

CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *Myrcia oblongata* DC AND POTENCIAL ANTIMICROBIAL, ANTIOXIDANT AND ACARICIDAL ACTIVITY AGAINST *Dermanyssus gallinae* (DEGEER, 1778)

COMPOSIÇÃO QUÍMICA DO ÓLEO ESSENCIAL DE *Myrcia oblongata* DC E POTENCIAL ANTIMICROBIANO, ANTIOXIDANTE E ACARICIDA CONTRA *Dermanyssus gallinae* (DEGEER, 1778)

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ABSTRACT: Essential oils have aroused interest in the industrial sector due to the multitude of potential applications, especially with respect their antimicrobial and antioxidant properties, among others. The objective of this study was to determine the chemical composition of the essential oil from the leaves of *Myrcia oblongata* DC by gas chromatography coupled to mass spectrometry (GC-MS). To evaluate the antioxidant potential of the oil by using the free radical capture method with 2,2-diphenyl-1-picryl hydrazyl (DPPH); to test the oil antimicrobial activity using the broth microdilution method; and to evaluate the repellency and fumigant potential of the oil on *Dermanyssus gallinae* (Degeer, 1778). The GC-MS analysis resulted in the identification of 30 oil constituents, with the bulk of the composition identified as caryophyllene oxide (22.03%) and *trans*-verbenol (11.94%). The oil presented moderate antioxidant activity compared to the synthetic antioxidant 2,6-di-tert-butyl-4-hydroxytoluene (BHT). Antimicrobial activity of the essential oil showed an inhibitory activity on Gram-positive bacteria, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus epidermidis* and for the yeast *Candida albicans*, and showed no activity against Gram-negative bacteria. All concentrations of the essential oil used in the fumigation test on *D. gallinae* resulted in mortality below 20%. For the repellency test, significant potential was observed for the concentration of 10%.

KEYWORDS: GC-MS. Caryophyllene oxide. *Dermanyssus gallinae*

INTRODUCTION

The main products of plant origin used in the industrial sector are essential oils. These oils contain compounds of the secondary metabolism and are extracted from various parts of plants, and their chemical composition varies between different species and among anatomical structures (SARTORATTO et al., 2004; OUSSALAH et al., 2007). The essential oils stand out for their antimicrobial and antioxidant properties (MAHMOUDI et al., 2016) in addition to presenting potential pesticide activity against mites that are considered pests (BASER; BUCHBAUER, 2015).

The bioactivity of the essential oils can be exerted by different compounds, notably terpenoids (BURT, 2004) and including hemiterpenes, monoterpenes, or sesquiterpenes in addition to their derivatives (XAVIER et al., 2016). Among the families that concentrate these elements, Myrtaceae (CIPRIANI et al., 2012) has been recognized not only through

investigations of the biological activity of the group but also due to the presence of essential oils (ACIOLE, 2001). This family has a natural distribution in all continents of the Southern Hemisphere, and is comprised of 145 genera and 5,970 species (THE PLANT LIST, 2013). In Brazil, the Myrtaceae occur mainly in the Atlantic forest and are represented by 1,025 species, belonging to 23 genera (SOBRAL et al., 2016).

Some *Myrcia* spp. (Myrtaceae) are used in folk medicine, especially *Myrcia amazonica* in which the leaves are used to treat leukemia (MORS et al., 2000), and *Myrcia bracteata*, used to treat rashes and cases of diarrhea (SIMÕES; SPITZER, 2004; Sá et al., 2012). However, there are few reports in the literature of the bioactivity and chemical composition of the essential oil of *Myrcia oblongata* DC. (CASCAES et al., 2015; SANTANA, 2017).

Despite the lack of reports on the bioavailability of *M. oblongata*, the essential oils of the family have recognized importance

especially for their antimicrobial activity (SIMONETTI et al., 2016). Studies the antimicrobial properties of plant products have increased in the last decade due to the extensive and undue use of antimicrobials. There has been a significant increase in the frequency of resistant bacteria, which were previously known to be sensitive to routinely used drugs. Today, these bacteria are resistant to various drugs available in the market (KASPER; FAUCI, 2015), thus highlighting the importance of research on new compounds.

