

Chemical composition, phytotoxic potential, biological activities and antioxidant properties of *Myrcia hatschbachii* D. Legrand essential oil

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Myrcia hatschbachii D. Legrand (Myrtaceae) is a native and endemic species from Brazil. This study investigated the essential oil composition, phytotoxic potential, *in vitro* toxicity, antioxidant properties and antibacterial activity of species. Chromatographic analysis of essential oil identified trans-calamanene, (E)-caryophyllene and spathulenol as major components. Antioxidant capacity was determined by the DPPH• scavenging method and phosphomolybdenum complex formation assay. Antibacterial activity was evaluated using the Minimal Inhibitory Concentration test, demonstrating that the essential oil was active toward *Enterococcus faecalis* and *Staphylococcus aureus*. Phytotoxic activity of essential oil was analyzed by testing interference on germination and growth of *Lactuca sativa*, demonstrating significant inhibition of the hypocotyls and radicles of seeds. Preliminary toxicity studies were determined using *Artemia salina*, resulting in an LC₅₀ of 409.92 µg/mL, and through hemolytic activity. The results of the phytotoxic activity point to a possible application for *Myrcia hatschbachii* in the development of natural herbicides and the *in vitro* toxicity assays suggests the performance of antitumoral activity tests, having in mind the prospection of antineoplastic drugs.

Keywords: Essential oil. Phytotoxic. Antioxidant. Antibacterial. Hemolysis. *Artemia salina*.

INTRODUCTION

Plants are important sources of biologically active substances and have become industrial models for the development of new drugs, whether of natural origin or chemical synthesis planned from natural products (Barreiro, Bolzani, 2009). Interest in aromatic species has grown steadily in recent years, particularly with regard to essential oils, which are of great economic interest and are widely described in the literature (Stefanello *et al.*, 2011). Essential oils may be alternatives for synthetic compounds used in pharmaceutical formulations, chemistry applications, foods, cosmetics, agricultural implements and cleaning materials.

Myrtaceae is one of the largest families of angiosperms and comprises approximately 140 genus and 5500 species, located on continents in the southern

hemisphere (Retamales, Scharaschkin, 2015). The species of the family are abundant in Brazil and present an economic importance, as a source of fruits, spices, timber and paper, and utilization as a therapeutic agent, folk remedy, and as an ornamental plant (Moresco *et al.*, 2014).

The *Myrcia* genus comprises a large portion of economically useful species of the family. *Myrcia* species are important sources of essential oils and are widely used in folk medicine, primarily for treatment of diabetes, an illness of worldwide importance. Essential oils are produced in leaves, stems, flowers and fruits, and are comprised mainly of sesquiterpenes (Cascaes *et al.*, 2015).

Several studies have highlighted the main properties of essential oils derived from the *Myrcia* species. *Myrcia ovata* leaf essential oil (EO) extracts exert anti-inflammatory (Santos *et al.*, 2014) and larvicidal (Lima *et al.*, 2011) activities. EO extracts of *Myrcia fallax* flowers (Alarcón *et al.*, 2009), *Myrcia myrtillifolia* leaves (Cerqueira *et al.*, 2007), and *Myrcia splendens* stems (Jiménez *et al.*, 2012) exhibit antimicrobial

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activities. EO of *Myrcia tomentosa*, *Myrcia bella*, and *Myrcia lingua* leaves have antioxidant properties (Takao, Imatomi, Gualtieri, 2015). EO of *Myrcia laruotteana* fruits have antiproliferative properties (Stefanello *et al.*, 2011).

The species *Myrcia hatschbachii* D. Legrand is native and endemic to Brazil, with confirmed occurrences in the Southern Region (Sobral *et al.*, 2015). The aim of this study was to investigate the composition of essential oil, and evaluate, for the first time, its phytotoxic potential, toxicity aspects using *in vitro* models, antioxidant properties and antibacterial activity. This species can be cultivated for extraction of essential oil and may have unidentified beneficial properties.

MATERIAL AND METHODS

Plant material

Leaves of *Myrcia hatschbachii* D. Legrand were collected at the Federal University of Parana, Curitiba, Brazil (25° 26' S, 49° 14' W) in autumn (April). Species identification was performed by comparison with the specimen held at the herbarium of the Municipal Botanical Museum of Curitiba, under registry number 72379, by the curator José Tadeu Weidlich Motta.

Essential oil extraction

Plant material was dried at room temperature for 30 days, and 600 g of leaves were extracted using hydrodistillation by water vapor drag for 6 hours using Clevenger apparatus. EO yield was 0.17%.

