

RESEARCH ARTICLE

Open Access



Essential oils: in vitro activity against *Leishmania amazonensis*, cytotoxicity and chemical composition

Milene Aparecida Andrade^{1,2}, Clênia dos Santos Azevedo¹, Flávia Nader Motta^{1,2}, Maria Lucília dos Santos³, Camila Lasse Silva¹, Jaime Martins de Santana¹ and Izabela M. D. Bastos^{1*}

Abstract

Background: The current chemotherapy for cutaneous leishmaniasis (CL) has a series of drug limitations such as toxic side effects, long duration, high costs and drug resistance, which requires the development of new drugs or effective alternatives to the CL treatment. Essential oils (EOs) are complex mixtures of secondary metabolites from various plants. It has been shown that several EOs, or their constituents, have inhibitory activity against protozoa. Thus, this study aims to evaluate the biological activity of different essential oils (EOs) on *Leishmania (L.) amazonensis* promastigotes forms, as well as their cytotoxicity on mammalian cells and chemical composition.

Methods: Sixteen EOs were evaluated by mean of IC₅₀/24 h and cytotoxicity against L6 cells (CC₅₀/24 h) using Resazurin assay. Only those EOs that presented better results for IC₅₀/24 h were submitted to GC–MS analysis to determine their chemical constitution.

Results: The EO from *Cinnamodendron dinisii*, *Matricaria chamomilla*, *Myroxylon peruiferum*, *Salvia sclarea*, *Bulnesia sarmientoi*, *Ferula galbaniflua*, *Siparuna guianensis* and *Melissa officinalis* were the most active against *L. amazonensis* with IC₅₀/24 h ranging from 54.05 to 162.25 µg/mL. Analysis of EOs by GC–MS showed mainly the presence of β-farnesene (52.73 %) and bisabolol oxide (12.09 %) for *M. chamomilla*; α-copaene (13.41 %), saffrole (8.35 %) and δ-cadinene (7.08 %) for *M. peruiferum*; linalool (28.80 %) and linalyl acetate (60.08 %) for *S. sclarea*; guaïol (48.29 %) and 2-undecanone (19.49 %) for *B. sarmientoi*; ethyl phthalate (13.09 %) and methyl-8-pimaren-18-oate (41.82 %) for *F. galbaniflua*; and neral (37.18 %) and citral (5.02 %) for *M. officinalis*.

Conclusion: The EO from *F. galbaniflua* showed to be effective against *L. amazonensis* promastigotes forms and presented low cytotoxic activity against L6 cells. Thus, it represents a strong candidate for future studies aiming its molecular activity on these pathogenic parasites.

Keywords: Secondary metabolites, Anti-*Leishmania*, Natural products, *Ferula galbaniflua*

Background

Leishmaniasis, the third most important vector-borne diseases, is caused by a protozoan parasite of the genus *Leishmania*, which is transmitted to human by the bite of sand flies. Leishmaniasis represents a complex disease with diverse clinical manifestations and poses a public health problem since it is a neglected tropical disease

with current high worldwide incidence [1, 2]. Globally, more than 12 million individuals are infected, with another 350 million at risk of infection, and nearly 2 million new cases are reported annually worldwide [3]. The disease is prevalent in 16 developed and 72 developing countries; nevertheless 90 % of cases are reported in three regions: Sudan/Ethiopia/Kenya, India/Bangladesh/Nepal and Brazil with as many as 0.02 to 0.04 million deaths every year [3, 4].

Leishmaniasis can be divided into three forms, varying in severity from self-healing cutaneous lesions, dermatological ulcers in cutaneous leishmaniasis (CL), destructive

* Correspondence: dourado@unb.br

¹Pathogen-Host Interface Laboratory, Department of Cell Biology, The University of Brasília, Campus Darcy Ribeiro, Bloco I, Brasília, DF CEP 70910-900, Brazil

Full list of author information is available at the end of the article



form of mucocutaneous leishmaniasis, to deadly form of visceral leishmaniasis (VL) [5]. CL is characterized by ulcers on the skin that are often formed at the site of the insect vector bite. Those ulcers can undergo metastasis of the nasopharyngeal mucosa developing to tissue destruction, depending on the species of *Leishmania* involved [6]. *Leishmania (Viannia) braziliensis* and *Leishmania (V.) panamensis* are responsible for cases of mucocutaneous leishmaniasis in the Americas, although *L. (V.) guyanensis* and *L. (L.) amazonensis* have been identified, especially, in immuno-compromised hosts [6].

The first-line drugs for systemic treatment of leishmaniasis are parenterally administered antimonials such as the sodium stibogluconate (Pentostam®) and the N-methyl glucamine antimoniate (Glucantime®) [7, 8] generally required for the treatment of CL in the New World due to the risk of mucosal involvement [9]. This current chemotherapy presents several issues such as high cost, difficult administration and elevated toxicity, associated with serious side effects [10], for instance musculoskeletal pain, gastrointestinal disturbances, mild to moderate headache, electrocardiographic QTs interval prolongation and mild to moderate increase of liver and pancreatic enzymes [11]. Second-line drug Pentamidine and amphotericin B are not widely used due to their high toxicity and cost. Miltefosine, the first oral antileishmanial drug, is the treatment of choice for diffuse cutaneous leishmaniasis and New World cutaneous leishmaniasis caused by *Leishmania braziliensis* but increasing resistance to this drug has been notified [12].

