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Original Article

Essential Oils of *Rosmarinus officinalis* and *Eucalyptus globulus* Cultivated in the Mountainous Region of the State of Rio de Janeiro (Brazil): Chemical Profile and Antileishmanial Activity

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ABSTRACT

The present study aimed to characterize the chemical profile of essential oils of *Rosmarinus officinalis* (EORo) and *Eucalyptus globulus* (EOEg) cultivated in the mountainous region of the state of Rio de Janeiro, Brazil, and to verify the leishmanicidal potential, as well as to determine the cytotoxicity *in vitro* in mammalian cells. EORo had α-Pinene (37.99%), Cineol (21.48%), D-Verbenone (5.78%) and Limonene (3.28%) as major constituents. The EOEg had as major components Cineol (75.52%), Limonene (8.82%) and o-Cymeno (7.81%). The EOEg had a lower IC_{50} value of 14.03 ± 2.08 µg/mL, while EORo had an IC_{50} value of 31.12 ± 4.6 µg/mL, both during the 24-hr exposure period. In the 48-hr period, the IC_{50} value was almost twice as high with rosemary oil, while in eucalyptus oil there was no significant difference when exposed in this period. The selectivity of EOs was demonstrated in murine cells, showing almost 30 times less toxic to the cell than to the parasite. It can be concluded that this is a pioneering study on the potential of these essential oils against the leishmania parasite. Furthermore, the low toxicity effect on the host cell encourages future studies and *in vivo* applications.

Keywords: *R. officinalis*, *E. globulus*, Essential oil, Cytotoxicity, Leishmanicidal.

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INTRODUCTION

Leishmaniasis is a spectrum of infectious parasitic diseases caused by protozoa of the genus *Leishmania*. The disease is considered as Neglected by the World Health Organization (WHO) because it mainly affects low-income people, there is a lack of investment in research, and few interest from the pharmaceutical industries in the development of new and more effective treatments.[1] Leishmaniasis is endemic in 98 (sub)tropical countries, with about 12 million people infected and more than 350 million people at risk.[2] Approximately 95% of Cutaneous Leishmaniasis (CL) cases occur in the Americas, the Mediterranean region, the Middle East and Central Asia. In 2020, more than 85% of new cases of CL occurred in 10 countries, including the Old World and the Americas.[2]

The use of chemotherapy drugs is still the main option for the treatment of leishmaniasis. Pentavalent antimony is the first choice in the treatment of infection, although greater resistance has been shown in some endemic areas.[3,4] As a second treatment step, miltefosine, amphotericin B and their liposomal formulations have also been used. Several disadvantages limit the use of these chemotherapeutics, such as high cost, serious adverse effects (e.g. cardiotoxicity and hepatotoxicity) and increased parasite resistance.[4,5] Thus, it is necessary and urgent to develop new drugs and therapeutic alternatives for the treatment of leishmaniasis.

Natural products have been used for the treatment of several diseases over the centuries.[6] In this context, plants have been

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Table 1: Instrumental parameters of analysis.

Parameters	Conditions
Column	NA–5MS (5% diphenyl 95% dimethylpolysiloxane)
Column dimensions	60 m x 0.25 mm x 0.25 µm film thickness
Oven programming	50°C (5 min); 5°C min $^{-1}$ till 280°C (10 min)
Flow gas	Helium / 1 mL min^{-1}
Injection volume /Split	1 µL / 1:50 (Split)
Injector temperature	280°C
Interface temperature	280°C
Ion source temperature	300°C
Electron ionization	70 eV
Analysis time	61 min

one of the main sources of drug discovery and development of new compounds for several diseases due to the production of secondary metabolites and bioactive molecules,[7,8] such as Essential Oils (EOs). Chemically, EOs are characterized as a complex mixtures of low molecular weight compounds, being highly volatile and responsible for aromas.[9] The volatile compounds can be easily extracted from secretory glands present on the surface of leaves and flowers or inside roots and stems.[10] Different plant species have shown EOs with potential biological activities, including antiprotozoal,[11,12] which makes the evaluation of its leishmanicidal activity interesting.

Secondary metabolites represent a chemical interface between plants and the surrounding environment, and the biosynthesis of these compounds is often affected by environmental conditions. Thus, in this work, essential oils extracted by hydrodistillation from *Rosmarinus officinalis* and *Eucalyptus globulus* cultivated in the mountainous region of the state of Rio de Janeiro (Brazil) were obtained and the a effect were evaluated *in vitro*.

Rosmarinus officinalis Linnaeus (Lamiaceae), is a woody perennial herb, routinely known as Rosemary, native to the Mediterranean region, but is now cultivated all over the world. It is used as an ornamental and aromatic plant, condiment but also has a pharmacological potential. In folk medicine it is widely used as an anti-inflammatory agent and in pain relief, it is a perennial plant popularly cultivated around the world.[13-15]

Eucalyptus globulus Labill. is an evergreen tree, native to Australia and widely cultivated for the pulp industry.[16] Belonging to the Myrtaceae family, eucalyptus is commonly used to treat asthma and bronchitis. Recent studies have demonstrated antimicrobial, antifungal, anthelmintic and antidiabetic properties for leaf extracts and essential oils.[17]

Therefore, the objective of this study was to characterize the chemical profile of essential oils produced from plants grown in the mountainous region of the state of Rio de Janeiro, Brazil, and evaluate the potential of the antileishmania activity/selectivity, as well as to determine the *in vitro* cytotoxicity of *Rosmarinus officinalis* (EORo) and *Eucalyptus globulus* (EOEg) essential oils.

