

***In vitro* antileishmanial and antioxidant activities of essential oils from different parts of *Murraya paniculata* (L.) Jack: a species of Rutaceae that occur in the Cerrado biome in Brazil**

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**Abstract**

In Brazilian folk medicine, *Murraya paniculata* has been used for treating intestine disorders, rheumatism and cough. This paper aims to investigate the *in vitro* antileishmanial and antioxidant activities of essential oils (EO) from *M. paniculata* leaves and fruits (ripe and unripe ones). Natural antioxidants may be very beneficial to improve quality of life, since they are capable of protecting the body against damage caused by free radicals and, consequently, either preventing or postponing many diseases from starting their cycles. One of the techniques which has been widely used for detecting antioxidant compounds is the method based on the elimination of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). It has been considered easy, precise, fast, simple, economical and appropriate to determine antioxidant activity of pure substances and complex mixtures, such as EO. Thus, antioxidant potential of EO was evaluated by using the method of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). EO from *M. paniculata* leaves and fruits exhibited weak potential, since EC<sub>50</sub> values were above 700 µg/mL. Several reports have stated that volatile oils from plants show promising leishmanicidal activity against promastigote forms of *Leishmania amazonensis*; in relation to this activity, leaf oil was highly active (IC<sub>50</sub> = 7.33±2.07 µg/mL) while ripe and unripe fruit oils were active, with values of IC<sub>50</sub> = 30.77±2.07 µg/mL and 13.04±1.64 µg/mL, respectively. Both GC-FID and GC-MS analyses revealed that the major components determined in EO from *M. paniculata* were sesquithujene (25.0%), *trans*-β-caryophyllene (23.8%), α-zingiberene (21.0%), α-ylangene (13.3%), germacrene D (13.1%), α-copaene (12.7%), and β-cubebene (10.2%). *In vitro* antileishmanial and antioxidant activities of EO from *M. paniculata* have also been described for the first time.

**Keywords:** *Murraya paniculata*, essential oils, *Leishmania amazonensis*, DPPH•, Cerrado

**Abbreviations:** GC-FID - gas chromatography-flame ionization detector; GC-MS -gas chromatography-mass spectrometry; EO - essential oils; DPPH• - 2,2-diphenyl-1-picrylhydrazyl; BHA - butylated hydroxyanisole; BHT - butylated hydroxytoluene; TBHQ - tertiary butylhydroquinone; PG - propyl gallate

**Introduction**

Due to its great diversity, the Brazilian Cerrado is considered the savanna with the largest biological richness worldwide and ranks second in the list of Brazilian biomes. Strong and important effort has been made to preserve this natural asset, since it stretches over 25% of the Brazilian territory and is the second largest biome in South America (the Atlantic Forest ranks first). It comprises 4,400 endemic plants which correspond to 1,5% of the global endemic flora and 11,806 plant species that represent 5% of the world's biodiversity (Gonçalves et al., 2016). It should be highlighted that further studies are needed to develop the sector that produces essential oils (EO) while associating preservation of the Cerrado, sustainable exploration of forest products and

social inclusion of populations that depend on forest production and exploration.

A large number of plants found in the Cerrado biome is responsible for the synthesis of antioxidant secondary metabolites that absorb in the range from 300 to 400 nm. It is significantly increased by UV radiation and provides high protection level against harmful oxidants generated by heat or by light (Morais et al., 2006). Besides, medicinal plants have been widely used as antioxidants in traditional medicine. Their therapeutic characteristics are mostly supported by their free radical scavenging ability, since radicals may be involved in several diseases (Chirag J et al., 2013). Cancer, emphysema, arteriosclerosis and arthritis

have been correlated with oxidative stress. In general, organisms have been protected against damage caused by free radicals of enzymes, such as superoxide dismutase and catalase, and by the ones of certain compounds, such as ascorbic acid, tocopherol and glutathione (Meena et al., 2012).

When mechanisms of antioxidant protection become inefficient as the result of age, deterioration of physiological functions may take place, thus leading to diseases and acceleration of the aging process. However, antioxidant food supplements may be used for helping the body mitigate oxidative damage (Yang et al., 2000). Antioxidants have been usually applied to oils and fat food to delay their autoxidation. Some synthetic antioxidants, such as BHA, BHT, TBHQ and PG, are toxic at high doses, even though they have often been applied to food. As a result, researches on natural antioxidants have increased a lot lately (Andrade et al., 2013).