All antileishmanial drugs except miltefosine have to be administrated parenterally. Most of these drugs are toxic, requires prolonged hospitalization and close monitoring, which makes the treatment costly and beyond the reach of most patients. Consequently, the development of alternative therapies is a priority for the treatment of leishmaniasis. As a strategy, the investigation of extracts and compounds, with biological activity, isolated from plants and used in traditional medicine is a promising in the research field for compounds with potential action for the prophylaxis and chemotherapy of CL [13].

Essential oils (EOs) are complex mixtures of secondary metabolites isolated from plants. In these mixtures, there are 10–60 constituents at different concentrations, but usually only 2–3 major constituents determine the biological properties of the EO [14]. Those compounds and their constituents present a broad pharmacological spectrum, and they are used as analgesics, sedatives, anti-inflammatory, and anti-spasmodic drugs, as well as antimicrobials, antiprotozoals and antihelmintics [13, 15, 16]. It has been shown that several EOs or their constituents have inhibitory activity on protozoa, especially *Leishmania* [17–19]. For instance, Santos and colleagues demonstrated that copaiba oil from *Copaifera martii* is a safer,

shorter, less-expansive, and more easily administered antileishmanial drug [18]. Therefore, the purpose of this present work was to analyze the effect of sixteen EOs biological potential on *L. amazonensis* promastigotes forms and L6 cells and chemical constitution, by GC-MS, of those EOs that showed better leishmanicidal results.

Methods

Essential oils

EOs of *Litsea cubeba* fruits; *Lavandula officinalis*, *Matricaria chamomilla* and *Cananga odorata* flowers; *Elettaria cardamomum* seeds; *Cinnamomum camphora*, *Myroxylon peruiferum* and *Bulnesia sarmientoi* barks; *Ferula galbaniflua* resin; *Salvia sclarea*, *Foeniculum officinalis*, *Cordia verbenaceae*, and *Melissa officinalis* leaves; *Pelargonium graveolens* leaves and stems were purchased from QUINARI Cosmetic and Fragrances Inc. (Maringá-PR, Brazil) with lot number 0717/05209/F. EOs of *Cinnamodendron dinisii* and *Siparuna guianensis* leaves were obtained as described by Andrade et al. [20].

Firstly, EOs and Amphotericin B 250 µg/mL (Sigma-Aldrich, St. Louis, USA) were diluted in dimethylsulfoxide (DMSO) at 100 mg/mL and 50 µg/mL, respectively. For use, the stock was diluted 5:100 in either Schneider (Sigma-Aldrich) or RPMI-1640 (Sigma-Aldrich) media (sub stock). DMSO final concentration in the experiments never exceeded 0.5 %, a concentration that is not harmful to parasites and L6 cells [13]. Stocks were stored at 4 °C in the dark, to avoid degradation [21]. The sub stock was freshly prepared before use.

Chemical composition of EOs

Gas chromatography–mass spectrometry (GC–MS) analysis was performed using a Shimadzu GC-2010 gas chromatograph coupled with GCMS-QP2010 Plus equipped with auto sampler (model AOC-20i, Shimadzu, Columbia, MD, USA) and GC–MS Solution software. Investigation was performed with a Rtx-5MS capillary column (30 mm × 0.25 mm × 0.25 µm) at programmed temperature ranging from 60 to 250 °C at 3 °C/min. Analysis conditions were: injector temperature 250 °C, ion source interface temperature 300 °C, analysis of masses between 40–350 m/z, electron impact at 70 eV, column head pressure at constant pressure of 59 kPa, column flow 1.02 mL/min, gas linear velocity: 36.8 cm/s, carrier gas: helium, injected volume 1 µL (1:1000 in hexane) in splitless. Constituents of EOs were identified by comparing their mass spectral pattern and retention indexes (RI) relative to a standard n-alkane series (C₉–C₂₄) with those known in the literature and the Wiley W9N08 database [13, 22].

Parasites and culture conditions

The promastigotes forms of *L. amazonensis* (strain MHOM/BR/77/LTB0016) were maintained at 28 °C in Schneider medium supplemented with 10 % fetal bovine serum (FBS) and 100 µg/mL gentamicin, with weekly passages. For the screening of EOs biological potential, promastigotes were collected from cultures at the mid-log phase of growth (3-day-old culture). The parasite strain was obtained from Fiocruz-COLPROT (Coleção de Protozoários da Fiocruz).

Antileishmanial activity of essential oils *in vitro*

EOs serial dilutions, from 500 to 31.25 µg/mL, were prepared on a 96-well cell culture plate. Afterwards, 1.35×10^6 parasite/mL culture resuspended in 150 µL were added to the plates and incubated for 24 h at 28 °C. After this period, 20 µL of Resazurin solution were added to a 2 mM final concentration in all wells [23, 24]. The plates were incubated for further 4 h at 37 °C followed by the fluorescence measurement under 570 nm_{ex}/595 nm_{em} in the microplate reader SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA).

In vitro experiment was performed in triplicate and repeated twice independently. DMSO was used as control in the same final concentration found in each dilution. Amphotericin B was used as positive control at final concentration ranging from 312.5 to 19.56 ng/mL.