Another growing agricultural sector in Brazil is poultry farming, in which there are various problems associated with pests (PALERMO, 2015) such as *Dermanyssus gallinae* (Acari: Dermanyssidae), the chicken mite. This parasite can cause losses in poultry production due to its reproductive potential, and as a result producers utilize various types of synthetic products to combat the pest. This causes severe problems including pest resistance, high levels of residues, environmental and human intoxication, and destruction of natural enemies (SOTO et al., 2011). Due to this scenario, the use of plant-based repellents and acaricides deserves attention among alternative methods to conventional chemical pest control (SPARAGANO et al., 2014).

In order to develop new products with antimicrobial, acaricidal and other bioavailability potential, toxicity tests must be carried out to verify the safety of the essential oils; it is for this reason that evaluation of antioxidant activity is also of great importance (ROSA et al., 2016). In addition, the search for new antioxidant agents for use in industries has increased the resistance of pathogenic microorganisms to synthetic products (TEPE et al., 2004; XAVIER et al., 2016).

The objective of this study was to determine the chemical composition of the essential oil from the leaves of *M. oblongata*, to evaluate the antimicrobial and antioxidant activity of the essential oil and to verify its repellent and acaricidal activity against *D. gallinae*.

MATERIAL AND METHODS

Collection, drying and identification of plant material

Leaves of *M. oblongata* were collected from March to June 2016 in the autumn season at the Parque Ecológico Paulo Gorski (24°56'14" to 24°58'17" S, 53°25'14" to 53°27'06" W) in the

municipality of Cascavel, Paraná, Brazil. Species identifications were carried out in the Herbarium of the Universidade Estadual do Oeste do Paraná (UNIOESTE), and the samples were registered under the number UNOP 1816.

The leaves were dried at 40 °C and milled in a Willy-type laboratory grinder to 0.42 mm. The resulting powder was stored in glass containers protected from light at room temperature until extraction of the essential oil (CEYHAN et al., 2012; WEBER et al., 2014).

Essential oil extraction

Following the methodology proposed by Weber et al. (2014), 140 g of the dried *M. oblongata* plant material was added to 1.4 L of distilled water. The solution was placed in Clevenger apparatus following the hydrodistillation technique for approximately 3 hours. The extracted oil was stored in a freezer at 4 °C for later assays.

Gas chromatography coupled to mass spectrometry (GC-MS)

The identification of the compounds present in the essential oil was performed using a FOCUS GS (Thermo Electron) gas chromatograph coupled to a DSQ II mass spectrometer (Thermo Electron) and detector with 70 V electronic ionization impact and a quadrupole mass analyzer. For the chromatographic separation a capillary column of fused silicon DB-5 (internal diameter of 30 m x 0.25 mm, film thickness 0.25 µm) and stationary phase of 5% phenyl: 95% dimethylpolysiloxane was used.

The initial temperature was 50 °C for 2 min with the injector temperature at 250 °C, followed by an increase to 180 °C at 2 °C min⁻¹ then 290 °C at 5 °C min⁻¹. The interface between the GC and MS was maintained at 270 °C, and the ionization source temperature for mass spectrometric analysis was 250 °C. The support gas helium flow was kept constant at 1 mL.min⁻¹. The sample and C7-C28 alkane standards were injected at a separation ratio of 1:25. The compounds were identified by comparison to the retention times in the literature (ADAMS, 2007) and through their retention indices.

Antimicrobial activity

The antimicrobial activity of essential oils was evaluated following methods by Pandini et al. (2015), with some modifications. The following bacteria used were: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25933), *Klebsiella pneumoniae* (ATCC 13883), *Salmonella Enteritidis* (ATCC 13076), *Salmonella Gallinarum* (ATCC 1138),

Staphylococcus epidermidis (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 19433) and *Bacillus subtilis* (CCD-04); the yeast *Candida albicans* (ATCC 10231) was also used. The microorganisms were recovered in brain heart infusion broth (BHI) and incubated for 24 h at 37 °C. The final concentrations of the bacteria were standardized to 1×10^5 CFU.mL⁻¹ in 0.85% saline solution.

The minimum inhibitory concentration (MIC) was determined according to the standards of the Clinical and Laboratory Standards Institute (2007) in 96-well microdilution plates. The essential oil was diluted to a concentration of 7000 µg.mL⁻¹. Briefly, an aliquot of 70 mg essential oil was diluted in 1 mL of methanol (10%). Then, 500 µL solution was homogenized in 4.5 mL of Muller-Hinton broth (MH). Serial dilutions ranged from 7000 to 3.4 µg.mL⁻¹. An aliquot of 10 µL of each microorganism was added to the wells containing 150 µL of MH broth and incubated for 24 h at 37 °C. After the incubation period, 10 µL of 1% triphenyl tetrazolium chloride (CTT) was added to each well and the microplates were incubated for additional three hours. The presence of red staining in the wells was interpreted as the absence of inhibitory effects of the essential oil.