MATERIALS AND METHODS

Plant Material and Essential oil

Rosmarinus officinalis and *Eucalyptus globulus* were grown at Nova Friburgo, State of Rio de Janeiro, Brazil, and the EOs were produced by Entrefolhas Óleos Essenciais e Produtos Naturais LTDA, in July 2019. Nova Friburgo has a tropical climate of altitude, with cool and dry winters and pleasant and humid summers. The average temperature of the municipality is 18°C, relative humidity of 80% and annual precipitation of 1279.8mm.[18]

Essential Oil Chemical Identification

The analyses were performed using a gas chromatograph (GC/MS from Thermo Scientific, Bremen, Germany), model TRACE 1310 / spectrometer TSQ-9000 mass model, with the TriPlus RSH self-sampling under the chromatographic analysis conditions described in Table 1. The data was acquired in scan mode (SCAN) in the range of 40 to 550 Da *m/z*, and cutting the solvent made at 5.5 min. The characterization of the oils and the attribution of the data was performed from the Thermo software Scientific Chromeleon Chromatography Data System (CDS) (version 7.2.10), The characterization of the oils and the attribution of the data was performed from the Thermo software Scientific Chromeleon Chromatography Data System (CDS) (version 7.2.10), and the compounds were experimentally identified by comparison of spectra, with the databases of the library NIST 05 and based on the comparison of the retention indices of the compounds, calculated from a pattern of n-alkanes from C7 to C24, with the indices available in the literature.

Parasites culture and macrophages Raw 264.7

Leishmania (*Leishmania*) *amazonensis* (MHOM/BR/1973/M2269) was kindly provided by Dra. Clara Lúcia Barbieri from UNIFESP, São Paulo, Brazil. Promastigote forms were cultured at 26°C in 199 medium (Gibco, Life Technologies Brand, Grand Island, NY, United States) supplemented with 4.2 mM sodium bicarbonate, 4.2 mM HEPES, 1 mM adenine (Sigma, St. Louis, MO, United States), 5 µg/ml hemin (bovine type I) (Sigma) plus 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, United States). All tests were performed with promastigotes in the log growth phase.

Murine macrophages lineage Raw 264-7 was cultured at 37°C in the presence of 5% CO_2 in RPMI1640 medium (Gibco, Life Technologies Brand, Grand Island, NY, United States) supplemented with 10% FBS.

In vitro leishmanicidal Assay

Leishmanicidal assays were performed with *L. amazonensis* promastigotes (2 × 10^6/mL) according to Rodrigues *et al.*[19] Parasites were distributed into 96 wells plates, 1 x 10^5 parasites per well. EOs were diluted in dimethylsulfoxide (DMSO) and 199 medium at maximal DMSO concentration of 0.05%. Then, EOs were submitted to serial dilution 125 to 0.5 µg/mL. As a positive control, the parasites were cultured without extracts and/or DMSO. Pentamidine (Sigma) was diluted at 15.6 to 0.5 µg/mL concentrations and was used as the standard drug. All tests were performed in three independent experiments. Plates were incubated for 24 hr or 48 hr of 26°C, until the analysis of parasites viability with Presto Blue (Invitrogen) 10µL/well. After 2hr of incubation with the reagent, plates were analyzed at 530 nm with plate reader equipment (BioTek, Winooski, VT, United States).

The percentage of growth inhibition was calculated as follow: The EOs were always tested in triplicates of each concentration, and the parameters for calculating the 50% inhibitory concentration (IC_{50}) were obtained by the Origin Lab 8.0 software, applying nonlinear regression to a sigmoidal curve.

Raw 264.7 macrophages Cytotoxic Assays

The cytotoxic effects of the EOs were evaluated in Raw 264.7 cells, according to Lall *et al.* with modifications.[20] Macrophages were seed in RPMI1640 medium supplemented with 10% FBS in 96-weel plates culture at 1 x 10^5 macrophages per well. Cells were treated with EOs at 125 to 0.5 µg/mL concentrations for 24 hr at 37°C in the presence of 5% CO_2. Then, Presto Blue (10 µL/well) was added, and after 2hr the cells viability was analyzed as described in promastigote assays. The 50% cytotoxic concentration (CC_{50}) was calculated with the Origin Lab 8.0 software and the selectivity indices (SI) were calculated according to Rodrigues *et al.*[19]

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM) of two independent experiments performed in triplicate of each concentration point. Statistical analyses were performed using GraphPad Prism software version 9.0. Concentrations capable

Table 2: Chemical composition (%) of the essential oil *Rosmarinus officinalis*.