The percentage of viable promastigotes cells was determined by the equation [25]:

$$\%P = (100 \times Fa)/Fc$$

Where %P: percentage of viable promastigotes cells; Fc: control fluorescence units; Fa: fluorescence units emitted by the analyzed samples (with inhibitor).

Cytotoxic activity of essential oils *in vitro*

Uninfected L6 cell monolayers were washed with Phosphate Buffered Saline (PBS) for 5 min at 37 °C, washed with RPMI medium pH 7.4 + 2.5 % FBS, centrifuged at 200 g for 10 min at 4 °C, resuspended in the same medium and, finally, seeded into 96-well plates (5×10^4 cells/well). Plates were incubated at 37 °C for 24 h, then the medium was removed and cells were washed with PBS. Diluted EOs were added to overnight-adhered L6 cells and incubated for more 24 h at 37 °C. Cell viability was assessed by 2 mM Resazurin as described above.

The percentage of viable cells was determined by the equation [25]:

$$\%V = (100 \times Fa)/Fc$$

Where %V is the percentage of viable cells, Fc: control fluorescence units; Fa: fluorescence units emitted by the

analyzed samples (with inhibitor). The selectivity index (SI) was calculated by dividing CC₅₀ for the IC₅₀.

Statistical analysis

For both *in vitro* EOs biological potential, a randomized complete block design (RBD) test was used, with 5 concentrations, 3 repetitions and 2 experiments (blocks) for each sample. The statistical program used was SISVAR [26]. Data were submitted to analysis of variance and the averages compared by Scott-Knott test and regression, both 5 % probability. The adjusted equations were used to calculate the concentration needed to cripple 50 % of L6 cells (CC₅₀) or 50 % of the parasites (IC₅₀).

Results

Antileishmanial and cytotoxic activity of essential oils *in vitro*

Growth inhibitory activity by the selected EOs was performed on *L. amazonensis* promastigotes forms at concentrations ranging from 30 to 500 µg/mL. In the test, the EOs of *L. cubeba*, *E. cardamomum*, *L. officinalis*, *C. camphora* and *C. odorata* did not show activity at 500 µg/mL (Table 1). Lower concentrations of the remaining EOs were then evaluated to estimate the IC₅₀/24 h (Table 1). The most effective EO was of the one from *S. guianensis* (48.55 ± 3.64 µg/mL), followed by *C. dinisii* (54.05 ± 4.88 µg/mL), *M. chamomilla* (60.16 ± 4.24 µg/mL), *C. verbenaceae* (64.75 ± 2.04 µg/mL), *B. sarmientoi* (85.56 ± 3.38 µg/mL), *F. galbaniflua* (95.70 ± 1.82 µg/mL), *M. officinalis* (132.02 ± 3.14 µg/mL), *M. peruiferum* (162.25 ± 1.57 µg/mL), *S. sclarea* (325.92 ± 8.58 µg/mL), *F. officinalis* (328, 28 ± 6,80 µg/mL) and *P. graveolens* (363.71 ± 6.77 µg/mL). The IC₅₀/24 h of Amphotericin B was 0.83 ± 0.03 µg/mL (Table 1).

The cytotoxicity against L6 cells and *L. amazonensis* were compared using the selectivity index (SI) (Table 1). Higher values of SI means more promising compounds for developing antileishmanial drugs. The SI measures the compound's level of selectivity towards *L. amazonensis*. Evaluation of cytotoxicity showed that the least cytotoxic EO was that of *C. camphora* (CC₅₀/24 h = > 500.00 µg/mL), followed by *E. cardamomum* (439.57 ± 2.27 µg/mL), *L. officinalis* (377.56 ± 8.91 µg/mL), *F. galbaniflua* (377.26 ± 2.71 µg/mL), *S. sclarea* (375.37 ± 3.62 µg/mL), *P. graveolens* (368.39 ± 3.90 µg/mL), *F. officinalis* (368.27 ± 3.81 µg/mL), *M. officinalis* (297.45 ± 1.32 µg/mL), *L. cubeba* (180.72 ± 1.37 µg/mL), *M. chamomilla* (173.04 ± 1.24 µg/mL), *B. sarmientoi* (163.46 ± 1.77 µg/mL), *M. peruiferum* (160.80 ± 1.62 µg/mL), *C. odorata* (142.80 ± 1.76 µg/mL), *C. verbenaceae* (130.00 ± 1.08 1.77 µg/mL), *C. dinisii* (106.31 ± 2.23 µg/mL) and the most cytotoxic EO was of the one from *S. guianensis* (78.02 ± 1.19 µg/mL) (Table 1).

