compounds whose concentration could not be measured were considered as traces and for purposes of calculation equal to 0%.

Data for the geographic region were provided by CIRAM/EPAGRI (Florianopolis-Brazil) and used for qualitative correlations between phytochemicals and climatic data.

Determination of Anticholinesterase Activity: The enzymatic activity was measured using the method described by Mata *et al*., (2007) [12]. Briefly, 325 µL of 50 mM Tris-HCl buffer (pH 8), 100 µL of a buffer solution of extracts (1.0 mg/mL in methanol) and 25 µL of AChE solution containing 0.28 U/mL (50 mM Tris-HCl, 0.1% BSA) were incubated for 15 min. Subsequently, 75 µL of a solution of acetylthiocholine iodide (0.023 mg/L in water) and 475 µL of DTNB (3 mM in Tris-HCl) were added and the final mixture incubated for another 30 min at room temperature. The absorbance of the mixture was measured at 405 nm.

A control mixture was prepared, using 100 µL of methanol instead of the sample. Inhibition (%) was calculated as follows: I (%) = 100 – ($A_{sample}/A_{control}$) × 100; where A_{sample} and $A_{control}$ are the absorbance values of the respective terms. Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against the sample solution concentrations. Galantamine was used as positive control.

Determination of Total Phenolic Content: Total phenolic content was determined with Folin-Ciocalteau reagent, based on the method proposed by Anagnostopoulos colaboradores (2006) [17].

First, 5.0 mL of deionized water and 250 µL of Folin-Ciocalteau reagent were added to 500 µL of the extract solutions (1 mg/mL). After 3 min, 1.0 mL of saturated sodium carbonate solution was added and the solution was stored for 1 h. The absorbance was measured at 725 nm. A blank solution was prepared according to the above procedure, without adding the sample. A standard curve was performed using gallic acid ($y = 0.0074x – 0.0844$, $R^2 = 0.9939$.). The total phenolic content was expressed in mg of gallic acid (GA) per g of sample (mg GA/g). Analyses were performed in triplicate.

Determination of Flavonoid Content: The flavonoid content was determined based on the method proposed by Woisky and Salatino (1998) [18], adding 2.5 mL of ethanol and 0.5 mL of $AlCl_3$ 2% solution to 500 µL of the extract solutions (1 mg/mL). After 1 h, the measurement of the absorbance was performed at 415 nm. A blank solution was prepared according to the above procedure, without adding the sample; the negative control was prepared with 0.5 mL of sample, 2.5 mL of ethanol and 0.5 mL of distilled water. A standard curve was performed using quercetin ($y = 0.0074x – 0.0347$, $R^2 = 0.9967$). The flavonoid content was expressed in mg of quercetin (QUE) per g of sample (mg QUE/g). Analyses were performed in triplicate.

Evaluation of Antioxidant Activity:

Determination of DPPH Free Radical Scavenging Activity: The assay for the determination of antioxidant activity using the DPPH free radical is based on the method described by Cavin *et al*., (1998) [19], with slight modifications. Two mL of a DPPH solution (0.004 % in methanol) was added to 1 mL of the test solutions at different concentrations. The absorbance of the solutions was measured at 517 nm, after 30 min. A blank solution (1 mL of test solution + 2 mL methanol) was used to discharge the possible interference of the extract.

Gallic acid was used as positive control and methanol as a negative control. Inhibition of DPPH radical was calculated by the following formula:

DPPH scavenging effect (%) = $(A_0 – A_1/A_0) \times 100$; where A_0 and A_1 are the absorbances of the negative control and the sample, respectively. The antiradical activity was expressed as EC_{50} (µg/mL), and the tests were performed in triplicate.

Determination of the Inhibitory Potential of the Lipid Peroxidation: The inhibition of lipid peroxidation was assessed by the model of β-carotene/linoleic acid [20]. An emulsion was prepared with 3.0 mg of β-carotene, 1.0 mL of chloroform, 45 mg of linoleic acid and 215 mg of Tween-80. The solvent was removed in a rotatory evaporator under 45 ℃ and 6.0 mL of distilled water were added under stirring. The obtained emulsion was then diluted to 100 mL with 0.01 M of hydrogen peroxide. Aliquots of this emulsion (4 mL) were transferred to tubes containing 0.2 mL of the test solutions at 1.0 mg/mL, in methanol. BHT (1.0 mg/mL) and methanol were used as a positive and negative control, respectively.