Rosmarinus officinalis					
Compound	**RT (min)**	**Relative area (%)**	**RI (Experim.)**	**RI (Literat.)**	**References**
TricIiclene	14.210	0.12	929	928	https://dx.doi.org/10.3923/rjphyto.2011.66.69 "> https://dx.doi.org/10.3923/rjphyto.2011.66.69
α-tujene	14.270	0.11	931	929	https://dx.doi.org/10.1007/s10661-011-2158-8 "> https://dx.doi.org/10.1007/s10661-011-2158-8
α-Pinene	14.630	37.99	941	940	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Canfene	15.290	4.30	959	959	https://doi.org/10.5897/JMPR.9000344 "> https://doi.org/10.5897/JMPR.9000344
2,4-Tujadiene	15.410	0.58	962	960	https://dx.doi.org/10.2298/HEMIND0704272R " > https://dx.doi.org/10.2298/HEMIND0704272R
Sabinene	16.070	0.03	980	977	https://dx.doi.org/10.1080/10412905.2010.9700290 "> https://dx.doi.org/10.1080/10412905.2010.9700290
ß-Pinene	16.320	2.76	987	987	http://dx.doi.org/10.5539/jas.v4n12p75 "> http://dx.doi.org/10.5539/jas.v4n12p75
ß-Mircene	16.540	1.26	993	993	https://dx.doi.org/10.9734/EJMP/2013/1987 "> https://dx.doi.org/10.9734/EJMP/2013/1987
α- Phellandrene	17.290	0.15	1015	1010	https://dx.doi.org/10.1016/j.chroma.2006.02.034 "> https://dx.doi.org/10.1016/j.chroma.2006.02.034
α-Terpinene	17.660	0.40	1025	1022	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
o-cymene	17.960	1.66	1034	1027	https://dx.doi.org/10.1080/10412905.2006.9699039 "> https://dx.doi.org/10.1080/10412905.2006.9699039

Rosmarinus officinalis					
Compound	**RT (min)**	**Relative area (%)**	**RI (Experim.)**	**RI (Literat.)**	**References**
Limonene	18.100	3.28	1038	1036	https://dx.doi.org/10.1590/S1413-70542013000200004 "> https://dx.doi.org/10.1590/S1413-70542013000200004
β-cis-Ocimene	18.210	0.04	1041	1044	http://dx.doi.org/10.5539/jas.v4n12p75 "> http://dx.doi.org/10.5539/jas.v4n12p75
Cineol	18.290	2.48	1044	1046	https://doi.org/10.5897/JMPR.9000344 "> https://doi.org/10.5897/JMPR.9000344
γ-Terpinene	19.090	0.81	1067	1064	https://dx.doi.org/10.1016/j.foodchem.2005.09.084 "> https://dx.doi.org/10.1016/j.foodchem.2005.09.084
Trans-4-Thujanol	19.580	0.05	1081	1081	http://www.idosi.org/mejsr/mejsr13(6)13/12.pdf "> http://www.idosi.org/mejsr/mejsr13(6)13/12.pdf
Terpinolene	20.020	0.72	1094	1093	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Linalol	20.400	2.27	1105	1107	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Isocrisantenone	21.370	0.32	1135	1132	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Cis-verbenol	22.140	0.32	1159	1152	https://dx.doi.org/10.1016/j.chroma.2006.03.060 "> https://dx.doi.org/10.1016/j.chroma.2006.03.060
Limonene	18.100	3.28	1038	1036	https://dx.doi.org/10.1590/S1413-70542013000200004 "> https://dx.doi.org/10.1590/S1413-70542013000200004
β-cis-Ocimene	18.210	0.04	1041	1044	http://dx.doi.org/10.5539/jas.v4n12p75 "> http://dx.doi.org/10.5539/jas.v4n12p75
Cineol	18.290	21.48	1044	1046	https://doi.org/10.5897/JMPR.9000344 "> https://doi.org/10.5897/JMPR.9000344
γ-Terpinene	19.090	0.81	1067	1064	https://dx.doi.org/10.1016/j.foodchem.2005.09.084 "> https://dx.doi.org/10.1016/j.foodchem.2005.09.084
Trans-4-Thujanol	19.580	0.05	1081	1081	http://www.idosi.org/mejsr/mejsr13(6)13/12.pdf "> http://www.idosi.org/mejsr/mejsr13(6)13/12.pdf
Terpinolene	20.020	0.72	1094	1093	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Linalol	20.400	2.27	1105	1107	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Isocrisantenone	21.370	0.32	1135	1132	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Cis-verbenol	22.140	0.32	1159	1152	https://dx.doi.org/10.1016/j.chroma.2006.03.060 "> https://dx.doi.org/10.1016/j.chroma.2006.03.060
Unidentified	22.220	0.04	1161	-	-
Borneol, (1S, 2R, 4S)	22.330	2.06	1165	1166	https://dx.doi.org/10.1016/j.jpba.2007.08.030 "> https://dx.doi.org/10.1016/j.jpba.2007.08.030