The minimum bactericidal concentration (CBM) was determined by inoculating 10 µL of the solution that was present in each well of the microdilution plates in Petri dishes containing MH agar. The plates were incubated for 24 h at 37 °C. As a positive control, gentamicin (bacteria) and nystatin (*C. albicans*) were used at 30 mg.mL⁻¹. The CIM and CBM were performed in triplicate and classified according to the criteria proposed by Sartoratto et al. (2004) as having low activity (7.000-3.500 µg.mL⁻¹), moderate activity (1.700-875 µg.mL⁻¹), high activity (437.5 to 218.75 µg.mL⁻¹) or very high activity (< 109.375 µg.mL⁻¹).

Antioxidant activity

The antioxidant activity of the essential oils was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method (SCHERER et al, 2009; WEBER et al, 2014.). Briefly, a 0.1 mL aliquot of the 7.000 µg.mL⁻¹ essential oil was treated with 3.9 mL of 50% methanol solution and homogenized in a tube shaker. The sample absorbances were measured in a spectrophotometer at 515 nm. As a negative control, a solution composed of methanol, acetone and water (40 mL of 70% acetone solution, 40 mL of 50% methanol solution and 20 mL distilled water) was added with DPPH. As a positive control, the commercial

synthetic antioxidant butyl hydroxyl toluene (BHT) was used; 50% methanol was used for calibration.

The absorbance of DPPH was determined at concentrations of 34, 64, 100, 134, 166 and 200 µm ($\lambda = 515$ nm) to determine the linear function of the data dispersion (i.e., the DPPH absorbances).

The antioxidant activity was calculated as follows: the DPPH straight line equation was initially calculated (linear function). The sequestration rate of DPPH for essential oil and BHT (positive control) was calculated by the following equation: AA% = [(Abs0 - Abs1) / Abs0] x 100; where Abs0 is the absorbance of the negative control and Abs1 is the absorbance of the sample. The oil concentration required to reduce 50% of the DPPH free radical (IC50) was calculated by the absorbances of the different DPPH concentrations, which generated a linear function. The absorbance values were analyzed by the Chi-square test with a significance level of 0.05. Statistical analyses were performed using the statistical software R® version 3.3.2.

Fumigation test of *M. oblongata* essential oil on *D. gallinae*

The mites were collected in a commercial aviary located in the city of Medianeira, PR, Brazil and transported to the Laboratory of Agricultural Biotechnology at Unioeste in Cascavel. Only the engorged females were separated for the fumigation tests. The mites were used up to 3 days after collection.

The fumigation test was performed according to methodology proposed by Locher et al. (2010) and Tabari et al. (2015). The essential oil was diluted in acetone 10% at four concentrations: 10%, 5%, 2.5% and 1.25%, with five replicates of 20 mites at each concentration. The control negative was performed with distilled water and 10% acetone. As a positive control we used triazophos (Hostathion) (COSTA et al., 2003). Twenty mites were placed in a carton (1 cm x 1 cm) and then in an Eppendorf tube (2 mL) with the cap cut off. The tube opening was closed with voile fabric and tied with elastic. Each tube was placed in a flat glass bottom tube (4 cm x 7 cm) and sealed with parafilm to prevent the loss of volatiles from the oil. Each flat bottom tube contained a piece of Whatman no. 2 filter paper (2 cm x 2 cm) containing 50 µL of the essential oil or control solution. This method prevented direct contact of the mite with the filter paper. The mites were conditioned in a BOD (27 ° ± 2 °C, 14 h photophase and 70% R.H.) room and after 24 h, mortality rates were analyzed. The mites that did not move when touched with a fine-bristle

brush were considered dead. Analysis of variance (ANOVA) was used to test for differences in mortality between treatments groups and controls with a significance level of 0.05, and the Tukey test was used for comparisons of means. Statistical analyses were performed using the statistical software R® version 3.3.2.