All tubes were placed in a water bath at 50 °C; absorbances at 470 nm were determined at time zero and every 30 min, until 180 min. A blank emulsion was prepared without β-carotene. The antioxidant activity (inhibition on the lipid peroxidation in percentage - ILP %) was calculated using the formula: ILP (%) = $100 [1 – (A_0 – A_t) / (A_{00} – A_{0t})]$; where A_0 and A_t are the absorbances of the sample at time zero and after 180 min, respectively, and A_{00} and A_{0t} are the absorbances of the negative control at time zero and after 180 min. Analyses were performed in triplicate.

Determination of Iron-Reducing Power: The determination of the reduction potential was based on the method of Price & Butler, proposed by Waterman & Mole (1994) [21], with adaptations. To 100 µL of the test solutions (at 1.0 mg/mL, in methanol), 8.5 mL of deionized water was added. Then, 1 mL of $FeCl_3$ (0.1 M) was added, and after 3 min, 1 mL of potassium ferricyanide (0.08 M) was mixed. The absorbance was measured at 720 nm, after 15 min. A blank assay was performed without the sample. A standard curve using acid ascorbic solutions was performed ($y = 0.0019 x + 0.0698$, $R^2 = 0.9967$). The reduction potential was expressed in mg of ascorbic acid (AA) per g of sample (mg AA/g). Analyses were performed in triplicate.

Statistical Analysis: The data were recorded as means ± standard deviation. Linear regression was performed using Microsoft® Excel 2010.

RESULTS AND DISCUSSION:

Analyses of the Essential Oils: The analyses of the chemical composition from EOs collected in all seasons of the year (between 2013 and 2014) are presented in **Table 1** and the climatic data of this period, in **Table 2**.

TABLE 1: COMPOUNDS IDENTIFIED (%) IN THE ESSENTIAL OIL OF LEAVES FROM *MYRCIARIA GLAZIOVIANA* IN THE FOUR SEASONS OF THE YEAR

S. no.	Compounds	Relative Concentration (%)				RI[a]		CV[d]
		Winter	Spring	Summer	Autumn	E[b]	L[c]	
1	δ-elemene	0.9	0.8	1.3	0.6	1335	1336	32
2	α-copaene	0.9	1.6	1.3	0.5	1374	1374	43
3	β-elemene	6.2	11.6	8.6	4.9	1389	1390	38
4	β-caryophyllene	2.2	3.3	2.8	1.4	1417	1417	33
5	β-copaene	0.2	tr	tr	0.4	1430	1428	133
6	γ-elemene	8.6	9.8	8.8	7.6	1434	1432	11
7	α-humulene	1.9	2.7	2.2	1.5	1452	1452	24
8	Allo-aromadendrene	0.1	tr	tr	0.1	1458	1459	116
9	γ-muurolene	0.4	0.8	0.6	0.5	1478	1474	25
10	Germacrene D	10.2	9.2	14.0	10.0	1484	1479	20
11	β-selinene	0.5	0.5	0.5	0.5	1489	1484	3
12	Trans-muurola-4(14),5-diene	0.2	tr	0.2	0.2	1493	1489	67
13	Bicyclogermacrene	3.6	3.5	4.9	3.7	1500	1494	16
14	α-muurolene	0.4	0.6	0.4	0.4	1500	1498	21
15	Isodaucene	0.3	0.5	0.6	0.6	1500	1502	26
16	γ-cadinene	0.5	-	-	-	1513	1508	-
17	δ-amorphene	-	-	0.2	0.2		1507	3
18	δ-cadinene	2.4	1.5	2.7	2.8	1522	1521	26
19	Zonarene	0.3	0.4	0.3	0.4	1528	1533	11