Rosmarinus officinalis					
Compound	**RT (min)**	**Relative area (%)**	**RI (Experim.)**	**RI (Literat.)**	**References**
Isopulegol	22.570	0.03	1172	1167	https://dx.doi.org/10.3923/ajps.2008.779.781 "> https://dx.doi.org/10.3923/ajps.2008.779.781
Trans-3-Pinanone	22.710	0.08	1176	1177	https://dx.doi.org/10.1080/10412905.2007.9699247 "> https://dx.doi.org/10.1080/10412905.2007.9699247
Unidentified	22.770	0.06	1178	-	-
Isomentol	22.920	0.21	1183	1182	https://dx.doi.org/10.1248/bpb.28.1892 "> https://dx.doi.org/10.1248/bpb.28.1892
Borneol	23.060	2.36	1187	1186	https://dx.doi.org/10.1080/10412905.2010.9700402 "> https://dx.doi.org/10.1080/10412905.2010.9700402
Terpinen-4-ol	23.240	1.37	1193	1192	https://doi.org/10.5897/JMPR.9000344 "> https://doi.org/10.5897/JMPR.9000344
Unidentified	23.420	0.04	1198	-	-
α-Terpineol	23.680	1.27	1207	1200	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
Myrtenol	23.740	0.11	1209	1207	https://doi.org/10.5897/JMPR.9000344 "> https://doi.org/10.5897/JMPR.9000344
D-Verbenone	24.190	5.78	1224	1228	https://dx.doi.org/10.1007/s10886-007-9257-6 "> https://dx.doi.org/10.1007/s10886-007-9257-6
Citronellol	24.370	0.66	1231	1236	https://dx.doi.org/10.1590/S1413-70542013000200004 "> https://dx.doi.org/10.1590/S1413-70542013000200004
ß-Citral	24.880	0.04	1248	1249	https://dx.doi.org/10.1590/S1413-70542013000200004 "> https://dx.doi.org/10.1590/S1413-70542013000200004
Geraniol	25.100	2.11	1256	1255	https://dx.doi.org/10.1080/14786410902900085 "> https://dx.doi.org/10.1080/14786410902900085
α-Citral	25.720	0.12	1277	1278	https://dx.doi.org/10.1590/S1413-70542013000200004 "> https://dx.doi.org/10.1590/S1413-70542013000200004
Bornyl acetate	26.290	0.92	1297	1297	https://dx.doi.org/10.1016/j.jep.2008.11.004 "> https://dx.doi.org/10.1016/j.jep.2008.11.004
Citronellyl acetate	27.840	0.14	1352	1349	https://dx.doi.org/10.1365/s10337-006-0130-5 "> https://dx.doi.org/10.1365/s10337-006-0130-5
Geranyl acetate	28.640	0.20	1381	1383	https://dx.doi.org/10.9734/EJMP/2013/1987 "> https://dx.doi.org/10.9734/EJMP/2013/1987
β-Elemene	29.200	0.05	1401	1403	https://dx.doi.org/10.1016/j.bse.2008.09.006 "> https://dx.doi.org/10.1016/j.bse.2008.09.006
unidentified	29.370	0.10	1407	-	-
Caryophyllene	30.200	2.62	1439	1438	http://respiratory-research.com/content/13/1/87 "> http://respiratory-research.com/content/13/1/87
α-Caryophyllene	31.150	0.33	1476	1474	https://dx.doi.org/10.1016/j.bse.2008.09.006 "> https://dx.doi.org/10.1016/j.bse.2008.09.006

Rosmarinus officinalis					
Compound	**RT (min)**	**Relative area (%)**	**RI (Experim.)**	**RI (Literat.)**	**References**
Germacrene D	31.770	0.14	1500	1503	https://dx.doi.org/10.1016/j.chroma.2006.02.034 "> https://dx.doi.org/10.1016/j.chroma.2006.02.034
γ-Cadinene	32.560	0.19	1532	1534	https://dx.doi.org/10.1016/j.chroma.2006.02.034 "> https://dx.doi.org/10.1016/j.chroma.2006.02.034
Elemol	33.350	0.17	1564	1557	https://dx.doi.org/10.1590/S1413-70542013000200004 "> https://dx.doi.org/10.1590/S1413-70542013000200004
Cariophileno oxide	34.410	0.05	1609	1613	https://dx.doi.org/10.1016/j.chroma.2006.02.034 "> https://dx.doi.org/10.1016/j.chroma.2006.02.034
α-Cadinol	36.000	0.09	1679	1670	https://dx.doi.org/10.1016/j.phytochem.2006.08.003 "> https://dx.doi.org/10.1016/j.phytochem.2006.08.003

*RT: retention time; min: minutes; RI: Retention index; Experim.: experimental; Literat: literature.

of causing 50% of death (CC_{50} - cells or IC_{50} - parasites) were calculated by dose vs. nonlinear regression, using Origin 8.0.

RESULTS AND DISCUSSION

The chemical composition determined by GC/ MS are presented in Table 2 and 3. The chromatograms can be seen in the Figures 1 and 2. In the essential oil of *Rosmarinus officinalis* (EORo), it was possible to identify fifty-three compounds, representing 99.75% of the total detected constituents. The major components were α-Pineno (37.99%), Cineol (21.48%) D-Verbenone (5,78%) and Limonene (3.28%). Approximately 150 compounds have been described in the work carried out essential oils of *Rosmarinus officinalis*, and the majority compounds are 1,8-cineole (Cineiol), α-pinene, camphor, bornyl acetate, borneol, camphene, α-terpineol, limonene, β-pinene, β-caryophyllene and myrcene.[15] Thus, the compounds found in our study corroborate the data from the literature and even the compound Verbenone was described in some works.[21,22] So, the chemical composition of essential oils depends on the species studied age, variety, the part collected, origin, climate, soil, agrochemicals used, stocking time, preparation and other factors,[15] and *Rosmarinus* from different part of the world differ from each other quantitatively.