M. oblongata essential oil as a repellent for *D. gallinae*

Repellency tests were carried out in a climate-controlled room with a temperature of 23 ± 2 °C and 70% R.H. in dark phase because *D. gallinae* mites present nocturnal habits and during the day remain aggregated and hidden. In order to observe the behavior of mites, a red lightbulb (15 w, 100 v) was used because it does not interfere with vision in arthropods (EIRAS; MAFRA-NETO, 2001).

To evaluate whether the essential oils repelled mites, an experiment was carried out with Y olfactometer (EIRAS ; MAFRA NETO, 2001). The olfactometer consisted of a Y-shaped glass tube (22 × 14 × 2 cm) containing a main tube and two side tubes (arms) forming an angle of 120 ° with the main tube. The olfactometer was operated with a continuous air flow of 3L.min⁻¹, and was previously humidified and filtered with activated charcoal. Pieces of filter paper cut into rectangles (1 x 4 cm) and impregnated with 10 µL of the control (ethanol P.A.) or 10 µL of the essential oil diluted in three different concentrations were placed in the side tubes. Detergent was applied by brushing to the lower end of the main tube to prevent the mites from escaping.

Three essential oil concentrations were evaluated: 10%, 5% and 1%, using P.A. ethanol as solvent. For each oil concentration, 30 replicates were performed with five mites. The behavior of the

mites was observed for 5 minutes. The time needed for mites to reach the source of odor was recorded, and if no mites chose one of the sources, the test was stopped and the mites were discarded. Only the repetitions in which the mites chose one of the sources of odor were considered. Every 5 replicates, the olfactometer was washed, oven dried at 60 °C, and the position of the arm containing the control or repellent was inverted to avoid any influence of the external environment.

The choice of odor source was analyzed statistically using a Chi-square test with a significance level of 0.05. The time the mites took to reach the odor source was analyzed using an independent-samples t-test (p = 0.05). Statistical analyses were performed using the statistical software R® version 3.3.2.

RESULTS AND DISCUSSION

GC-MS

The chemical composition of the essential oil extracted from the leaves of *M. oblongata* revealed 30 compounds, representing 82.63% of the total area of the essential oil of the sample analysed, the main compounds obtained were caryophyllene oxide (22.03%) and the *trans*-verbenol (11.94%). It is important to note that the test of GC-MS is not able to quantify all the compounds present in the essential oil. Caryophyllene oxide is a bicyclic sesquiterpene, whereas *trans*-verbenol is a compound resulting from the bioconversion of α -pinene (SOUZA et al., 2011), the third most abundant compound (6.65%). These compounds are also present in many of the Myrtaceae (LIMBERGER et al., 2004), such as *Myrcia salzmannii* (CERQUEIRA et al., 2009), *Myrcia obtecta*, *Myrcia hatschbachii* and *Myrcia arborescens* (LIMBERGER et al., 2004).

Table 1. Chemical composition of essential oil extracted from *M. oblongata* leaves.

Nº	Compounds	Class of compounds	Tr ^a (min)	Area (%)	I _R ^b	*I _R ^c
1	δ-Pinene	Monoterpene	7.39	6.65	945	942
2	Camphene	Monoterpene	7.98	0.12	962	958
3	2,4-tujadiene	Monoterpene	8.12	0.44	966	959
4	P-cymene	Monoterpene	10.91	0.27	1038	1033
5	Crisantenone	Monoterpene	15.11	0.28	1134	1131
6	α-Campholenal	Oxygenated monoterpene	15.37	0.37	1140	1130
7	L-pinocarveol	Oxygenated monoterpene	15.96	0.98	1153	1154
8	Cis-verbenol	Oxygenated monoterpene	16.04	1.52	1154	1142
9	** <i>Trans</i> -verbenol	Oxygenated	16.21	11.94	1158	1150