EOs with higher selectivity indexes were those from *F. galbaniflua* (3.94), *M. chamomilla* (2.87) and *M. officinalis*

Table 1 EOs biological potential and selectivity indexes (SI) for *L. amazonensis* (IC₅₀/24 h) promastigotes and L6 cells (CC₅₀/24 h)

Essential oils	<i>L. amazonensis</i> IC ₅₀ ^a ± DP (µg/mL)	L6 cells CC ₅₀ ^f ± DP (µg/mL)	SI ^g
<i>Litsea cubeba</i>	NI ^e	180.72 ± 1.37	-
<i>Matricaria chamomilla</i>	60.16 ± 4.24	173.04 ± 1.24	2.87
<i>Elettaria cardamomum</i>	>500.00 ^b	439.57 ± 2.27	-
<i>Lavandula officinalis</i>	>500.00	377.56 ± 8.91	-
<i>Cinnamomum camphora</i>	>500.00	>500.00	-
<i>Myroxylon peruiferum</i>	162.25 ± 1.57	160.80 ± 1.62	0.99
<i>Salvia sclarea</i>	325.92 ± 8.58	375.37 ± 3.62	1.15
<i>Bulnesia sarmientoi</i>	85.56 ± 3.38	163.46 ± 1.77	1.91
<i>Ferula galbaniflua</i>	95.70 ± 1.82	377.26 ± 2.71	3.94
<i>Pelargonium graveolens</i>	363.71 ± 6.77	368.39 ± 3.90	1.01
<i>Cananga odorata</i>	NI	142.80 ± 1.76	-
<i>Foeniculum officinalis</i>	328.28 ± 6.80	368.27 ± 3.81	1.12
<i>Cordia verbenaceae</i>	64.75 ± 2.04	130.00 ± 1.08	2.01
<i>Melissa officinalis</i>	132.02 ± 3.14	297.45 ± 1.32	2.25
<i>Siparuna guianensis</i>	48.55 ± 3.64	78.02 ± 1.19	1.60
<i>Cinnamodendron dinisii</i>	54.05 ± 4.88	106.31 ± 2.23	1.97
Anfotericina B ^c	(0.083 ± 0.003 µg/mL)	NI	-
DMSO ^d	NI	NI	-

^a IC₅₀ ± DP: the concentration able to cripple 50 % of the parasites ± standard deviation

^b > 500.00: IC₅₀ greater than the highest concentration tested

^c Amphotericin B - positive control

^d DMSO - negative control

^e NI: no inhibition

^f CC₅₀: the concentration able to cripple 50 % of cells after 24 h of treatment ± standard deviation

^g Selectivity index - SI = CC₅₀ L6 / IC₅₀ promastigotes

(2.25), but all were more cytotoxic and less selective than Amphotericin B, because the reference drug did not show CC₅₀/24 h value.

Chemical composition

The GC-MS analyses were performed for EOs that showed the lower values of IC₅₀ and/or the higher SI values (Table 2). The analysis identified the main constituents as β-farnesene (52.73 %), bisabolol oxide (12.09 %), α-farnesene (10.34 %) for *M. chamomilla*; α-copaene (13.41 %), guaial (9.35 %), safole (8.35 %) and δ-cadinene (7.08 %) for *M. peruiferum*; linalool (28.80 %) and linalyl acetate (60.08 %) for *S. sclarea*; guaial (48.29 %) and 2-undecanone (19.49 %) for *B. sarmientoi*; ethyl phthalate (13.09 %) and methyl-8-pimaren-18-oate (41.82 %) for *F. galbaniflua*; and neral (37.18 %) and geranial (5.02 %) for *M. officinalis*. According to Andrade et al. [20], EO from *C. dinisii* fresh leaves is composed mainly by α-pinene (35.41 %), β-pinene (17.81 %), sabinene (12.01 %) and bicyclogermacrene (7.59 %). EO from *S. guianensis* fresh leaves contains β-myrcene (13.14 %), germacrene-D (8.68 %) and bicyclogermacrene (16.71 %).

Discussion

According to the classification of cytotoxicity and antileishmanial activity for extracts and fractions derived from plants and natural products defined by Study Program and Disease Control [27], the evaluated EOs are classified as moderately toxic (100 < CC₅₀ ≤ 1000 µg/mL), except the EO from *S. guianensis*, which was classified as toxic (10 < CC₅₀ ≤ 100 µg/mL). Regarding the antileishmanial activity only EOs from *S. guianensis*, *C. dinisii*, *M. chamomilla*, *C. verbenaceae*, *B. sarmientoi*, *F. galbaniflua* and *M. officinalis* are considered moderately active (50 < IC₅₀ ≤ 150 µg/mL). The others are considered not active.

Considering the chemical composition of the EO from *M. chamomilla*, (E)-β-farnesene and (E,E)-α-farnesene were found as the major compound group representing 73.07 % of the total composition. These results corroborate with those reported by Machado et al. [28] that found farnesene derivatives as the most representative constituents (22 %) and their bioassays using EO from *Lantana camara* revealed a significant leishmanicidal activity against *L. amazonensis* (IC₅₀/72 h = 0.25 µg/mL), except for the cytotoxic activity, in which the authors