In the essential oil of Eucalyptus Globulus (EOEg) fourteen compounds have been identified, representing 100% of the compounds present in the sample. The major components were Cineol (75.52%), Limonene (8.82%) and o-Cymeno (7.81%). Considering the studies already conducted with Eucaliptus, it can be observed that the essential oil of this species are usually rich in monoterpenes and in some cases sesquiterpenes and when we evaluate the chemical composition, these oils are complex mixtures ranging from 20 to 80 compounds, differing in their concentrations.[16] However, the common characteristic among studies is that the majority compound is 1,8-cineole (Cineol), and in most of these studies, cineol represents more than 80% of the chemical composition. In this study, cineol was around 75%, followed by compounds Limonene, o-Cineno and γ-Terpineno. In a study with essential oil extracted from Eucaliptus globulous in the state of São Paulo, Brazil, the chemical composition was 1,8 Cineol (83.89%), (+)-limonen (8.16%), α-pineno (4.15%), o-cymeno (2.93%), and γ-terpinene (0.87%).(23) Thus, 1.8 cienol is the majority compound but minority compounds may alter among some components already described in the genus.

In Figure 3A, treatment with the two essential oils tested showed a dose-dependent inhibition effect on the proliferation of *L. amazonensis* promastigotes. The EOEg had a lower IC_{50} value of 14.03 ± 2.08 μg/mL, followed by the EORo with an IC_{50} value of 31.12 ± 4.6 μg/mL, all during the 24-hr exposure period. In the 48-hr exposure period, the IC_{50} value was about twice as high with rosemary oil, showing no significant difference as compared to EOEg at that time (Figure 3B).

Essential oils were also evaluated for their potential toxic effects on the host cell, the macrophage strain Raw 264.7. The 50% cytotoxic concentration (CC_{50}) was evaluated in the 24-hr treatment period, with CC_{50} values of 79.96 ± 7.16 and 908.60 ± 14.2 μg/mL. In this case, the host cell toxicity values were five to almost thirty times higher than the 50% parasite inhibition concentration (Table 4). The efficiency of the EO activity was evaluated by the Selectivity Index (SI). The SI results for *L. amazonensis* showed that EORo was 29.19 times less toxic to the macrophage than to the protozoan. While the reference drug used (Pentamidine) had a lower SI of 9.39 (Table 4). Demonstrating then, the selectivity of the oils tested against this *Leishmania* species, one of the

Table 3: Chemical composition (%) of the essential oil *Eucalyptus globulus*.

Eucalyptus globulus					
Compound	**RT (min)**	**Relative area (%)**	**RI (Experim.)**	**RI (Literat.)**	**References**
α-Pinene	14.600	1.97	940	940	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
ß-Pinene	16.320	0.39	987	987	http://dx.doi.org/10.5539/jas.v4n12p75 "> http://dx.doi.org/10.5539/jas.v4n12p75
ß-Myrcene	16.540	0.79	993	993	https://dx.doi.org/10.9734/EJMP/2013/1987 "> https://dx.doi.org/10.9734/EJMP/2013/1987
α-Phellandrene	17.290	0.50	1015	1010	https://dx.doi.org/10.1016/j.chroma.2006.02.034 "> https://dx.doi.org/10.1016/j.chroma.2006.02.034
α-Terpinene	17.660	0.08	1025	1022	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
o-Cimene	17.970	7.81	1034	1027	https://dx.doi.org/10.1080/10412905.2006.9699039 "> https://dx.doi.org/10.1080/10412905.2006.9699039
Limonene	18.120	8.82	1038	1036	https://dx.doi.org/10.1590/S1413-70542013000200004 "> https://dx.doi.org/10.1590/S1413-70542013000200004
β-cis-Ocimene	18.190	0.04	1041	1044	http://dx.doi.org/10.5539/jas.v4n12p75 "> http://dx.doi.org/10.5539/jas.v4n12p75
Cineol	18.330	75.52	1045	1046	https://doi.org/10.5897/JMPR.9000344 "> https://doi.org/10.5897/JMPR.9000344
β-trans-Ocimene	18.570	0.04	1051	1048	http://www.idosi.org/mejsr/mejsr13(6)13/12.pdf "> http://www.idosi.org/mejsr/mejsr13(6)13/12.pdf
γ-terpinene	19.090	3.72	1067	1064	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
terpinolene	20.030	0.10	1094	1093	https://doi.org/10.5897/JMPR09.506 "> https://doi.org/10.5897/JMPR09.506
p-Mentan-3-one	22.480	0.08	1169	1166	https://dx.doi.org/10.1021/jf062508c "> https://dx.doi.org/10.1021/jf062508c
Borneol	23.110	0.15	1188	1186	https://dx.doi.org/10.1080/10412905.2010.9700402

*RT: retention time; min: minutes; RI: Retention index; Experim: experimental; Literat: literature.

etiologic agents of most common form of the disease, cutaneous leishmaniasis.

The leishmanicidal effects of essential oils from species of the genus *Eucalyptus* and *Rosmarinus* have been little reported in the world and absent in Brazil. A study carried out with commercial EO produced in the USA and France was cited with EO from *Eucalyptus radiata* with an IC_{50} value of 164.7 ± 8.3 µg/mL, CC_{50} of 100.2 ± 8.4 µg/mL and SI<1 versus *L. amazonensis*.[24] As for the EO of *Rosmarinus officinalis*, in this same study, the values were 89.7 ± 2.0 (IC_{50}), 83.4 ± 7.3 (CC_{50}) µg/mL and SI<1. OERo collected in a region in Morocco has been identified as a potent leishmanicidal agent showing effects against strains of *L. infantum, L. major* and *L. tropica*, presenting IC_{50} values of 1.2 ± 0.36, 2.6 ± 0.64 and 3.5 ± 0.83 µg/mL, respectively.[21] In Colombia, EORo showed activity against *L. braziliensis* promastigotes with an IC_{50} of 17.4 µg/mL.[25] Likewise, Tunisian rosemary essential oil (43.8% 1,8-cineole, 12.0% camphor, 11.5% α-pinene, 8.6% β-pinene, 4.8% camphene) was effective against promastigotes of L. infantum (IC_{50} 16.3 µg/mL) and L. major (IC_{50} 20.9 µg/mL).[26] On the other hand, in Germany a commercial rosemary essential