20	Selina-3,7(11)-diene	0.3	0.4	0.4	0.4	1545	1540	15
21	Elemol	0.2	0.4	-	0.8	1548	1548	56
22	Germacrene B	27.1	26.8	29.5	30.1	1559	1557	6
23	Maaliol	0.4	0.4	0.3	0.4	1566	1566	20
24	Spathulenol	0.2	1.0	tr	0.4	1577	1576	116
25	Globulol	1.6	1.5	1.2	1.6	1,590	1582	13
26	Viridiflorol	2.5	2.0	1.6	2.2	1592	1590	20
27	Guaiol	0.4	0.3	0.3	0.4	1600	1596	17
28	Rosifoliol	0.5	0.3	0.2	0.4	1600	1600	40
29	1-epi-cubenol	0.3	0.3	0.2	0.3	1627	1627	18
30	γ-eudesmol	1.3	0.9	0.8	1.3	1630	1631	23
31	Epi-α-muurolol	6.0	3.6	3.4	5.5	1640	1643	28
32	α-muurolol	0.8	0.7	0.5	0.8	1644	1646	20
33	β-eudesmol	0.4	0.3	0.3	0.4	1649	1649	22
34	α-cadinol	12.1	8.0	6.1	12.0	1652	1654	32
35	Valerianol	tr	0.1	0.2	-	1656	1655	97
36	Eudesm-7(11)-em-4-ol	1.4	2.0	1.2	2.8	1700	1695	39
	Monoterpene hydrocarbons	0.0	0.0	0.0	0.0			0
	Oxygenated monoterpenes	0.0	0.0	0.0	0.0			0
	Total monoterpenes	0.0	0.0	0.0	0.0			0
	Sesquiterpene hydrocarbons	67.2	74.0	79.3	66.8			8
	Oxygenated sesquiterpenes	28.1	21.8	16.3	29.3			25
	Total sesquiterpenes	95.3	95.8	95.6	96.1			0
	TOTAL	95.3	95.8	95.6	96.1			

[a]RI = Retention index; [b]E = Experimental data; [c]L = Adams, 2007; [d]CV = Coefficient of variation in %; tr = Traces, not quantified.

TABLE 2: CLIMATIC CONDITIONS IN THE REGION AND SEASONS OF COLLECTION*

	Winter	Spring	Summer	Autumn
Total rainfall (mm)	490	275	451	447
Number of rainy days	28	29	35	33
Absolute maximum temperature (°C)	33.0	36.8	37.4	31.2
Minimum absolute temperature (°C)	2.1	10.6	13.9	6.2
Average temperature (°C)	18	24	28	21
Average relative humidity (%)	79	77	76	83

* Data obtained from CIRAM/EPAGRI (Florianopolis, Brazil)

No relevant difference in the yields of the EOs was observed along the seasons. The highest yield was obtained in the winter (0.1585%), followed by the autumn (0.1409%), spring (0.1324%) and summer (0.1280%). Thirty-six compounds were identified accounting 95.3, 95.8, 95.6 and 96.1% of the EOs obtained in winter, spring, summer, and autumn, respectively. Just sesquiterpenes were identified in all EOs, and non-functionalized sesquiterpenes comprised the major fractions (from 66.8 to 79.3%).

An interesting variation between the functionalized and non-functionalized compounds was observed, with an increase of the latter in the warmer seasons. This fact differs from that was generally observed in the literature. An increase in functionalized compounds was observed in the warmer seasons in some studies with Myrtaceae, and the predominance of non-functionalized was reported in colder seasons [22, 23]. There was no clear influence of precipitation and humidity in the essential oil composition.

The compounds are mostly characterized by cadinene, germacrene, muurolene, and elemene skeletons, which are common to species of Myrtaceae [24]. Germacrene B was the major compound in all seasons (from 26.8 to 30.1%). Germacrene D (10.2 and 10.0%) and α-cadinol (12.1 and 12.0%) were also identified in high contents, respectively, in winter and autumn; germacrene D (14.0%) was also among the major compounds in summer and β-elemene (11.6%) in spring. The large variation in the relative content of the individual compounds was observed among the seasons. The calculated coefficients of variation (CV) for single compounds in EOs ranged from 3 to 133%. The compounds with higher CV (> 50%) were β-copaene, allo-aromadendrene, spathulenol,

valerianol, trans-muurola-4(14), 5-diene, and elemol. All these compounds are present as minor components, with relative contents under 2%. Furthermore, only γ-cadinene, δ-amorphene, elemol, and valerianol were not present in all seasons.