Analysis of chemical composition of essential oil

Identification of EO components was performed using a gas chromatograph coupled to a mass spectrometer (Shidmazu® CG-EM QP2010 Plus), equipped with a capillary column (RTX-5MS; 30 m × 0.25 mm × 0.25 μm). The instrument was operated in split mode with the injector set at 250 °C. The interphase and ion source were maintained at 250 °C. Scan data were collected across the range of 40-350 m/z. Helium was used as the carrier gas with a constant flow of 1.02 mL/minute. Initial column pressure was 59 KPa, and the programmed temperature rose from 60 °C to 250 °C at a rate of 3 °C/minute. Following data collection, chemical constituents were characterized using the Kovats retention index and comparison to existing literature (Adams, 2007).

Antioxidant activity

Antioxidant activity was evaluated by the DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) scavenging method (adapted from Mensor *et al.*, 2001) and the phosphomolybdenum complex formation assay (adapted from Prieto, Pineda, Aguilar, 1999). For both methods, EO solutions and standards were prepared (ascorbic acid, rutin and butylated hydroxytoluene [BHT]) at 200 μg/mL in methanol, with the addition of polysorbate 80. Data were evaluated by ANOVA and statistical differences between results were determined using Tukey's test (p<0.05).

The following procedure was performed for the DPPH• radical scavenging method. The reaction was performed in a microplate by mixing 142 μL of standard or EO solution with 58 μL of methanolic solution of DPPH•. A blank was prepared to account for background color of the sample and methanol was used as a negative control. The reaction was incubated for 30 minutes in the dark at room temperature. Absorbance (Abs) was measured at 540 nm using a spectrophotometer. Antioxidant activity percentage (AA%) of the EO was calculated as follows: $AA\% = 100 - [(Abs\ sample - Abs\ blank) \times 100] - Abs\ control$.

Phosphomolybdenum complex formation assay was performed as follows. An aqueous reactive solution of sulfuric acid (0.6 mol/L), sodium phosphate (28 mmol/L), and ammonium molybdate (4 mmol/L) was prepared. In test tubes, 0.3 mL of standard or EO solution was pipetted, followed by addition of 3 mL of the aqueous reactive solution. A blank was prepared using the solvents used to prepare samples. The tubes were closed and placed in a water bath at 95 °C for 90 minutes. Following incubation, samples were allowed to equilibrate to room temperature. Absorbance (Abs) was measured at 690 nm using a spectrophotometer. Results were expressed as antioxidant activity (AA%) of the sample compared to ascorbic acid, BHT, and rutin (standards with 100% antioxidant activity) as follows: $AA\% = [(Abs\ sample - Abs\ blank) / (Abs\ standard - Abs\ blank)] \times 100$.

Lethality against brine shrimp

Evaluation of toxicity against brine shrimp (*Artemia salina*) was performed as previously described by Meyer *et al.* (1982). Artificial saline was prepared by dissolving 14.31 g of sea salt in 400 mL of distilled water, with pH between 8.0 and 10.0. Two hundred milligrams of *Artemia salina* eggs were added to the saline solution, and the mixture was maintained at 27 °C to 30 °C, and constantly agitated and illuminated (20 W) for 48 h

to facilitate hatching. EO samples were solubilized in polysorbate 80 and prepared at concentrations of 1000, 750, 500, 250, 100, 50 and 10 $\mu\text{L}/\text{mL}$ in methanol. The positive control, quinidine sulfate, was prepared at the same concentrations. Samples were placed in a laboratory oven at 40 °C to evaporate solvent. Following evaporation, the samples were resuspended in saline solution and 10 nauplii were incubated. Live and dead nauplii were counted after 24 hours. Data were analyzed using the Probit method, using SPSS version 23.0. The lethal concentration, LC_{50} , and the confidence interval at 95% were defined.