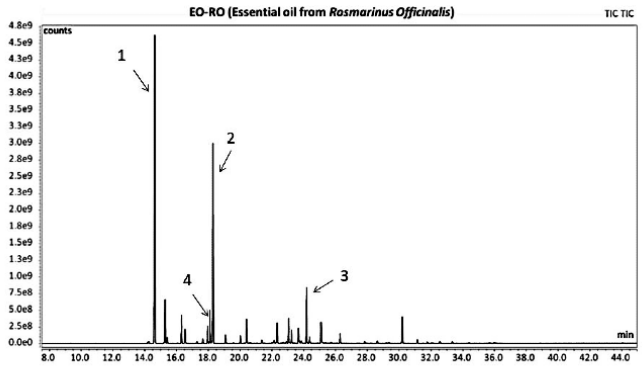

Figure 1: Chromatogram of the essential oil *Rosmarinus officinalis:* 1- α-Pineno, 2- Cineol, 3- D-Verbenone, 4-Limonene.

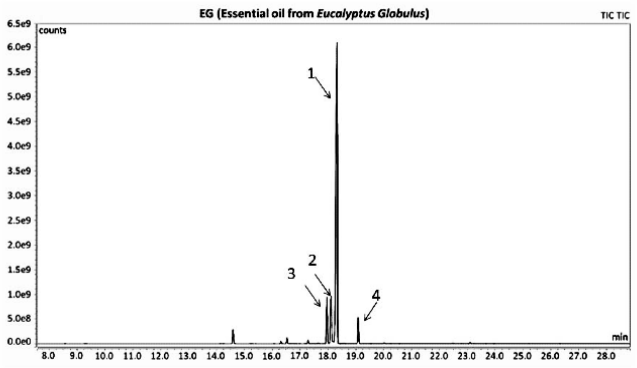

Figure 2: Chromatogram of the essential oil *Eucalyptus globulus*: 1-Cineol, 2-Limonene, 3- o-Cineno and 4- γ-Terpineno.

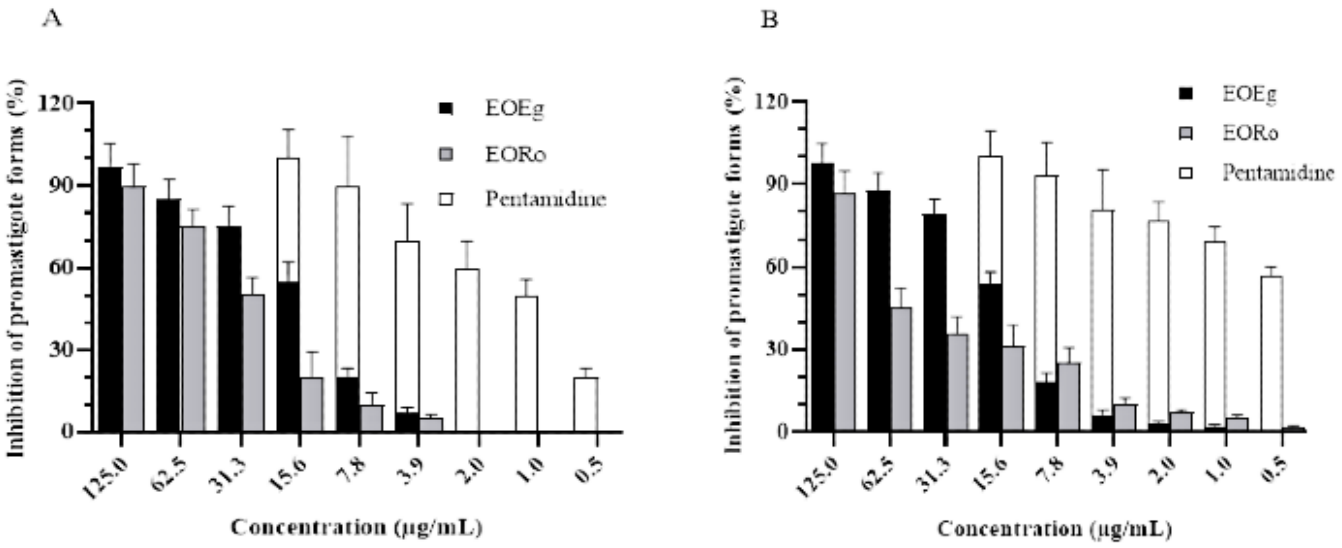

Figure 3: Effects of EOEg, EORo, and Pentamidine on the survival of *L. amazonensis* promastigotes. The cell viability of promastigotes was evaluated after (A) 24 hr or (B) 48 hr of incubation with different concentrations of EOEg, EORo, and Pentamidine. The IC_{50} was obtained through non-linear regression of means and the percentage of growth inhibition was calculated as described in material and methods. Cultures were tested in triplicates, and the results shown are the average of at least three independent experiments.