These advantages justify researchers' increasing interest in the development of antioxidant substances, mainly from natural products, such as plants. One of the techniques that has been used for detecting antioxidant capacity of several compounds is the method based on the elimination of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). The DPPH• molecule has been well-known as a stable organic free radical which has many advantages, such as good stability in the absence of light, applicability, simplicity and viability. The DPPH• method has been used in about 90% of preliminary studies which aim at evaluating antioxidant activity of pure substances, as well as complex mixtures and matrices. Even though it enables the evaluation of antioxidant capacity, it should not be the only methodology used for this purpose; other methods are needed to thoroughly characterize a compound as an antioxidant (Oliveira, 2015).

Since free radicals are molecules with unpaired electrons, they are unstable and quite reactive, but DPPH•, due to its chemical structure, is a stable free radical with three aromatic rings that bestow the resonance effect which is important to stabilize its electronic charge. Stabilization of DPPH• has also been attributed to the displacement of unpaired electrons on it, attracted by three NO<sub>2</sub> groups and by two atoms of nitrogen, which also enable electrons to displace (Oliveira, 2015).

DPPH• is either purple or violet due to the location of the free electron in its molecule; absorption takes place in ethanol or methanol solutions at wavelength of 515-520 nm. Any antioxidant substance may either donate an atom of hydrogen or transfer an electron to a receptive molecule, such as DPPH•, which accepts an atom of hydrogen to become a stable diamagnetic molecule and originate the reduced form DPPH-H. In this form, the violet solution turns into pale yellow or light violet after some time. Changes in color – from dark to light violet –, the result of decrease in absorbance of DPPH•, may be monitored by a UV-visible spectrophotometer to determine antioxidant capacity. This monitoring must always be carried out in the dark, since light is a factor that directly interferes in the reaction of DPPH• with a certain substance and leads to decrease in absorbance and, consequently, affects final results (Oliveira, 2015).

Leishmaniasis is a tropical disease which is caused by an intracellular parasite, a protozoan of the genus *Leishmania*,

whose vector is phlebotomine, a small insect that belongs to the genus Diptera. In Brazil, this sandfly is called “mosquito palha” (straw mosquito) (Blanco et al., 2017). The clinical presentation of the disease depends on the complexity of the interaction between the immunological system of the host and the type of protozoan. There are three different forms of leishmaniasis: cutaneous, mucocutaneous and visceral (the most serious one) (Andrade et al., 2016). The World Health Organization (WHO) estimates that it is the second insect-borne disease that kills the largest number of people in the world. Regarding its geographic occurrence, leishmaniasis is classified into two types: the “old world” one occurs in Asia, Africa, the Mediterranean region and the Middle East, and the “new world” leishmaniasis is found in Central America, South America and in southern Texas (Blanco et al., 2017).

In this context, plants are valuable sources to maintain human health. Their use has been emphasized lately, since several studies of therapeutic products from medicinal plants have been carried out. The World Health Organization (WHO) states that medicinal plants are the best sources of a great variety of medication and that 80% of the world's population uses traditional medicine in the search for relief from some painful or unpleasant symptoms (Dutra et al., 2009). It is worth highlighting that the selection of plants which produce EO has been the focus of relevant studies, since these oils have exhibited promising *in vitro* activity against *Leishmania amazonensis* (Andrade et al., 2016).

EO extracted from different plant species are naturally volatile and constitute a complex mixture of monoterpenes, sesquiterpenes and phenylpropanoids, which are responsible for the strong odor of oils. Their extraction may be carried out by several methods, such as steam distillation and hydrodistillation, which have been often used in laboratories of natural products (Pandey and Singh, 2017). EO are secondary metabolites extracted from distinct parts of plants; they have complex chemical composition and provide adaptative advantages to plants in their environment. Chemical composition of volatile oils varies among species and parts of plants, since any species may be affected by its place of cultivation, harvest conditions, stabilization and storage, besides biotic and abiotic factors (Miranda et al., 2016).