		monoterpene				
10	Pinocarvone		16.93	0.52	1173	1168
11	α -Felandren-8-ol	Oxygenated monoterpene	17.44	1.80	1185	1170
12	Mirtenol	Oxygenated monoterpene	18.49	0.75	1208	1202
13	<i>Cis</i> -verbenone	Oxygenated monoterpene	18.96	3.32	1218	1218
14	<i>Trans</i> -carveol	Oxygenated monoterpene	19.63	0.31	1233	1229
15	α -Cubebene	Hydrocarbon	25.13	0.36	1356	1355
16	α -Copaene	Sesquiterpene hydrocarbon	26.33	1.12	1384	1383
17	β -Cubebene	Sesquiterpene hydrocarbon	26.87	0.37	1396	1392
18	β -Caryophyllene	Sesquiterpene hydrocarbon	28.16	3.70	1426	1428
19	(+)-Epi-bicyclosesquipelandene	Sesquiterpene hydrocarbon	29.39	0.35	1456	1470
20	α -Caryophyllene	Sesquiterpene hydrocarbon	29.65	0.70	1462	1458
21	(+)-Aromadendrene	Sesquiterpene hydrocarbon	29.81	0.46	1466	1463
22	β -eudesmane	Sesquiterpene hydrocarbon	31.03	3.47	1495	1496
23	Elixene	Sesquiterpene hydrocarbon	31.30	2.66	1502	1511
24	\square -Cadinene	Sesquiterpene hydrocarbon	32.25	2.94	1525	1524
25	Calamene	Sesquiterpene hydrocarbon	32.37	3.11	1528	1529
26	α -Calacorene	Sesquiterpene hydrocarbon	33.14	1.30	1548	1548
27	Caryophyllene alcohol	Oxygenated sesquiterpene	33.49	2.04	1557	1563
28	(-) - Epatulenol	Oxygenated sesquiterpene	34.53	4.22	1583	1582
29	Caryophyllene oxide	Oxygenated sesquiterpene	34.69	22.03	1587	1583
30	Humulene Epoxide II	Oxygenated sesquiterpene	35.76	2.75	1614	1607
TOTAL			41.60	82,63%		
Monoterpenes				7.76%		
Oxygenated monoterpene				21.51%		
Sesquiterpene				20.54%		
Oxygenated sesquiterpene				32.82%		

^aT_R: retention time; ^bI_R: calculated retention index values; ^c*I_R: retention index values from NIST/EPA/NIH Mass Spectral Library, version 2.0 d, april 2005

Comparison of data from the present study with the data described in the literature revealed a variation in the number of compounds present in the

essential oil of *M. oblongata* (Table 1) when compared to other species of the Myrtaceae family. This variation may be attributed to environmental

variables such as leaf collection time, circadian rhythm, plant development, temperature, water availability, ultraviolet radiation, soil nutrient content, altitude, pollution, and pathogen attack, among other factors (GOBBO-NETO; LOPES, 2007). These variables demonstrate a significant influence of external factors on the composition of essential oils (DUDAREVA et al., 2004).

Essential oils of 17 species of the genus *Myrcia* were studied and there was predominance of cyclic sesquiterpenes (CERQUEIRA et al., 2007; SILVA et al., 2007; STEFANELLO et al., 2010), except in *Myrcia myrtifolia*, *Myrcia acuminatissima* and *Myrcia bombycina* whose levels of monoterpenes were more abundant (CERQUEIRA et al., 2009; HENRIQUES et al., 2011) the oil of *M. oblongata* presented significant amounts of sesquiterpenes and monoterpenes, as well as the species of the same family.

Caryophyllene derivatives have been identified in large numbers in *M. oblongata* in this work and also by other authors, this substance has also been isolated in species of the same genus, such as *Myrcia acuminatissima*, *Myrcia alagoensis*, *Myrcia arborescens*, *Myrcia cuprea*, *Myrcia fallax*, *Myrcia glabra*, *Myrcia hatschbachii*, *Myrcia multiflora*, *Myrcia pubiflora*, *Myrcia pubipetala*,

Myrcia richardiana, *Myrcia rostrata*, *Myrcia rufipila*, *Myrcia salzmännii*, *Myrcia selloii* and *Myrcia splendens*. Derived from the terpene pinene were also isolated in most of these species, but the compound verbenol was only identified in *M. oblongata* (CASCAES et al., 2015).