Hemolytic activity

In vitro hemolytic activity was performed as previously described by Banerjee *et al.* (2008). Phosphate buffered (PBS) solution, sample solutions and controls were prepared. Sheep red blood cells solution (2% v/v) was prepared in cold PBS. EO samples were taken from a stock solution, in which the oil was solubilized in 50 μL of DMSO (Dimethyl sulfoxide) and 10% methanol in PBS. Dilutions were made in PBS to obtain 250, 500, 750, and 1000 $\mu\text{g}/\text{mL}$ solutions. Triton (1%) in PBS and clean water were used as positive controls. Phytochemical standards were prepared by diluting saponin to the same concentrations as the samples. The reactions for the sample, phytochemical standard, positive controls, and negative controls (solvent dilution) consisted of 200 μL of each respective solution with addition of 200 μL of 2% red blood cells. A blank was prepared to account for the color of the sample by combining 200 μL of each sample solution with 200 μL of PBS. Samples were incubated at 37 °C for 3 hours and centrifuged at 3000 rpm for 5 minutes. The supernatant was transferred to a microplate and absorbance was measured at 540 nm using a spectrophotometer. The percentage of the hemolytic activity was calculated using triton (1%) and clean water as 100%. Data were analyzed by ANOVA and the statistical difference between results was determined using Tukey's test ($p < 0.05$).

Antibacterial activity

Antibacterial activity of EO was evaluated by determining the Minimal Inhibitory Concentration (MIC), using the microdilution method, according to CLSI – Clinical and Laboratory Standards Institute (2008). EO were tested against strains of *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25913, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhimurium*

ATCC 14028. Bacterial suspensions were prepared at 1.0×10^8 UFC/mL in saline solution, corresponding to 0.5 on the McFarland scale, and inoculated in microplates (5 μL) with a final concentration of 10^4 UFC/mL. EO samples were prepared in 5% polysorbate and tested in the range of 1000–3.9 $\mu\text{g}/\text{mL}$. Negative controls were prepared by adding 100 μL of 5% polysorbate solution to 100 μL of Mueller-Hinton Broth (MHB) and 5 μL of the bacterial preparation. The positive control was composed of 100 μL of MHB and 5 μL of bacterial preparation. The sterility control was prepared by combining 100 μL of MHB and 100 μL of EO. Microplates were placed in a bacteriological incubator at 35 °C for 16 to 20 hours. After this period, 20 μL of 0.5% triphenyltetrazolium chloride solution (TTC) was added and the microplates were incubated for 3 hours. MIC results were characterized as follows: good antibacterial activity (up to 100 $\mu\text{g}/\text{mL}$), moderate activity (between 100–500 $\mu\text{g}/\text{mL}$), weak activity (between 500–1000 $\mu\text{g}/\text{mL}$), and inactive (greater than 1000 $\mu\text{g}/\text{mL}$) (Ayres *et al.*, 2008; Santos *et al.*, 2008).

Phytotoxic activity

Phytotoxic activity was evaluated as described by Silva, Overbeck and Soares (2014), in which the influence of EO on germination and growth of *Lactuca sativa* was evaluated. In gerbox boxes, two paper filters were added at the base and one paper filter was placed on the box cover. A stock solution was prepared by addition of polysorbate 80 to EO. From this stock solution, dilutions were made in distilled water to obtain concentrations of 1%, 0.1%, 0.01%, 0.001%. Controls consisted of 1% polysorbate 80 in water. In a laminar flow cabinet, 5 mL of distilled water was added to the paper filter at the base of the gerbox boxes. The boxes were divided into 4 quadrants, each containing 5 lettuce seeds (*Lactuca sativa*, variety Grand Rapids). After addition of seeds, 3 mL of each sample solution was added to the paper filter glued to the box cover. The gerbox boxes were closed and maintained in BOD incubators at 20 ± 5 °C, for 7 days. During germination, daily readings were collected for 7 days with withdrawal of germinated seeds. Growth verifications were made on the last day through measurement of hypocotyl and radicle growth. Mean differences were determined using the Scott-Knott ($p < 0.05$) method.

RESULTS AND DISCUSSION

In the identification of the chemical constituents of EO (Table I) 40 compounds were detected, and among these, 34 terpenes were identified (corresponding to