*Murraya paniculata*, which belongs to the family Rutaceae, is a tree native to India that was brought to Brazil, where it has been widely used for urban afforestation in São Paulo, SP. This species is considered medicinal in tropical and subtropical Asian regions, such as China and Indonesia. In these countries, its leaves and roots have been used for treating intestinal disorders, rheumatism and cough (Mesquita et al., 2008). Therefore, this study aimed to determine *in vitro* antioxidant and antileishmanial activities of EO from *M. paniculata* leaves and fruits (ripe and unripe ones) for the first time (Fig. 1).

## Results and Discussion

### **Constituents of EO from *M. paniculata* leaves and fruits (ripe and unripe ones)**

EO from *M. paniculata* leaves, ripe fruits and unripe fruits were extracted in Rio Verde, Goiás, Brazil, at 0.7, 0.6 and 0.5% yields (w/w), respectively. Volatile compounds were

identified by gas chromatography-flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC–MS). The three major compounds identified in EO from leaves are *trans*- $\beta$ -caryophyllene (23.8%, **1**),  $\alpha$ -zingiberene (21.0%, **2**) and  $\beta$ -cubebene (10.2%, **3**). The major components in EO from unripe fruits are sesquithujene (25.0%, **4**),  $\alpha$ -zingiberene (18.2%), germacrene D (13.1%, **5**) and  $\alpha$ -copaene (12.7%, **6**). EO from ripe fruits are mainly composed of *trans*- $\beta$ -caryophyllene (23.1%),  $\alpha$ -ylangene (13.3%, **7**), germacrene D (10.9%) and  $\alpha$ -zingiberene (9.7%) (Table 1 and Fig. 2). Previous reports of EO from leaves of other *M. paniculata* specimens have shown that terpenes predominate in their chemical composition, which varies significantly, depending on the origin of the plant. For example, EO from leaves of plants cultivated in Bangladesh had the following seven major constituents: caryophyllene oxide,  $\beta$ -caryophyllene, spathulenol,  $\beta$ -elemene, germacrene D, cyclooctene and 4-methylene-6-(1-propenylidene) (Chowdhury et al., 2008), whereas EO collected in Nepal provided methyl palmitate, isospathulenol, (*E,E*)-geranyl linalool, benzyl benzoate, selin-6-en-4-ol,  $\beta$ -caryophyllene, germacrene B, germacrene D and  $\gamma$ -elemene as their major constituents (Dosoky et al., 2016). In EO from leaves collected in mountains in Central Cuba,  $\beta$ -caryophyllene was found to be the only major constituent (Rodríguez et al., 2012). However, in Nigeria, EO from leaves had seven major constituents, i. e.,  $\beta$ -cyclocitral, methyl salicylate, *trans*-nerolidol,  $\alpha$ -cubebene, (-)-cubenol,  $\beta$ -cubebene and isogermacrene (Olawore et al., 2005). Olawore and collaborators (2005) reported that the major components found in EO from *M. paniculata* fruits were  $\beta$ -caryophyllene (43.4%), (-)-zingiberene (18.9%), germacrene D (8.3%),  $\alpha$ -copaene (5.5%) and  $\alpha$ -humulene (5.1%), even though they did not mention the maturation conditions of the fruits. Therefore, the study described by this paper pioneers the analysis of chemical profiles of EO extracted from *M. paniculata* unripe and ripe fruits collected in the Cerrado in Goiás state, Brazil. By comparison with the previously reported chemical composition of *M. paniculata* fruits, compositions of EO from *M. paniculata* ripe and unripe fruits found by this study were similar, since their major constituents were also  $\alpha$ -copaene,  $\beta$ -caryophyllene, germacrene D,  $\alpha$ -zingiberene and  $\alpha$ -humulene, even though concentrations were different (Olawore et al., 2005). It should be highlighted that  $\alpha$ -ylangene (13.3%) and sesquithujene (25.0%), the other major constituents of EO from ripe and unripe fruits, were not found in EO from fruits collected in Nigeria (Olawore et al., 2005). In Brazil, both terpenes  $\beta$ -caryophyllene and  $\alpha$ -zingiberene were the major constituents of EO from *M. paniculata* leaves collected in Espírito Santo state (Neta et al., 2017). The chemical composition of EO from *M. paniculata* leaves collected in Goiás state was similar to the one reported by Neta et al. (2017), but it differs regarding  $\beta$ -cubebene (10.2 %), which was identified as the third major component of EO from leaves and it had not been found in those EO extracted in Espírito Santo.