Table 2 Chemical composition of selected essential oils

Content (%)										
IRc	IRI	Component	<i>Matricaria chamomilla</i>	<i>Myroxylon peruiferum</i>	<i>Salvia sclarea</i>	<i>Bulnesia sarmientoi</i>	<i>Ferula galbaniflua</i>	<i>Melissa officinalis</i>	<i>Siparuna guianensis</i> ^a	<i>Cinnamodendron dinisia</i> ^a
930	932	α-pinene	–	–	–	–	–	–	1.83	35.41
958	963	sabinene	–	–	–	–	–	–	–	12.01
961	970	β-pinene	–	–	–	–	17.34	–	–	17.81
969	971	6-metil-5-hepten-2-one	–	–	–	–	–	3.88	–	–
975	0980	β-myrcene	–	–	1.23	–	–	–	13.14	1.46
1018	1024	1,8-cineole	–	–	–	8.71	3.71	–	–	4.37
1086	1092	linalool	–	–	28.80	2.67	–	–	–	–
1135	1130	α-terpineol	–	–	5.14	–	–	–	–	–
1231	1235	neral	–	–	–	–	–	37.18	–	–
1247	1252	linalyl acetate	–	–	60.08	2.64	–	–	–	–
1263	1264	geranial	–	–	–	–	–	52.02	–	–
1282	1285	safrole	–	8.35	–	–	–	–	–	–
1287	1293	2-undecanone	–	–	–	19.49	–	–	1.69	–
1374	1374	α-copaene	–	13.41	–	–	–	–	–	–
1428	1432	trans-α-bergamotene	–	3.48	–	–	–	–	–	–
1443	1439	(+)-aromadendrene	–	2.27	–	1.26	–	–	–	–
1451	1455	(E)-β-farnesene	52.73	–	–	–	–	–	–	–
1455	1458	allo-aromandrendene	–	5.24	–	–	–	–	–	–
1470	1474	γ-gurjunene	–	5.29	–	–	–	–	–	–
1476	1482	germancrene-D	3.42	–	–	–	–	–	8.68	–
1478	1479	ar-curcumene	–	5.05	–	–	–	–	–	–
1481	1487	β-selinene	–	3.27	–	–	–	–	–	–
1488	1497	bicyclogermancrene	–	–	–	–	–	–	16.71	7.59
1504	1505	(E,E)-a-farnesene	10.34	–	–	–	–	–	–	–
1504	1507	β-bisabolene	–	2.09	–	–	–	–	–	–
1510	1511	δ-amorfene	–	6.59	–	–	–	–	–	–
1520	1522	δ-cadinene	–	7.08	–	–	–	–	1.04	0.14
1576	1577	(–)-spathulenol	–	–	–	5.79	–	–	4.16	1.88
1594	–	diethyl phthalate	–	–	–	–	13.09	–	–	–
1601	1600	Guaiol	–	9.35	–	48.29	–	–	–	–
1630	–	(–)-sinularene	–	5.81	–	–	–	–	–	–
1652	1649	β-eudesmol	–	2.10	–	–	–	–	–	–
1656	1656	bisabolol oxide B	12.09	–	–	–	–	–	–	–
1657	1658	t-cadinol	–	–	–	1.35	–	–	4.14	–
1685	1685	α-bisabolol	9.83	–	–	–	–	–	3.35	–
1732	–	camazulene	2.30	–	–	–	–	–	–	–
–	–	elixene	–	–	–	–	5.87	–	–	–
–	–	methyl 8 (14)-pimaren-18- oate	–	–	–	–	41.82	–	–	–
–	–	NI	–	–	–	–	9.39	–	–	–
		Total identified (%)	90.72	79.38	94.05	88.85	81.83	93.08	55.25	80.67

IRI literature retention rate [22], IRc retention ratio calculated by Kovats' equation. a) Described by Andrade et al. [20]

obtained high values on Brine shrimp (CC_{50} 10 $\mu\text{g/mL}$). Subsequently, Gawde et al. [29] observed that the chemical composition of *M. chamomilla* was similar to the one found in our study (β -farnesene, α -bisabolol oxide B, chamazulene) but no leishmanicidal activity on *L. donavani* was observed.

Studies on the chemical composition and biological activity of *M. peruiiferum* EO are scarce. The literature reports (E) and (Z)-nerolidol, α -bisabolol and (E, E)-farnesol as its major components [30] but those compounds were not identified in the present study. Santos et al. [18] reported high levels of α -copaene in EO from *Copaifera reticulata* as well as for EO from *M. peruiiferum*. The last one showed growth inhibitory activity for *L. amazonensis* with $IC_{50}/72$ h values of 5 $\mu\text{g/mL}$ for promastigotes and low cytotoxicity on J774G8 macrophages.

Ghannadi and Amree [31] have already described the EO composition obtained from the fresh oleogum resin and latex of Iranian *F. galbaniflua* (synonym *F. gummosa*) and the main constituents of this monoterpene rich oil were β -pinene (58.8 %). Other studies also indicate β -pinene as the major compound from the fresh oleogum resin and latex of this same specie [32, 33], which corroborates our results. The presence of methyl 8-(14)-pimaren-18-ate, a diterpene esters hydrocarbons, has been reported on rosin, a solid form of resin obtained from pines and some other plants; and also in the Cretaceous resins from India and Myanmar [34, 35]. To our knowledge, there is no antileishmanial activity reports related to this EO to date.

Rodilla et al. [36] determined the chemical composition of EO from *B. sarmientoi*. In accordance with our work, they identified guaiol as its major component. Studies with EO from *Endlicheria bracteolata*, which has 72.12 % of guaiol in its composition, showed IC_{50} of 7.93 $\mu\text{g/mL}$ for *L. amazonensis* and presented a CC_{50} of 15.14 $\mu\text{g/mL}$ for J774.G8 macrophages [37]. The antileishmanial activity may be attributed to the presence of a hydroxyl group of alcohol characteristics in the guaiol, especially in the exocyclic portion of the molecule [36].