TABLE I - Chemical composition of essential oil from *Myrcia hatschbachii*

RT	RI _{cal}	RI _{lit}	Compound	Classification	%
5.600	928	932	α -Pinene	Monoterpene	1.46
6.653	967	974	β -Pinene	Monoterpene	1.18
7.017	980	988	Myrcene	Monoterpene	2.48
8.180	1018	1025	β -Phellandrene	Monoterpene	1.18
8.227	1019	1024	Limonene	Monoterpene	0.90
10.533	1083	1095	Linalool	Oxygenated monoterpene	1.91
13.543	1157	1174	Terpinen-4-ol	Oxygenated monoterpene	0.76
14.000	1168	1186	α -Terpineol	Oxygenated monoterpene	3.33
21.517	1342	1348	α -Cubebene	Sesquiterpene	1.01
22.613	1368	1373	α -Ylangene	Sesquiterpene	2.36
23.180	1381	1389	β -Elemene	Sesquiterpene	0.62
24.033	1400	1409	α -Gurjunene	Sesquiterpene	0.42
24.303	1407	1417	(E)-Caryophyllene	Sesquiterpene	10.96
25.667	1440	1452	α -Humulene	Sesquiterpene	0.87
25.967	1447	1458	Allo-aromadendrene	Sesquiterpene	1.49
26.617	1462	1464	9-epi-(E)-Caryophyllene	Sesquiterpene	0.66
26.747	1466	1480	Germacrene D	Sesquiterpene	1.52
26.963	1471	1489	β -Selinene	Sesquiterpene	0.72
27.417	1482	1496	Viridiflorene	Sesquiterpene	2.50
27.597	1486	1500	α -Muurolene	Sesquiterpene	0.76
28.240	1502	1521	trans-Calamenene	Sesquiterpene	19.10
28.480	1508	1511	δ -Amorphene	Sesquiterpene	4.22
28.943	1519	1544	α -Calacorene	Sesquiterpene	0.60
30.260	1552	1577	Spathulenol	Oxygenated sesquiterpene	5.03
30.453	1557	1582	Caryophyllene oxide	Oxygenated sesquiterpene	2.54
30.653	1562	1590	Globulol	Oxygenated sesquiterpene	2.38
30.927	1569	1600	Guaiol	Oxygenated sesquiterpene	3.18
31.053	1572	1595	Cubeban-11-ol	Oxygenated sesquiterpene	1.77
31.383	1580	1602	Ledol	Oxygenated sesquiterpene	2.95
31.957	1595	1618	Junenol	Oxygenated sesquiterpene	0.79
32.357	1605	1627	1-epi-Cubenol	Oxygenated sesquiterpene	1.08
32.793	1616	1640	epi- α -Muurolol	Oxygenated sesquiterpene	3.34
33.133	1625	1651	Pogostol	Oxygenated sesquiterpene	3.40
33.230	1628	1644	α -Muurolol	Oxygenated sesquiterpene	3.59
Monoterpene					7.20
Oxygenated monoterpene					6.00
Sesquiterpene					47.81
Oxygenated sesquiterpene					30.05
Total identified					91.06

RT = Retention time (min), RI_{cal} = Kovats retention index experimentally calculated, RI_{lit} = Kovats retention index (Adams, 2007), % = Composition.

91.06% of identified components present in the EO). Among the 34 identified compounds, 47.81% were sesquiterpenes, 30.05% oxygenated sesquiterpenes, 7.20% monoterpenes and 6.00% oxygenated monoterpenes. Sesquiterpenes are prominent in the majority of species from the *Myrcia* genus (Cerqueira *et al.*, 2009; Rosa *et al.*, 2016, Silva, Uetanabro, Lucchese, 2013).

The major compounds in *Myrcia hatschbachii* EO were the sesquiterpenes trans-calamenene (19.10%), (E)-caryophyllene (10.96%) and spathulenol (5.03%).

Trans-calamenene was also the main constituent of the species *Myrcia obtecta* (Stefanello *et al.*, 2010). (E)-caryophyllene was previously found in leaves of *Myrcia sylvatica* (Rosa *et al.*, 2016), *Myrcia cuprea* (Zoghbi *et al.*, 2003) and *Myrcia salzmännii* (Cerqueira *et al.*, 2009), and is associated with antibacterial (Huang *et al.*, 2012; Lang, Buchbauer, 2012) and anticarcinogenic activities (Alcântara *et al.*, 2010). The oxygenated sesquiterpene, spathulenol, also has antibacterial (Lang and Buchbauer, 2012) and anticarcinogenic activities (Alcântara *et al.*, 2010), and has been found in the species *Myrcia bracteata* and *Myrcia sylvatica* (Zoghbi *et al.*, 2003).

A previous study of composition of EO of fresh leaves from the species *Myrcia hatschbachii*, collected between the months of November and January, was made for comparison to other species within the same genus. The predominant compounds identified were (E)-caryophyllene (23.3%), δ -cadinene (8.1%), and bicyclogermacrene (6.9%) (Limberger *et al.*, 2004). The two latter compounds were not identified in our study.

On the other hand, trans-calamenene (major constituent) has not been reported by Limberger (2004). The variation between the compositions of EO might be related to season and collection site, growth conditions, age of the plant, weather conditions, intensity of the solar radiation, soil composition or genetic variability (Rosa *et al.*, 2016; Sá *et al.*, 2012).