Antimicrobial activity

The MIC and MBC of *M. oblongata* essential oils for microorganisms ranged from 218.75 to 7.000 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table 2). The best results were observed for Gram-positive bacteria, with the highest inhibitory and bactericidal activity for *E. faecalis* (MIC / MBC: 218.75 / 875 $\mu\text{g}\cdot\text{mL}^{-1}$), followed by *S. aureus* and *B. subtilis*, both with MIC 875 and MBC 1.700 $\mu\text{g}\cdot\text{mL}^{-1}$, and *S. epidermidis* (1,700/1,700 $\mu\text{g}\cdot\text{mL}^{-1}$). The activity against Gram-negative bacteria was considered low, with the best result for *E. coli* (MIB and MCB = 7,000 $\mu\text{g}\cdot\text{mL}^{-1}$), followed by *P. aeruginosa* and *S. enteritidis*, both with MIC of 7,000 $\mu\text{g}\cdot\text{mL}^{-1}$ and no activity for MBC. No activity was observed for *P. mirabilis*, *K. pneumoniae* and *S. Gallinarum*. For *C. albicans*, minimal fungicidal and inhibitory activity (MIC and MBC, respectively) were both observed at 3,500 $\mu\text{g}\cdot\text{mL}^{-1}$.

Table 2. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (CFM) of *M. oblongata* essential oil against standard microorganisms.

Microorganism	MIC/MIB or MFC ($\mu\text{g}\cdot\text{mL}^{-1}$)
Gram-negative	
<i>E. coli</i>	7000/7000
<i>P. aeruginosa</i>	7000/—
<i>P. mirabilis</i>	—
<i>K. pneumoniae</i>	—
<i>S. Enteritidis</i>	7000/—
<i>S. Gallinarum</i>	—
Gram-positive	
<i>S. epidermidis</i>	1700/1700
<i>S. aureus</i>	875/1700
<i>E. faecalis</i>	218.75/875
<i>B. subtilis</i>	875/1700
Fungi	
<i>C. albicans</i>	3500/3500

The activity of the essential oils against *C. albicans* was considered low. There are no reports

in the literature for plants of the same genus, but similar results have been found for *Eucalyptus*

citriodora and *Eugenia uniflora*, which also belong to the family Myrtaceae. The essential oils from the leaves of these plants were tested on different species of *Candida* and presented moderate and low activity (LIMA et al., 2006; CASTRO; LIMA, 2010). The low susceptibility of the fungal cells to the essential oil may be due to the low hydrophobicity of the chemical constituents. The lower the hydrophobicity, the lower the permeability of the oil into the cell, and this decreases the efficacy of the essential oil (PRABUSEENIVASAN et al., 2006).

Gram-positive bacteria were more susceptible to the action of essential oils than Gram-negative bacteria. This was also observed for the essential oils of *Myrcia tomentosa* and *Myrcia myrtifolia*, which showed activity against *B. subtilis*, *S. aureus* and other Gram-positive bacteria (SÁ et al., 2012; YOKOMIZO; NAKAOKA-SAKITA, 2014; SIMONETTI et al., 2016). This susceptibility of Gram-positive bacteria can be explained by the presence of a bacterial cell wall that does not restrict the penetration of toxic molecules, whereas Gram-negative bacteria have a barrier system in the outer membrane of the bacterial wall formed by phospholipids, lipopolysaccharides and proteins that are impermeable to many microorganisms (LAMBERT, 2002; MADIGAN et al., 2010).

Antioxidant activity

The DPPH free radical sequestration index presented as linear function with the equation $y = 0.0105x - 0.0249$. The absorbances (for essential oil and BHT) were analyzed by the chi-square test for adherence, with significance level of 0.05. We found no significant statistical difference between the absorbances of the positive control (BHT) and the essential oil of *M. oblongata* ($\chi^2 = 0.21356$; GL = 1; $p = 0.644$). There was also no significant statistical difference in IC₅₀ ($\chi^2 = 1.8639$, GL = 1, $p = 0.1722$).

Although the essential oil of *M. oblongata* did not present antioxidant activity as high as BHT according to the classification of Scherer et al. (2009), it has moderate activity in this same classification, indicating that this oil at the concentration of 7,000 $\mu\text{g}\cdot\text{mL}^{-1}$ has antioxidant potential.

The percentages of DPPH sequestration through the absorbance equation and the IC₅₀ are shown in Table 3. The percentage of DPPH sequestration was 88.33% and the IC₅₀ was 2.80 for the essential oil, while those values were 94.58% and 1.39, respectively, for BHT.

Table 3. Antioxidant activity of essential oil from the leaves of *M. oblongata*

Test Solution	% Sequestration of DPPH	IC ₅₀
Positive control (BHT)	94.58%	1.39
<i>M. oblongata</i> essential oil	88.33%	2.80

BHT= synthetic commercial antioxidant; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC₅₀ = concentration of oil required to reduce DPPH radicals by 50%.