#### **Antioxidant activity of EO from *M. paniculata* leaves and fruits (ripe and unripe ones)**

DPPH• scavenging activity is usually expressed as an EC<sub>50</sub> value, which is defined as the concentration of the

antioxidant needed to scavenge 50% of DPPH• found in the test solution. The effect of antioxidants on DPPH• radical scavenging is thought to be due to their hydrogen donating ability or radical scavenging activity. Antioxidants in a sample lead to the disappearance of DPPH• radical chromogens, which can be detected spectrophotometrically at 515 nm. A low EC<sub>50</sub> value indicates high antioxidant activity (Medini et al., 2011; Oliveira et al., 2016).

The DPPH• scavenging activity assay resulted in EC<sub>50</sub> = 932.55  $\mu$ g/mL, 1123.72  $\mu$ g/mL and 716.72  $\mu$ g/mL, in the cases of EO from *M. paniculata* leaves, ripe fruits and unripe fruits, respectively. The comparison between the EC<sub>50</sub> value of EO under study and the standard BHT (EC<sub>50</sub> = 85.20  $\mu$ g/mL) shows that these EO have low antioxidant potential. These data demonstrate that the antioxidant potential of EO from *M. paniculata* leaves collected in Brazil is different from the one of the same plant found in mountains in Cuba (Rodríguez et al., 2012). Differences found in the antioxidant potential of EO from the same parts of plants of the same species may be justified by their distinct chemical composition. Variation in concentrations of chemical constituents of EO may be justified when several factors, such as seasonality, circadian rhythm, developmental stage, age, temperature, harvest time, water availability, UV radiation, soil nutrients, altitude, atmospheric composition and tissue damage, are taken into account, since all affect secondary metabolism and influence the total number of metabolites and their relative proportions (Andrade et al., 2013). Antioxidant activity of EO from ripe and unripe fruits has been investigated for the first time by this study.

Antioxidant potential of EO has been widely investigated by researchers all over the world (Silva et al., 2016). The low antioxidant activity of EO from *M. paniculata* may be explained by their low concentration of compounds with this potential. In their composition, they do not have compounds with well-known antioxidant activity at significant amounts, except *trans*- $\beta$ -caryophyllene, which has its antioxidant activity increased by synergistic effects in association with phenolic compounds (Shahidi et al., 1992).

#### **Antileishmanial activity of EO from *M. paniculata* leaves and fruits (ripe and unripe ones)**

In the search for new leishmanicidal agents, the effect of EO from *M. paniculata* (Rutaceae) leaves and fruits (ripe and unripe ones) on promastigote cultures of *Leishmania amazonensis* was evaluated.

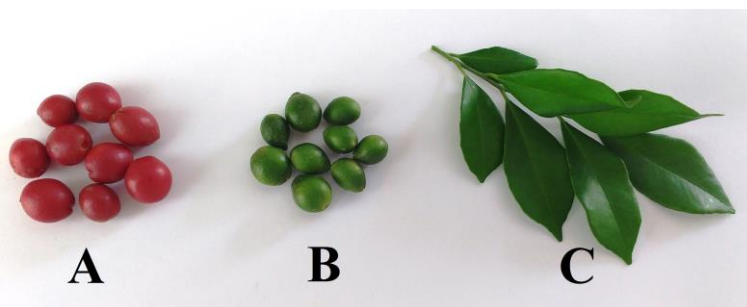
Leishmanicidal potential of EO has been well studied (Cardoso et al. 2015) and EO from *M. paniculata* leaves have exhibited high leishmanicidal activity when tested against promastigote forms of *L. amazonensis*. Increase in parasite lysis was observed with increase in EO concentration, i. e., IC<sub>50</sub> = 7.33 $\pm$ 2.07  $\mu$ g/mL (Table 2). Even though EO from ripe and unripe fruits had higher IC<sub>50</sub> values, they were active against the parasite. Their IC<sub>50</sub> values were 13.04 $\pm$ 1.64 and 30.77 $\pm$ 2.07  $\mu$ g/mL, respectively (Table 2). EO from *M. paniculata* inhibited parasite growth at a concentration/dose-dependent manner. Amphotericin B (IC<sub>50</sub> = 0.011 $\pm$ 0.34  $\mu$ g/mL) was used as positive control.