Table 4: ***In vitro*** **antileishmanial activity, cytotoxicity and selectivity index. The concentration needed to inhibit 50% of the** ***Leishmania*** **promastigotes (IC_{50}) viability, as well as the selectivity index (SI), which was calculated by the ratio between the CC_{50} and IC_{50} values.**

Sample (EO)	IC_{50} µg/mL±SD 24hr	IC_{50} µg/mL±SD 48hr	CC_{50} µg/mL±SD 24hr*	SI
Eucalyptus globulus (Eucalyptus)	14.03 ± 2.08	11.93 ± 1.07	79.96 ± 7.16	5.70
Rosmarinus officinalis (Rosemary)	31.12 ± 4.6	70.37 ± 5.53	908.60 ± 14.2	29.19
Pentamidine	0.71 ± 0.03	< 0.48	6.67 ± 0.83	9.39

*Raw 264.7 cells; EO Essential Oil; SD Standard Deviation.

oil had no activity against *L. major* promastigotes (IC_{50} 282 µg/mL).[27]

The potential leishmanicidal effects of EOEg and Ro observed in this work may be due to the presence in greater amounts of Cineol compounds in the case of EOEg and α-Pinene in the case of EORo. Santana *et al.*,[28] demonstrated that these compounds inhibited the survival of intracellular parasites of *L. amazonensis* in a dose-dependent manner with IC_{50} of 48.4 µg/mL and 37 µg/mL, respectively. In contrast, the pure compound 1,8 Cineol had no *in vitro* effect against *L. infantum, L. tropica* and *L. major.*[29] Leishmanicidal activity has also been described in the literature with compounds found here in smaller amounts, such as Limonene,[30] and Cimeno,[28] against different species of the parasite.