The antioxidant activity of *Myrcia* spp. were verified in the literature for *Myrcia splendens*, *Myrcia bella* and *Myrcia lingua*, and in the family (Myrtaceae) for *Blepharocalyx salicifolius*, *Eugenia bimarginata*, *Eugenia dysenterica*, *Eugenia klotzschiana*, *Hexachlamys edulis*, *Psidium australe*, *Psidium cinereum*, *Psidium laruotteanum* and *Psidium guajava*; all these species have high or moderate antioxidant activity (TAKAO et al., 2015) as did *M. oblongata* in this study.

The antioxidant potential of Myrtaceae species may be associated with the presence of caryophyllenes, verbenols and phenolic compounds, whose antioxidant activity has been verified through various chromatographic techniques (SHAHIDI et al., 1992). However, the results of antioxidant activity assays reported in the literature cannot

always be directly compared since different methods generate different responses (MOLYNEUX, 2004). Among the most commonly used methods, the free radical DPPH reduction method used in this study is as a faster and less expensive alternative compared to other common spectrophotometry techniques (MOLYNEUX, 2004; ALVES et al., 2010). The spectrophotometer used in this study was composed of the radiation source of tungsten filament lamp, a monochromator, the sample holder (quartz cuvettes) and the coupled charge devices.

M. oblongata essential oil fumigation of *D. gallinae*

Comparison of means of the four concentrations (10%, 5%, 2.5% and 1.25%) of essential oil showed no significant differences from

control negative according to the Tukey test ($F = 0.21$, $p = 0.92$, $GL = 4$). The positive control, triazophos, killed 100% of the mites. All essential oil concentrations resulted in mortality lower than 20% (Table 4). Similar results were observed for *Eugenia uniflora* (Myrtaceae), which did not cause

significant mortality for *Ornithonyssus bursa* (Acari: Dermanyssidae) after fumigation treatment (VILLAÇA, 2012). There are currently no reports of activity against pests after fumigation with essential oils for any species of the genus *Myrcia*.

Table 4. Fumigation with different concentration of *M. oblongata* essential oil against *D. gallinae* (n = 100).

	Control water, Tween and 10% acetone)	(distilled 10%	5%	2.5%	1.25%
Mean number	0.6~1 ± 0.5 a	3.8~4 ± 1.8	2.4~3 ± 1.8	2.4~3±2.6	2 ± 1.7 a
Mortality (%)	5	20	15	15	10

Means ± standard deviation of mite mortality at 24h. Values followed by the same letter do not differ other according to the Tukey test.

However, the exposure time of the mites in this experiment was 24 h and according to Pauliquevis and Fávero (2015), lethal effects of fumigation may be related to the time of exposure to essential oil; these authors found no mortality in mites fumigated with essential oil of *Eucalyptus urograndis* (Myrtaceae) after 24h, but did observe mortality after 48h. Although the fumigation test in this experiment resulted in mortality lower than 20%, acaricides with plant essential oils as the active ingredients are already available on the market. These products are mainly obtained from plants of the family Myrtaceae such as *Syzygium aromaticum* (ISMAN, 2010). The volatility of the essential oils has proven fumigant action and may serve as an alternative to conventional acaricides (ASLAN et al., 2004). However, the type of application influences the effectiveness against mites, and for this reason different methods of application of essential oils and the resulting acaricidal effects should be investigated for *M. oblongata*.

Repellency of *M. oblongata* essential oil against *D. gallinae*

The activity of *M. oblongata* essential oil as a repellent for chicken mites was analyzed at three different concentrations and was evaluated according to the number of responses (Chi-square goodness-of-fit test) and response time (independent-samples t-test). At concentrations of 1% and 5%, the number of mites attracted to the essential oil was similar to that of the control (1%: $\chi^2 = 0.926$, $GL = 1$, $p = 0.336$; 5%: $\chi^2 = 3.24$, $GL = 1$, $p = 0.0719$) (Table 5). These concentrations also led to similar average times between treatments ($t = 1.52$, $p = 0.93$) and controls ($t = 2.51$, $p = 0.99$). These results demonstrate that at the lowest concentrations, the essential oil did not repel mites. At the 10% concentration, there was also no statistical difference in response time between the control and oil treatment ($t = 2.78$, $p = 0.99$). However, there were fewer mites attracted to the essential oil (5) than to the control (17) ($\chi^2 = 6,545$; $GL = 1$; $p = 0.0105$). This result demonstrates the repellent action potential of *M. oblongata* essential oil at 10% concentration.