The presence of linalyl acetate and linalool as the major compounds in *S. sclarea* EO (total of 88.88 %) corroborate to the results presented by Pitarokili et al. [38] that evaluated the EO composition of *S. sclarea* originated from two localities in Greece, and by Kuźma et al. [39] that evaluated the EO composition from *S. sclarea* plants generated *in vitro*. On the other hand, antileishmanial activity of linalool-rich EO from leaves of *Croton cajucara* against *L. amazonensis* was previously evaluated by Rosa et al. [40], they were able to demonstrate morphological changes in *L. amazonensis* promastigotes when treated with 15 ng/mL of that EO. In this study the cell lysis was observed within 1 h, indicating that the antileishmanial activity observed is

directly related to the presence of linalool, due to the existence of a hydroxyl group in the organic alcohol function.

As in our study, the presence of the isomers of citral, nerol and geranial are constantly reported in the chemical composition of the EO from *M. officinalis* [41–43]. Regarding the antileishmanial activity, Mikus et al. [44] reported an $IC_{50}/72$ h of 7 $\mu\text{g/mL}$ for *L. major*, a $CC_{50}/72$ h of 25.5 $\mu\text{g/mL}$ in HL-60 cells and SI of 3.6, higher than those observed in our study. Another study has already showed that citral presents activity against *T. cruzi*, possibly by inducing cell membrane lysis with leakage of cytoplasm [45].

The EO from *C. dinisii* and *S. guianensis* showed weak inhibitory effect on the protozoan *T. cruzi* with values of $IC_{50}/24$ h = 209.30 $\mu\text{g/mL}$ and 282.93 mg/mL , respectively. These values are higher when compared to those obtained in the study for *L. amazonensis*, 54.05 and 48.55 $\mu\text{g/mL}$, respectively [25].

The mechanism of action by which EOs inhibits parasite growth is still not well known, but previous studies have suggested that structural and morphological changes are caused by drugs that inhibit ergosterol synthesis, or interact with the membrane ergosterol [19, 46]. Other studies indicated that the activity of essential oils on parasites is mainly due to terpene composition. Terpenes are responsible for the hydrophobic characteristic of EOs, thus allowing their diffusion through the parasite cell membrane, affecting intracellular metabolic pathways and organelles [47].

Conclusion

F. galbaniflua EO is effective against *L. amazonensis* promastigotes forms and has low cytotoxic activity. Thus, it represents a strong candidate for future studies in order to comprehend its biological activity against *L. amazonensis*.

The promising results of this study offer prospects for further research, as the evaluation of the antileishmanial potential of the major compounds and the elucidation of their molecules may, in the future, contribute to the discovery of effective drugs derived from plants for the treatment of parasitic diseases.

Acknowledgements

The authors are indebted with Central Analítica do Instituto de Química da Universidade de Brasília (CAIQ/UnB) for GC-MS analysis.

Funding

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES): Programa Nacional de Incentivo a Pesquisa em Parasitologia Básica (CAPES grant no: 23038.005298/2011-83); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Chamada 79/2013-MCTI/CNPq/FNDCT); Fundação de Amparo à Pesquisa do Distrito Federal (FAP-DF- 193.001.076/2015); PGCTS-FCE/UNB; Financiadora de Estudos e Projetos (FINEP); Decanato de Pesquisa e Pós-graduação/UnB (DPP/UnB).

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

IMDB and JMS conceived the research idea. MAA, CSA and CLS conducted the experiment, analyzed and interpreted the data as well as prepared the first draft. MLS supported the conduct of the analysis and interpreted the data of CG-MS. IMDB, JMS, FNM, and CSA critically read and revised the paper. All authors have read and approved the paper before its final submission.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Author details

¹Pathogen-Host Interface Laboratory, Department of Cell Biology, The University of Brasília, Campus Darcy Ribeiro, Bloco I, Brasília, DF CEP 70910-900, Brazil. ²Faculty of Ceilândia, The University of Brasília, Brasília, Brazil. ³Institute of Chemistry, The University of Brasília, Brasília, Brazil.