Regarding leishmanicidal activity (IC<sub>50</sub> values), the literature describes that samples whose IC<sub>50</sub> < 10  $\mu$ g/mL are considered highly active. When IC<sub>50</sub> > 10 < 50  $\mu$ g/mL, it is active, IC<sub>50</sub> > 50 < 100  $\mu$ g/mL is moderately active and IC<sub>50</sub> >

**Table 1.** Chemical composition of EO from *M. paniculata* ripe fruits (RF-EO), unripe fruits (UF-EO) and leaves (LM-EO).

Compounds	RI <sub>exp</sub>	RI <sub>lit</sub>	RA%		
			RF-EO	UF-EO	LM-EO
Bicycloelemene	1334	1336	0.3	1.4	-
Elemene isomer	1341	1344	2.7	-	-
α-Cubebene	1351	1352	0.9	6.1	-
β-Bourbonene	1377	1384	-	-	0.6
α-Copaene	1379	1377	6.1	12.7	1.6
β-Cubebene	1385	1390	-	-	10.2
α-Ylangene	1405	1406	13.3	1.1	5.6
Sesquithujene	1415	1417	0.4	25.0	-
α-Gurjunene	1419	1419	0.1	-	-
<i>trans</i> -β-Caryophyllene	1425	1423	21.3	1.1	23.8
Isogermacrene D	1437	1439	0.8	-	-
β-Gurjunene	1439	1440	0.7	-	-
β-Humulene	1446	1440	-	-	6.4
γ-Muurolene	1448	1449	0.4	6.4	-
α-Humulene	1456	1455	5.3	1.0	-
Aromadendrene	1465	1463	1.1	-	1.8
Germacrene D	1480	1480	10.9	13.1	9.8
α-Zingiberene	1499	1496	9.7	18.2	21.0
Bicyclogermacrene	1503	1501	5.3	0.5	-
β-Bisabolene	1508	1506	0.8	1.7	1.2
β-Cadinene	1528	1527	5.5	5.6	-
Cadina-1.4-diene	1534	1533	0.3	-	-
<i>trans</i> -Nerolidol	1557	1565	-	-	1.4
Germacrene-D-4-ol	1574	1574	1.0	1.0	-
Spathulenol	1576	1576	-	-	2.5
Caryophyllene oxide	1590	1589	1.2	1.1	1.5
Lauryl acetate	1608	1606	0.2	-	-
Octil 2-methylbutanoate	1624	1623	0.2	-	-
t-Cadinol	1634	1638	-	-	0.4
10-epi-α-Muurolol	1640	1641	-	-	2.6
Isovaleric acid. decyl ester	1657	1659	0.1	-	-
τ-Muurolol	1659	1660	0.5	-	-
α-Cadinol	1662	1663	0.3	-	1.0
Decyl senecioate	1720	1719	4.0	-	-
Isovaleric acid. dodecyl ester	1844	1845	2.5	-	-
Total			95.9	96.1	97.7

RI<sub>exp</sub>: Retention index relative to *n*-alkanes (C<sub>8</sub>-C<sub>20</sub>) on the Rtx-5MS column. RI<sub>lit</sub>: Retention Index from the literature. RA%: relative area. (-) = not detected.

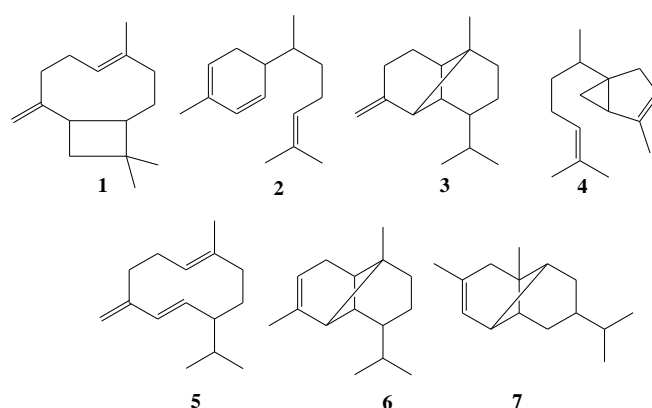


**Fig 1.** *Murraya paniculata* (Rutaceae): ripe fruits (A), unripe fruits (B) and leaves (C)

**Table 2.** Leishmanicidal activity of EO from *M. paniculata* leaves (LM-EO), ripe fruits (RF-EO) and unripe fruits (UF-EO) against *L. amazonensis* promastigote forms.