Table 5. Total number of *D. gallinae* females attracted to an odor source and mean time (h: m: s) to choosing Y-olfactometer arms for three concentrations of *M. oblongata* essential oil (n = 150).

	Total number of responses	Time (h:m:s)
Control	16	00:45:16
Essential oil 1%	10	00:26:24
Control	17	00:41:12
Essential oil 5%	8	00:15:59
Control	17*	00:45:54
Essential oil 10%	5	00:15:07

*Indicated a statistically significant difference in the number of responses between the control and essential oil treatment (Chi-square test, $p < 0.05$). For time, there were no statistically significant differences between any treatments (t-test, $p > 0.05$).

George et al., (2009) evaluated the acaricidal activity of essential oils of four species of Myrtaceae on *D. gallinae*: *Eucalyptus globulus*, *Eucalyptus radiata*, *Eucalyptus staigeriana* and *Eucalyptus citriodora*. The authors found that oils with the highest activity had a greater number of chemical constituents in their composition. The species *M. oblongata*, tested in the present work, contains 30 compounds (Table 1) that can interact and that repel the mites. In addition, the activity of terpenes such as δ -pinene and caryophyllene oxide, the latter being one of the major compounds of *M. oblongata*, are known to act as repellents against *Sitophilus zeamais*, *Tribolium confusum* (Coleoptera: Curculionidae) and the mite *Tetranychus urticae* (Acari: Tetranychidae) (TAPONDJOU et al., 2005; LIMA et al., 2009).

CONCLUSIONS

We identified 30 chemical compounds in the essential oil of *M. oblongata*, with caryophyllene oxide (22.03%) and *trans*-verbenol (11.94%) as the majority constituents.

The antimicrobial activity of the essential oil was higher for Gram-positive bacteria than for Gram-negative bacteria. The highest activity was

against *E. faecalis*, followed by *S. aureus*, *B. subtilis* and *S. epidermidis*.

The antimicrobial activity against Gram-negative bacteria was considered low, with the best results observed for *E. coli*, *P. aeruginosa*, and *S. Enteritidis*. The oil showed no activity against *P. mirabilis*, *K. pneumoniae* and *S. Gallinarum*. Activity against *C. albicans* was also considered low. At a concentration of 7,000 $\mu\text{g.mL}^{-1}$, the oil showed antioxidant potential and repellent potential against *D. gallinae* at 10% concentration, but did not repel mites when applied at concentrations below 10%.

The fumigation test of *M. oblongata* on *D. gallinae* showed that essential oil concentrations between 1.25% and 10% promoted low mortality.

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RESUMO: Os óleos essenciais tem despertado interesse no setor industrial por apresentarem multiplicidade de aplicações, destacando-se por suas propriedades antimicrobianas, antioxidantes dentre outras. Diante disto, o objetivo deste estudo foi determinar a composição química do óleo essencial das folhas de *Myrcia oblongata* DC pelo método de cromatografia gasosa acoplada à espectrometria de massas (CG-EM), avaliar o potencial antioxidante do óleo pelo método de captura do radical livre 2,2- difenil-1-picril hidrazil (DPPH), testar a atividade antimicrobiana pelo método de microdiluição em caldo, bem como avaliar a repelência e potencial fumigante deste óleo sobre *Dermanyssus gallinae* (Degeer, 1778). As análises de CG-EM resultaram na identificação de 30 constituintes, sendo majoritários o óxido de cariofileno (22.03%) e o *trans*-verbenol (11.94%). O óleo apresentou atividade antioxidante moderada quando comparada ao antioxidante sintético 2,6-di-tert-butil-4-hidroxitolueno (BHT). Em relação à atividade antimicrobiana, o óleo essencial demonstrou atividade inibitória para as bactérias Gram-positivas, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis* e *Staphylococcus epidermidis*, e para levedura *Candida albicans* e não apresentou atividade para bactérias Gram negativas. Todas as concentrações do óleo essencial testadas no teste de fumigação sobre *D. gallinae* apresentaram mortalidade inferior a 20%. Para o teste de repelência, observou-se um potencial significativo para a concentração de 10%.

PALAVRAS-CHAVE: CG-EM. óxido de cariofileno. *Dermanyssus gallinae*

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