The composition of the essential oils of some other *Myrciaria* species has been analyzed. Among them, *M. tenella* and *M. cauliflora* also presented sesquiterpenes as major fraction, especially germacrene D, β-caryophyllene, and α-, β-, γ-eudesmol. However, monoterpenes such as α- and β-pinene, myrcene, 1, 8-cineole and limonene were reported to the essential oils as the minor fraction [25-28]. Therefore, the present study represents the first report of the seasonal variation in the essential oil composition from this genus.

Total Phenolic and Flavonoid Content: Results from the assessment of total phenolic and flavonoid contents of the extracts are in **Table 3**. In general, dichloromethane extracts presented higher contents of flavonoids, highlighting the DEL extract with 56.31 ± 3.36 mg QE/g of extract. This fact may suggest that these compounds are present as aglycones from flavones, flavonols, flavanones, and other isoflavone aglycones, with a high degree of methylation [29]. On the other hand, methanolic extracts showed higher phenolic contents, especially MES and MEF extracts, with 351.01 ± 27.66 and 313.16 ± 15.92 mg GA/g, respectively. This fact indicates that besides flavonoids, other phenolic compounds such as phenolic acids and glycosides, hydroxycinnamates and stilbenes may be present.

Phenolic compounds are produced in plants as secondary metabolites *via* the shikimic acid pathway and present redox property. Therefore, among phytochemicals possessing the antioxidant capacity, phenolic compounds belong to the most important groups. Among them, flavonoids are the most numerous and are found throughout the plant kingdom [30]. Some studies report the phenolic, flavonoid and anthocyanin composition of fruits from *Myrciaria* species, with high amounts of these compounds [31].

Analyses of the Antioxidant Activity: The reduction of ferric ions to ferrous ions is indicative of the antioxidant potential. It is directly proportional to the green/blue color produced, due to the reduction of Fe^{3+}/ferricyanide complex to the ferrous (Fe^{2+}) form [32]. The results are expressed in ascorbic acid equivalent. The methanol extracts (especially MES and MEF, with respectively 806.15 ± 15.79 and 798.26 ± 15.26 mg AA/g of extract) showed higher power reduction than the apolar extracts, what was also observed in studies with *M. dubia* [31].

DPPH free radical scavenging assay is useful for rapid analysis of free radical-quenching efficacies of various plant extracts. DPPH is a stable free radical with an unpaired electron with violet color. In the presence of an antioxidant (electron/ hydrogen donor), the electron becomes paired off, and the colour vanishes [32]. Results of the DPPH scavenging activity indicated remarkable activity for the methanolic extracts (EC_{50} = 14.06 ± 0.32, 9.77 ± 1.64 and 13.52 ± 0.81 µg/mL to MEL, MES, and MEF extracts, respectively).

Some previous studies reported the DPPH scavenging potential of *Myrciaria* species. The polar extracts of fruits from *M. cauliflora*, *M. dubia*, and *M. vexator* showed EC_{50} = 19.4, 57.2 and 38.6 µg/mL [33]; a better result was obtained to the methanolic extract of *M. caulifora* fruits (EC_{50} = 6.2 µg/mL) [34]. It has long been recognized that high levels of free radicals may induce direct damage to lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids as glycolipids, phospholipids and cholesterol [35].

Phenolic compounds inhibit the lipid oxidation caused by the chain-propagating lipid peroxyl radicals and ferrous generating enzymatic systems [32]. In the present study, the extracts were tested for its protective effect against peroxyl radical-induced lipid peroxidation on linoleic acid.

The data indicated that MEL and MEF extracts were the most effective, with 61.94 ± 1.23 and 66.80 ± 1.96% of the inhibition values, respectively. However, these extracts were less active than the positive control BHT (93.54 ± 0.56 %). High inhibition (90.6%) of the β-carotene bleaching was found to the polar extract of dry fruits from *M. dubia*[31]; for *M. cauliflora*, the skin of the fruits showed strong antioxidant potential [36].