	% of lysis ± S.D /Concentration (µg.mL <sup>-1</sup> )					IC <sub>50</sub> (µg/mL)
	50	25	12.5	6.25	3.12	
LM-EO	100±0.00	98.28±1.01	72.87±22.16	39.10±2.03	19.22±14.74	7.33±2.07
RF-EO	100±0.00	74.55±4.06	40.25±21.63	25.24±34.68	17.49±16.70	13.04±1.64
UF-EO	78.87±29.8	31.56±8.73	17.21±7.54	12.39±3.54	2.88±4.08	30.77±2.07
	<b>0.19</b>	<b>0.095</b>	<b>0.047</b>	<b>0.023</b>	<b>0.011</b>	
Amph. B	99.88±0.60	78.33±24.43	68.74±21.97	54.67±17.77	42.44±20.97	0.011±0.34

**Negative Control:** RPMI Medium + 0.1% DMSO. **Amph. B:** Amphotericin B (positive control); **S.D:** Standard Deviation.



**Fig 2.** Structures of the major constituents identified in EO from *M. paniculata* leaves, ripe fruits and unripe fruits: *trans*- $\beta$ -caryophyllene (1);  $\alpha$ -zingiberene (2);  $\beta$ -cubebene (3); sesquithujene (4); germacrene D (5);  $\alpha$ -copaene (6); and  $\alpha$ -ylangene (7).

100 µg/mL is inactive (Andrade et al., 2018). According to Kauffmann et al. (2017), the observed antileishmanial activity may be attributed to the mixture of sesquiterpene constituents. The promising leishmanicidal activity exhibited by EO from *M. paniculata* leaves and ripe fruits can be attributed to the chemical constituent *trans*- $\beta$ -caryophyllene, which was identified at high concentration in EO under study.

EO from unripe fruits, although active, had the highest IC<sub>50</sub> value, a fact that can be explained by the low concentration of *trans*- $\beta$ -caryophyllene in the oils. The sesquiterpene *trans*- $\beta$ -caryophyllene should be mentioned because its promising leishmanicidal activity has already been reported in the literature (Soares et al., 2013).

Studies of promastigote cultures of *L. amazonensis* also revealed that the oxygenated sesquiterpene nerolidol (although at low concentrations in EO from leaves, it exhibits antileishmanial activity) may be associated with the inhibition of the biosynthesis of cellular isoprenoids (Arruda et al., 2005). It encourages an intensive investigation into the mechanisms of action of the EO terpene. It has also been suggested that the low density of EO contributed to the disruption of the cytoplasmic membrane, driving force of protons, electron flow, active transport and coagulation of cell contents (Dhifi et al., 2016). Thus, the search for new treatment options for this neglected disease is fundamental and EO and terpenes are alternatives. However, further studies are needed to explain the complete mechanisms of

these interactions, mainly in the specific case of leishmaniasis.

## Materials and methods

### Plant materials

The plant material was collected at the “Instituto Federal Goiano – Campus Rio Verde” in Rio Verde, Goiás state, Brazil (17°48’08.1’’S and 50°54’22.2’’W), in October 2017. The plant was identified by the botanist Luzia Francisca de Souza and a voucher specimen of *Murraya paniculata* (HJ 28760/MP) was deposited at the Herbarium Jataiense Professor Germano Guarim Neto.

### Extraction of EO

EO from *M. paniculata* were extracted from leaves and fruits (ripe and unripe ones) by hydrodistillation in a Clevenger-type apparatus for 2 h. Hydrodistillation was performed in triplicate. The plant material was divided into three 500-g samples and 500 mL distilled water was added to each sample. After manual collection of EO, traces of water remaining in the oil were removed with anhydrous sodium sulfate, which was followed by filtration. EO were stored in an amber bottle and kept in a refrigerator at 4°C until analysis. EO yield was calculated from the weight of the fresh leaves and fruits (ripe and unripe ones) and expressed as the average of the triplicate analyses.