Antioxidant activity as evaluated by the DPPH• radical scavenging method and phosphomolybdenum complex formation assay is summarized in Table II.

Results from the DPPH• radical scavenging method demonstrated that EO was less active than standards. However, due to the fact that this method is more sensitive to polar substances, and that EO has a lipophilic characteristic, composed majoritarily by sesquiterpenes, it was necessary to evaluate antioxidant activity by phosphomolybdenum complex formation assay. EO presented results statistically superior to rutin, which is a flavonoid with known antioxidant properties (Zhang *et al.*, 2016).

The LC₅₀ of EO of *Myrcia hatschbachii* is summarized in Table III.

According to Meyer *et al.* (1982), results are considered significant when LC₅₀ is less than 1000 µg/mL. According to Amarante *et al.* (2011), samples where LC₅₀ is between 100 and 500 µg/mL, such as the analyzed oil, are considered moderately toxic. In a study performed with the species *Myrcia myrtifolia*, EO treatment resulted in an LC₅₀ of 479.16 µg/mL, suggesting potential usage as antimicrobial (Cerqueira *et al.*, 2007).

TABLE II - Antioxidant activity of *Myrcia hatschbachii* essential oil

Method		Essential oil	Ascorbic acid	BHT	Rutin
DPPH• scavenging (%)		9.14 ± 0.33% ^a	96.66 ± 0.45% ^c	93.78 ± 1.08% ^b	94.86 ± 0.66% ^b
Phosphomolybdenum complex (%)	Related to Ascorbic acid	37.32 ± 1.24% ^a	100 ^b	-	-
	Related to BHT	65.09 ± 2.17% ^a	-	100 ^b	-
	Related to Rutin	132.46 ± 4.41% ^b	-	-	100 ^a

BHT = Butylated hydroxytoluene. Results are expressed as average ± standard deviation. Samples classified in the same group do not differ statistically (p < 0.05), as determined by Tukey's test.

TABLE III - Preliminary *in vitro* toxicity against *Artemia salina*

Sample	LC ₅₀ µg/mL (Confidence Limits)	Chi-square test		
		Chi-square	Degrees of freedom	Significance level
Quinidine sulfate	101.03 (59.52–154.92)	14.78	5	0.01
Essential oil	409.92 (353.36–465.23)	0.63	5	0.99

LC₅₀ = Concentration able to kill 50% of brine shrimp.

To evaluate hemolytic activity, the percentage of hemolysis was calculated against 1% triton and clean water, which were used as 100% positive controls. Linear regression was used to determine the concentration necessary to achieve 50% hemolytic activity. The regression equations were as follows: $y = 0.0334x + 19.149$ ($R^2=0.9902$) for 1% triton and $y = 0.0321x + 18.402$ ($R^2=0.9902$) for clean water. The abscissa represents the sample concentration and the ordinate is the percentage of hemolysis. The results of this determination were 923.68 $\mu\text{g/mL}$ for 1% triton and 984.36 $\mu\text{g/mL}$ for clean water. Hemolysis was dose-dependent.

The biological activities shown using *in vitro* toxicity tests against *Artemia salina* and hemolytic activity presented a toxic potential of *Myrcia hatschbachii* EO and indicate an opportunity of research and evaluation of antitumoral capacity.

EO toxicity might be related to properties of its major constituents. Previous studies have shown that (E)-caryophyllene and spathulenol have anticarcinogenic properties through apoptotic signaling and inhibition of tumor cell proliferation (Alcântara *et al.*, 2010). Moreover, the remaining constituents might act synergistically with the major components, influencing biological activity of the oil.

The toxicity assay against *Artemia salina* might be used as a model to evaluate acute oral toxicity of plant extracts. In a study using 20 species, toxicity against *Artemia salina* was compared to toxicity tests made in mice, demonstrating good correlation between the methods *in vitro* and *in vivo* (Parra *et al.*, 2001). This experiment is also an indication of potential anticancer activity, and can be used as a pre-screen to investigate activity against solid tumors. The concentration capable of causing lethality of 50% of the brine shrimp is usually ten times the effective dose of inhibition of the cellular growth in antitumoral tests (McLaughlin, Rogers, Anderson, 1998; Rosa *et al.*, 2016).

In vitro evaluation of hemolytic activity of natural products helps to determine possible damage caused to the membranes of erythrocytes by the constituents of EO. When red blood cells undergo lysis, hemoglobin is released (Sobrinho *et al.*, 2016). Thus, hemolytic activity is considered a preliminary test of toxicity of vegetal samples, especially when evaluating potential therapeutic activities.