A positive correlation between the content of phenolic compounds and their antioxidant activities have been demonstrated in some works. In present study, the total phenolic content showed a good correlation with reduction potential (R^2 = 0.9922) and DPPH scavenging activity (R^2 = 0.8398). However, flavonoids content seems not to be correlated with these activities (R^2 = 0.0971 and 0.0003, respectively). Both tests showed poor correlation with inhibition lipid peroxidation (R^2 = 0.0105 and 0.3805 to total phenolic and flavonoid content, respectively); this indicated that other phenolic compounds possessing antioxidant activity are also present in the analyzed extracts.

Analyses of the Anticholinesterase Activity: The acetylcholinesterase inhibition was determined using Ellman's reagent. The colorimetric test is based on the reaction of thiocholine (one of the products of enzymatic hydrolysis of acetylthiocholine) with 5,5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent) forming a yellow product, which was quantified spectrometrically [37]. As summarized in **Table 3**, all of the methanolic extracts exhibited inhibitory activity at the concentration of 1 mg/mL, especially MEL extract, which presented IC_{50} = 367.10 ± 92.26 µg/mL. However, the best inhibitory activity was recorded for the DEL extract, with IC_{50} = 249.25 ± 82.27 µg/mL.

Some compounds present in the extracts may be responsible for this activity. Alkaloids represent the main group of natural compounds with reported acetylcholinesterase activity. Furthermore, some studies have reported this activity to other classes as well, mainly flavonoids, stilbenes, terpenes and xanthones [11, 38]. There is only one report of anticholinesterase activity to *Myrciaria* genus. In this study, essential oils from the flowers and leaves of *M. floribunda* presented IC_{50} = 1583 and 681 µg/ml, respectively. Phytochemical analyses showed monoterpenes as the major compounds in these oils [39].

TABLE 3: RESULTS FOR THE ANTIOXIDANT AND ANTICHOLINESTERASE ASSAYS OF EXTRACTS FROM AERIAL PARTS OF *MYRCIARIA GLAZIOVIANA

	Total flavonoids (mg QE/g)	Total phenol (mg GA/g)	Reducing power (mg AA/g)	DPPH (EC_{50} – µg/mL)	Lipid peroxidation (% inhibition)	AchE (IC_{50} – µg/mL)
DEL	56.31 ± 3.36	133.16 ± 4.14	307.91 ± 21.89	199.51 ± 9.53	18.05 ± 0.40	249.25 ± 82.27
MEL	8.04 ± 1.99	270.19 ± 11.91	623.52 ± 23.45	14.06 ± 0.32	61.94 ± 1.23	367.10 ± 92.26
DES	9.78 ± 3.72	34.82 ± 5.12	34.43 ± 5.77	> 1000	49.78 ± 1.09	> 1000
MES	7.62 ± 3.29	351.01 ± 27.66	806.15 ± 15.79	9.77 ± 1.64	21.30 ± 0.43	562.19 ± 91.46
DEF	18.38 ± 2.51	32.57 ± 2.30	6.19 ± 1.03	> 1000	-	> 1000
MEF	8.61 ± 0.23	313.16 ± 15.92	798.26 ± 15.26	13.52 ± 0.81	66.80 ± 1.96	886.40 ± 54.97
Gallic acid	-	-	-	3.80 ± 0.04	-	-
BHT	-	-	-	-	93.54 ± 0.56	-
Galantamine	-	-	-	-	-	2.12 ± 0.01

* Experiments were performed in triplicate and expressed as mean ± standard deviation. DEL = dichloromethanic extract from leaves; MEL = methanolic extract from leaves; DES = dichloromethanic extract from stems; MES = methanolic extract from stems; DEF = dichloromethanic extract from flower buds; MEF = methanolic extract from flower buds

CONCLUSION: The extracts from aerial parts of *M. glazioviana* showed antioxidant and anticholinesterase activities. The high antioxidant activity of the methanolic extracts suggests *M. glazioviana* as a potential source of antioxidants, as well as natural phenolic compounds. Furthermore, the dichloromethane extract from leaves exhibited promising anticholinesterase activity, constituting the first report of anticholinesterase activity to extracts from *Myrciaria* genus. The chemical composition of the essential oil of leaves of *M. glazioviana* varied quantitatively throughout the year. The temperature seems to interfere in the quali-quantitative composition of EOs; sesquiterpenes were predominant with an increase of non-functionalized ones in the warmer seasons.

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CONFLICT OF INTEREST: Nil

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