### Analysis of EO

EO were dissolved in ethyl ether and analyzed by Gas chromatography-flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC–MS) with both Shimadzu QP5000 Plus and GCMS2010 Plus (Shimadzu Corporation, Kyoto, Japan) systems. The temperature of the column in GC-FID was programmed to rise from 60 to 240°C at 3°C/min and was held at 240°C for 5 min; the carrier gas was H<sub>2</sub> at a flow rate of 1.0 mL/min. The equipment was set to operate in the injection mode. The injection volume was 0.1 µL (split ratio of 1:10) and injector and detector temperatures were 240 and 280°C, respectively. Relative concentrations of the components were obtained by normalizing peak areas (%). Relative areas consisted of the average of triplicate GC-FID analyses. GC-MS conditions and the identification of EO have been previously reported (Melo et al., 2015). Identification of the volatile components of EO from *M. paniculata* (Table 1) was based on their retention indices on an Rtx-5MS (30 m X 0.25 mm; 0.250 µm) capillary column under the same operating conditions used for GC relative to a homologous series of *n*-alkanes (C<sub>8</sub>–C<sub>20</sub>). Structures were computer-matched with Wiley 7, NIST 08 and FFNSC 1.2 while their fragmentation patterns were compared with literature data (Adams, 2007).

### Antioxidant activity

Antioxidant activity was determined by the method described by Oliveira et al. (2016). It was evaluated by the DPPH• scavenging activity assay which makes use of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). Ethanol solutions from 0.5 to 10 g/L DPPH• were prepared for the whole assay. Absorbance readings were carried out 60 minutes after the addition of 0.1 mL EO solution at different concentrations and 3.9 mL DPPH• solution (0.06 mM) by a Bel Engineering digital UV-Vis spectrophotometer, model UV-M51, at wavelength of 515 nm in a quartz cuvette. Results were expressed as EC<sub>50</sub> µg/mL (the sample amount which was needed to decrease initial concentration of the radical DPPH• in 50%).

### Statistical analysis

Data were analyzed by repeated measures of one-way analysis of variance (ANOVA) and the Tukey's post test while correlation of total phenolic and antioxidant activities (DPPH•) was analyzed by the Pearson rank correlation coefficient with the use of SPSS Version 6.0. Significance was set at 5%.

### Antileishmanial activity

To evaluate antileishmanial activity, promastigote forms of *L. amazonensis* (MHOM/BR/PH8) were maintained in RPMI 1640 (Gibco) culture medium supplemented with 10% fetal bovine serum, penicillin (100 UI/mL) and streptomycin (100 µg/mL). Subsequently, about 1 x 10<sup>6</sup> parasites were distributed on 96-well plates and EO, previously dissolved in 100% dimethylsulfoxide (DMSO, stock solution 100 mM) (Synth), were added to the cultures at concentrations from 3.12 to 50 µg/mL. Amphotericin B (Sigma Aldrich, 97 % purity), at concentrations from 0.19 to 0.011 µg/mL, was

added to cultures and used as positive control. Cultures were incubated in a BOD (Quimis) incubator at 25 °C for 24 h and antileishmanial activity was determined by verifying whether the growth of the promastigote forms was inhibited, as revealed by counting the total number of live promastigotes in the Neubauer (Global Glass - Porto Alegre, BR) chamber on the basis of flagellar motility. RPMI 1640 medium (Gibco) with 0.1% DMSO (Synth) (highest concentration) was used. Results were expressed as mean of percentage of growth inhibition related to the negative control (0.1% DMSO). Experiments were performed in triplicate.

### Conclusion

Based on its findings, this study concludes that some major components of EO from *M. paniculata* are  $\alpha$ -zingiberene, germacrene D,  $\alpha$ -copaene,  $\beta$ -cubebene,  $\alpha$ -ylangene, sesquithujene and *trans*- $\beta$ -caryophyllene. The promising leishmanicidal activity of EO under investigation may be related to the presence and high concentration of the constituent *trans*- $\beta$ -caryophyllene. Therefore, EO from *M. paniculata* can be considered a source of bioactive compounds with leishmanicidal potential. It is noteworthy to mention that EO from *M. paniculata* exhibited weak antioxidant activity when evaluated by the DPPH• method. Thus, it is relevant to investigate whether natural products, such as EO, have high antioxidant potential to be employed in the areas of food science and complementary medicine. It is very difficult to attribute the activities to one or a few active principles found in these EO because minor compounds may also be effective regarding this potential. It is known that many compounds that are found at small amounts in a mixture can act as synergistic or antagonistic elements. In sum, further studies are needed to identify active chemical constituents of EO from *M. paniculata* and determine *in vivo* and *in situ* MIC.

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