Antioxidant activity also showed relevant results for *Myrcia hatschbachii* EO. Compounds with antioxidant capacity are of interest with regard to treatment and prevention of cancers. An example of these compounds is ascorbic acid. At higher concentrations, ascorbic acid might enhance production of ATP and induce apoptosis in tumor cells through a pro-oxidant mechanism (Mata *et al.*, 2016).

Regarding antibacterial activity, *Myrcia hatschbachii* EO treatment resulted in a MIC of 500 $\mu\text{g/mL}$ (moderate activity) for *Enterococcus faecalis* and 1000 $\mu\text{g/mL}$ (weak activity) for *Staphylococcus aureus*. These bacteria are important pathogens in foodborne illnesses (Jesus *et al.*, 2016). For the remaining microorganisms, EO showed MIC results greater than 1000 $\mu\text{g/mL}$. EO of *Myrcia alogenensis* (Silva *et al.*, 2013) and *Myrcia myrtifolia* (Cerqueira *et al.*, 2007) exhibited activity against *Staphylococcus aureus*. In contrast, EO from *Myrcia fallax* (Alarcón *et al.*, 2009) and *Myrcia splendens* (Jiménez *et al.*, 2012) were active against *Staphylococcus aureus* and *Enterococcus faecalis*.

Phytotoxic activity was evaluated by examining the influence of EO of *Myrcia hatschbachii* on germination and growth of *Lactuca sativa* seeds. Regarding the germination results, no statistical differences were observed compared to water and 1% polysorbate controls. However, at the concentration of 1%, EO negatively affected germination speed index (GSI). Results are summarized in Table IV.

TABLE IV - Influence of essential oil of *Myrcia hatschbachii* on GSI and growth of hypocotyls and radicles of *Lactuca sativa*

Control/ Sample	Concentration (% m/v)	GSI		Hypocotyl		Radicle	
		Average	Scott-Knott	Average (mm)	Scott-Knott	Average (mm)	Scott-Knott
Water	-	4.88	b	33.10	c	37.65	c
Polysorbate	-	4.63	b	30.20	c	34.60	c
Essential oil	0.001	5.00	b	26.60	b	30.00	b
	0.01	4.52	b	26.75	b	27.80	b
	0.1	4.46	b	24.85	b	35.20	c
	1	3.33	a	4.95	a	15.35	a

GSI = Germination Speed Index. Samples classified in the same group do not differ significantly ($p < 0.05$), as determined by the Scott Knott test.

Growth results of both hypocotyl and radicle, showed the most expressive activity in 1% preparations. All concentrations of EO tested inhibited growth of the hypocotyl of *Lactuca sativa*. Regarding the radicle, the oil presented more significant results growth inhibition results at the concentration of 1%, but the concentrations 0.001% and 0.01% also presented negative influence according to the statistical analysis, compared to the controls water and polysorbate at 1% in water.

A previous study examining *Myrcia guianensis* EO showed greater potential as an inhibitor of seed germination than radicle or hypocotyl growth in seeds of *Mimosa pudica* and *Senna obtusifolia* (Souza Filho *et al.*, 2006).

Our results showing inhibition of growth of the hypocotyl and radicle of *Lactuca sativa* demonstrate the phytotoxic potential of *Myrcia hatschbachii* and support further investigation of this species for development and production of natural herbicides.

Study of metabolites as allelochemicals has increased recently, focused mainly on production of bioherbicides. These agents might be used as alternatives for synthetic agrochemicals, which are associated with health risks and negative environmental impact. Thus, the search for bioherbicides becomes an alternative for sustainable agriculture with the utilization of medicinal plants at the farming of foods free of agrochemicals. It also contributes to the reduction of costs at the production chain, as they tend to be cheaper than the traditional herbicides (Santiago *et al.*, 2017).

A general assay to predict possible therapeutic or biological activity of plant species would aid in research and development of new products and new drugs (Rosa *et al.*, 2016).

This study demonstrates that EO of *Myrcia hatschbachii* could be used in agriculture for development of bioherbicides based on phytotoxic potential. *In vitro* toxicity tests point to the execution of antitumoral activity tests, searching for prospection of antineoplastic drugs. Finally, these EO also exhibit antibacterial activity against two important foodborne bacteria: *Staphylococcus aureus* and *Enterococcus faecalis*.

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