CONCLUSION

The results of this study showed the variety of chemical composition of the essential oils of *Rosmarinus officinalis* and *Eucalyptus globulus* collected in this Brazilian region, highlighting the compounds Cineol and α-Pineno. Appreciably, essential oils are complex mixtures of compounds, and biological activities cannot necessarily be attributed to individual components. Furthermore, it revealed the *in vitro* anti-*Leishmania* effect against promastigotes of the *L. amazonensis* species, with a high selectivity index, that is, a low cytotoxic effect against the host cell. This enables future studies and possible *in vivo* applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Molyneux DH, Savioli L, Engels D. Neglected tropical diseases: Progress towards addressing the chronic pandemic. Lancet. 2017;389(10066):312-25. doi: 10.1016/S0140-6736(16)30171-4, PMID 27639954.
2. WHO. Leishmaniasis; 2022 [cited May 14 2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis.
3. Kedzierski L. Leishmaniasis. Hum Vaccin. 2011;7(11):1204-14. doi: 10.4161/hv.7.11.17752, PMID 22048116.
4. Mohapatra S. Drug resistance in leishmaniasis: Newer developments. Trop Parasitol. 2014;4(1):4-9. doi: 10.4103/2229-5070.129142, PMID 24754020.
5. Altamura F, Rajesh R, Catta-Preta CMC, Moretti NS, Cestari I. The current drug discovery landscape for trypanosomiasis and leishmaniasis: Challenges and strategies to identify drug targets. Drug Dev Res. 2022;83(2):225-52. doi: 10.1002/ddr.21664, PMID 32249457.
6. Bernardini S, Tiezzi A, Laghezza Masci V, Ovidi E. Natural products for human health: An historical overview of the drug discovery approaches. Nat Prod Res. 2018;32(16):1926-50. doi: 10.1080/14786419.2017.1356838, PMID 28748726.
7. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 2016;79(3):629-61. doi: 10.1021/acs.jnatprod.5b01055, PMID 26852623.
8. Guerriero G, Berni R, Muñoz-Sanchez JA, Apone F, Abdel-Salam EM, Qahtan AA, *et al*. Production of plant secondary metabolites: examples, tips and suggestions for biotechnologists. Genes. 2018;9(6):309. doi: 10.3390/genes9060309, PMID 29925808.
9. Teixeira B, Marques A, Ramos C, Neng NR, Nogueira JMF, Saraiva JA, *et al*. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind Crops Prod. 2013;43:587-95. doi: 10.1016/j.indcrop.2012.07.069.
10. Pichersky E, Gershenzon J. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr Opin Plant Biol. 2002;5(3):237-43. doi: 10.1016/s1369-5266(02)00251-0, PMID 11960742.
11. Rottini MM, Amaral ACF, Ferreira JLP, Oliveira ESC, Silva JRA, Taniwaki NN, *et al*. *Endlicheria bracteolata* (Meisn.) essential oil as a weapon against *Leishmania amazonensis*: *in vitro* assay. Molecules. 2019;24(14):2525. doi: 10.3390/molecules24142525, PMID 31295880.
12. Monzote L, Scherbakov AM, Scull R, Satyal P, Cos P, Shchekotikhin AE, *et al*. Essential Oil from *Melaleuca leucadendra*: antimicrobial, Antikinetoplastid, antiproliferative and cytotoxic Assessment. Molecules. 2020;25(23):5514. doi: 10.3390/molecules25235514, PMID 33255562.
13. Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC Complement Altern Med. 2014;14:225. doi: 10.1186/1472-6882-14-225, PMID 25002023.
14. Pereira PS, Maia AJ, Tintino SR, Oliveira-Tintino CDdM, Raulino ISdS, Vega MC, *et al*. Trypanocide, antileishmania and cytotoxic activities of the essential oil from *Rosmarinus officinalis* L *in vitro*. Ind Crops Prod. 2017;109:724-9. doi: 10.1016/j.indcrop.2017.09.030.
15. Borges RS, Ortiz BLS, Pereira ACM, Keita H, Carvalho JCT. *Rosmarinus officinalis* essential oil: a review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J Ethnopharmacol. 2019;229:29-45. doi: 10.1016/j.jep.2018.09.038, PMID 30287195.
16. Barbosa LC, Filomeno CA, Teixeira RR. Chemical variability and biological activities of Eucalyptus spp. Essential oils. Molecules. 2016;21(12):1671. doi: 10.3390/molecules21121671, PMID 27941612.
17. González-Burgos E, Liaudanskas M, Viškelis J, Žvikas V, Janulis V, Gómez-Serranillos MP. Antioxidant activity, neuroprotective properties and bioactive constituents analysis of varying polarity extracts from *Eucalyptus globulus* leaves. J Food Drug Anal. 2018;26(4):1293-302. doi: 10.1016/j.jfda.2018.05.010, PMID 30249328.
18. Pinho GMd, Francisco CN, Salgado CM. Análise espacial dos movimentos de massa em Nova Friburgo/RJ: O caso do desastre natural de janeiro de 2011. Rev Tamoios. 2013;9(1):16-27. doi: 10.12957/tamoios.2013.5355.
19. Rodrigues CA, Dos Santos PF, da Costa MOL, Pavani TFA, Xander P, Geraldo MM, *et al*. 4-Phenyl-1,3-thiazole-2-amines as scaffolds for new antileishmanial agents. J Venom Anim Toxins Incl Trop Dis. 2018;24:26. doi: 10.1186/s40409-018-0163-x, PMID 30214457.
20. Lall N, Henley-Smith CJ, De Canha MN, Oosthuizen CB, Berrington D. Viability reagent, PrestoBlue, in comparison with other available reagents, utilized in cytotoxicity and antimicrobial assays. Int J Microbiol. 2013;2013:420601. doi: 10.1155/2013/420601, PMID 23653650.
21. Bouyahya A, Et-Touys, A., Bakri, Y., Talbaui, A., Fellah, H., Abrini, J. and Dakka, N. (2017) Chemical composition of Mentha pulegium and *Rosmarinus officinalis* essential oils and their antileishmanial, antibacterial and antioxidant activities. Microbial pathogenesis, 111, 41–49. https://doi.org/10.1016/j.micpath.2017.08.015.
22. Mouahid A, Dufour C, Badens E. Supercritical CO_2 extraction from endemic Corsican plants; comparison of oil composition and extraction yield with hydrodistillation method. J CO2 Util. 2017;20:263-73. doi: 10.1016/j.jcou.2017.06.003.
23. Macedo IT, Bevilaqua CM, de Oliveira LM, Camurça-Vasconcelos AL, Vieira Lda S, Oliveira FR, *et al*. Ovicidal and larvicidal activity *in vitro* of *Eucalyptus globulus* essential oils on Haemonchus contortus. Rev Bras Parasitol Vet. 2009;18(3):62-6. doi: 10.4322/rbpv.01803011, PMID 19772778.
24. Monzote L, Herrera I, Satyal P, Setzer WN. *In vitro* Evaluation of 52 Commercially-Available Essential Oils Against Leishmania amazonensis. Molecules. 2019;24(7):1248. doi: 10.3390/molecules24071248, PMID 30934998.
25. Arévalo Y, Robledo S, Muñoz L, Granados-Falla D, Cuca LE, Delgado G. *In vitro* evaluation of essentials oils activity from Colombians plants on *Leishmania braziliensis*. Rev Colomb Cienc Quim Farm. 2009;38(2):131-41. ISSN 0034-7418.
26. Essid R, Rahali FZ, Msaada K, Sghair I, Hammami M, Bouratbine A, *et al*. Antileishmanial and cytotoxic potential of essential oils from medicinal plants in Northern Tunisia. Ind Crops Prod. 2015;77:795-802. doi: 10.1016/j.indcrop.2015.09.049.
27. 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(4):366-8. doi: 10.1055/s-2000-8548, PMID 10865458.

28. Santana RC, Rosa ADS, Mateus MHDS, Soares DC, Atella G, Guimarães AC, *et al. In vitro* leishmanicidal activity of monoterpenes present in two species of Protium (Burseraceae) on *Leishmania amazonensis*. J Ethnopharmacol. 2020;259:112981. doi: 10.1016/j.jep.2020.112981, PMID 32442591.
29. Machado M, Dinis AM, Santos-Rosa M, Alves V, Salgueiro L, Cavaleiro C, *et al*. Activity of *Thymus capitellatus* volatile extract, 1,8-cineole and borneol against Leishmania species. Vet Parasitol. 2014;200(1-2):39-49. doi: 10.1016/j.vetpar.2013.11.016, PMID 24365244.
30. Carvalho RCV, Sousa VC, Santos LP, Santos ILD, Diniz RC, Rodrigues RRL, *et al*. Limonene-carvacrol: A combination of monoterpenes with enhanced antileishmanial activity. Toxicol *In vitro*. 2021;74:105158. doi: 10.1016/j.tiv.2021.105158, PMID 33823240.

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