Received: 20 April 2016 Accepted: 13 October 2016

Published online: 08 November 2016

References

- Homsy Y, Makdasi G. Leishmaniasis: a forgotten disease among neglected people. *Internet J Health*. 2009;11:1–5. <http://ispub.com/IJH/11/2/9151>. Accessed 04 Apr 2016.
- de Vries HJ, Reedijk SH, Schallig HD. Cutaneous leishmaniasis: recente developments in diagnosis and management. *Am J Clin Dermatol*. 2015;16:99–109.
- WHO. World Health Organization. Investing to overcome the global impact of neglected tropical diseases: 3rd WHO report on neglected diseases. 2015. http://apps.who.int/iris/bitstream/10665/152781/1/9789241564861_eng.pdf. Accessed 04 Apr 2016.
- Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*. 2012;7:e35671.
- Ejaz A, Raza N, Iftikhar N. Recurrent cutaneous leishmaniasis presenting as sporotrichoid abscesses: a rare presentation near Afghanistan border. *Dermatol Online J*. 2007; 13:15. <http://www.ncbi.nlm.nih.gov/pubmed/17498434>. Accessed 04 Apr 2016.
- WHO. World Health Organization. Technical Report Series 949—control of leishmaniasis. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis, Geneva, 22–26 March 2010. http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf. Accessed 04 Apr 2016.
- Name RQ, Borges KT, Nogueira LSC, Sampaio JHD, Tauil PL, Sampaio RNR. Clinical, epidemiological and therapeutic study of 402 patients with american cutaneous leishmaniasis attended at University Hospital of Brasília, DF, Brazil. *Ann Bras Dermatol*. 2005;80:249–54.
- González U, Pinart M, Rengifo-Pardo M, Macaya A, Alvar J, Tweed JA. Interventions for American cutaneous and mucocutaneous leishmaniasis. *Cochrane Database Syst Rev*. 2009;2:1–175. <http://www.ncbi.nlm.nih.gov/pubmed/19370612>. Accessed 04 Apr 2016.
- Minodier P, Parola P. Cutaneous leishmaniasis treatment. *Travel Med Infect Dis*. 2007;5:150–8.
- Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. *Lancet Infect Dis*. 2007;7:581–96.
- Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A, Ben Alaya N, Bettaieb J. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. *N Engl J Med*. 2013;368:524–32.
- Utaile M, Kassahun A, Abebe T, Hailu A. Susceptibility of clinical isolates of *Leishmania aethiopia* to miltefosine, paromomycin, amphotericin B and sodium stibogluconate using amastigote-macrophage *in vitro* model. *Exp Parasitol*. 2013;134:68–75.
- Azeredo CMO, Santos TG, Maia BHLNS, Soares MJ. *In vitro* biological evaluation of eight different essential oils against *Trypanosoma cruzi*, with emphasis on *Cinnamomum verum* essential oil. *BMC Complementary Altern Med*. 2014;14:309–15.
- Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - A review. *Food Chem Toxicol*. 2008;46:446–75.
- Singh G, Maurya S, Lampasona MP, Catalan CAN. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food Chem Toxicol*. 2007;45:1650–61.
- Victoria FN, Lenardão EJ, Savegnago L, Perin G, Jacob RG, Alves D, Da Silva WP, Motta AS, Nascente OS. Essential oil of the leaves of *Eugenia uniflora* L.: antioxidant and antimicrobial properties. *Food Chem Toxicol*. 2012;50:2668–74.
- Ueda-Nakamura T, Mendonça-Filho RR, Morgado-Díaz JA, Maza PK, Prado Dias Filho B, Cortez DAD, Alviano DS, Rosa MS, Lopes AH, Alviano CS, Nakamura CV. Antileishmanial activity of eugenol-rich essential oil from *Ocimum gratissimum*. *Parasitol Int*. 2006;55:99–105.
- Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Junior VF, Pinto AC, Nakamura CV. Effect of Brazilian copaiba oils on *Leishmania amazonensis*. *J Ethnopharmacol*. 2008;120:204–8.
- Medeiros MGF, Silva AC, Citó AMGL, Borges AR, Lima SG, Lopes JAD, Figueiredo RCBQ. *In vitro* antileishmanial activity and cytotoxicity of essential oil from *Lippia sidoides* Cham. *Parasitol Int*. 2011;60:237–41.
- Andrade MA, Cardoso MG, Andrade J, Silva LF, Teixeira ML, Resende JV, Figueiredo ACS, Barroso J. Chemical Composition and Antioxidant Activity of Essential Oils from *Cinnamodendron dinisii* Schwacke and *Siparuna guianensis* Aublet. *Antioxidants*. 2013;2:384–97.
- Guimarães LGL, Cardoso MG, Zacaroni LM, Lima RK, Pimentel F, Morais AR. Influência da luz e da temperatura sobre a oxidação do óleo essencial de capim-limão (*Cymbopogon citratus* (D.C.) STAPP). *Quim Nova*. 2008;31:1476–80.
- Adams RP. Identification of Essential oil Components by Gas Chromatography/Mass Spectroscopy. 4th ed. IL: Allured Publishing Corporation; 2007.
- O'Brien J, Wilson I, Orton T, Pognan F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur J Biochem*. 2000;267:5421–6.
- Raz B, Iten M, Grether-Buhler Y, Kaminsky R, Brun R. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T. b. rhodesiense* and *T. b. gambiense*) *in vitro*. *Acta Trop*. 1997;68:139–47.
- Andrade MA, Cardoso MG, Gomes MS, Azeredo CMO, Batista LR, Soares MJ, Rodrigues LMA, Figueiredo ACS. Biological Activity of the Essential Oils from *Cinnamodendron dinisii* and *Siparuna guianensis*. *Braz J Microbiol*. 2015;46:189–94.
- Ferreira DF. SISVAR: A computer statistical analysis system. *Cienc Agrotec*. 2008;6:36–41. doi:10.1590/S1413-70542011000600001. Accessed 04 Apr 2016.
- Rios YK, Otero AC, Muñoz DL, Echeverry M, Robledo SM, Yepes MA. Actividad citotóxica y leishmanicida *in vitro* del aceite de manzanilla (*Matricaria chamomilla*). *Rev Colomb Cienc Quím Farm*. 2008;37:200–11. <http://www.revistas.unal.edu.co/index.php/rccquifa/article/view/15254/16051>. Accessed 04 Apr 2016.
- Machado RRP, Valente Júnior W, Lesche B, Coimbra ES, Souza NB, Abramo C, et al. Essential oil from leaves of *Lantana camara*: a potential source of medicine against leishmaniasis. *Rev Bras Farmacogn*. 2012;22:1011–7.
- Gawde A, Cantrell CL, Zheljzakov VD, Astatkie T, Schlegel V. Steam distillation extraction kinetics regression models to predict essential oil yield, composition, and bioactivity of chamomile oil. *Ind Crops Prod*. 2014;58:61–7.
- Wanner J, Schmidt E, Bail S, Jirovetz L, Buchbauer G, Gochev V, et al. Chemical composition and antibacterial activity of selected essential oils and some of their main compounds. *Nat Prod Commun*. 2010;5:1359–64. <http://www.ncbi.nlm.nih.gov/pubmed/20922991>. Accessed 04 Apr 2016.
- Ghannadi A, Amree S. Volatile oil constituents of *Ferula gummosa* Boiss. from Kashan, Iran. *J Essent Oil Res*. 2002;14:420–42.
- Kouyakhi ET, Naghavi MR, Alayhs M. Study of the essential oil variation of *Ferula gummosa* samples from Iran. *Chem Nat Compounds*. 2008;44:124–6.
- Sahebkar A, Iranshahi M. Volatile Constituents of the Genus *Ferula* (Apiaceae): A Review. *J Essent Oil-Bear Plants*. 2011;14:504–31.
- Chang T, Mead TE, Zinkel DF. Mass Spectra of Diterpene Resin Acid Methyl. *J Am Oil Chem Soc*. 1970. [presented in part at the Meeting of the American Chemical Society, Chicago. 1970. http://link.springer.com/article/10.1007/BF02544660](http://link.springer.com/article/10.1007/BF02544660). Accessed 05 Apr 2016.
- Dutta S, Mallick M, Kumar K, Mann U, Greenwood PF. Terpenoid composition and botanical affinity of Cretaceous resins from India and Myanmar. *Int J Coal Geol*. 2011;85:49–55.
- Rodilla JM, Silva LA, Martinez N, Lorenzo D, Davyt D, Castillo L, Giménez C, Cabrera R, González-Coloma A, Zrostliková J, Dellacassa E. Advances in the identification and agrochemical importance of sesquiterpenoids from *Bulnesia sarmientoi* essential oil. *Ind Crops Prod*. 2011;33:497–503.

37. Rottini MM, Amaral ACF, Ferreira JLP, Silva JRA, Taniwaki, Souza CSF, d'Escoffier LN, Almeida-Souza F, Hardoim DJ, Costa SCG, Calabrese KS. *In vitro* evaluation of (–) α -bisabolol as a promising agent against *Leishmania amazonensis*. *Exp Parasitol*. 2015;148:66–72.
38. Pitarokili D, Couladis M, Petsikos-Panayotarou N, Tzakou O. Composition and antifungal activity on soil-borne pathogens of the essential oil of *Salvia sclarea* from Greece. *J Agric Food Chem*. 2002;50:6688–91.
39. Kuźma L, Kalemba D, Różalski M, Różalska B, Więckowska-Szakiel M, Krajewska U, Wysokińska H. Chemical composition and biological activities of essential oil from *Salvia sclarea* plants regenerated *in vitro*. *Molecules*. 2009;14:1438–47.
40. Rosa MSS, Mendonça-Filho RR, Bizzo HR, Rodrigues IA, Soares RMA, Souto-Padrón T, Alviano CS, Lopes AHCS. Antileishmanial activity of a linalool-rich essential oil from *Croton cajucara*. *Antimicrob Agents Chemother*. 2003;47:1895–901.
41. Sadraei H, Ghannadib A, Malekshahia K. Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. *Fitoterapia*. 2003;74:445–52.
42. Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and Antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. *J Agric Food Chem*. 2004;52:2485–9.
43. Silva S, Sato A, Lage CLS, Gil S, Silva RA, Azevedo, Almeida D, Esquibel MA. Essential oil composition of *Melissa officinalis* L. *in vitro* produced under the influence of growth regulators. *J Braz Chem Soc*. 2005;16:1387–90.
44. Mikus J, Harkenthal M, Steverding D, Reichling J. *In vitro* effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. *Planta Med*. 2000;66:366–8.
45. Santoro GF, Cardoso MG, Guimarães LGL, Freire JM, Soares MJ. Antiproliferative effect of the essential oil of *Cymbopogon citratus* (DC) Stapf (lemongrass) on intracellular amastigotes, bloodstream trypomastigotes and culture epimastigotes of *Trypanosoma cruzi* (Protozoa: Kinetoplastida). *Parasitology*. 2007;13:1649–56. <http://www.ncbi.nlm.nih.gov/pubmed/17686189>. Accessed 05 Apr 2015.
46. Brenzan MA, Nakamura CV, Filho BPD, Ueda-Nakamura T, Young MCM, Cortez DAG. Antileishmanial activity of crude extract and coumarin from *Calophyllum brasiliense* leaves against *Leishmania amazonensis*. *Parasitol Res*. 2007;101:715–22.
47. Borges AR, Aires JR, Higino TM. Trypanocidal and cytotoxic activities of essential oils from medicinal plants of Northeast of Brazil. *Exp Parasitol*. 2012;132:123–8.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

