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# Anti-*Leishmania* activity of essential oil of *Myracrodruon urundeuva* (Engl.) Fr. All.: Composition, cytotoxity and possible mechanisms of action

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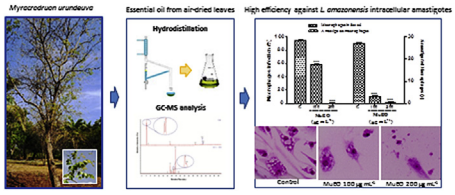
## HIGHLIGHTS

- MuEO inhibits the proliferation of *L. amazonensis* promastigotes and axenic amastigotes.
- MuEO exhibited selectivity indexes (SI) greater than reference drugs.
- MuEO reduced the infection index of macrophages by *L. amazonensis*.
- MuEO is more effective against intracellular amastigotes than promastigotes.
- MuEO increase phagocytic activity in macrophages.

## GRAPHICAL ABSTRACT

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## ABSTRACT

*Myracrodruon urundeuva* (Engl.) Fr. All., commonly known as "aroeira-do-sertão", is a medicinal plant from Anacardiaceae family. In this study, the chemical composition of *M. urundeuva* essential oil (MuEO) was evaluated by gas chromatography-mass spectrometry (GC-MS), as well as its anti-*Leishmania* potential, cytotoxicity, and macrophage activation capability as possible antiprotozoal mechanism of action were assessed. Fourteen compounds were identified, which constituted 94.87% of total oil composition. The most abundant components were monoterpenes (80.35%), with β-myrcene (42.46%), α-myrcene (37.23%), and caryophyllene (4.28%) as the major constituents. The MuEO inhibited the growth of promastigotes ($IC_{50}$ 205 ± 13.4 μg $mL^{-1}$), axenic amastigotes ($IC_{50}$ 104.5 ± 11.82 μg $mL^{-1}$) and decreased percentage of macrophage infection and number of amastigotes per macrophage ($IC_{50}$ of 44.5 ± 4.37 μg·$mL^{-1}$), suggesting significant anti-*Leishmania* activity. The cytotoxicity of MuEO was assessed by MTT test in Balb/c murine macrophages and by human erythrocytes lysis assay and low cytotoxicity for these cells was observed. The $CC_{50}$ value against macrophages were 550 ± 29.21 μg $mL^{-1}$, while cytotoxicity for erythrocytes was around 20% at the highest concentration assessed, with $HC_{50}$ > 800 μg $mL^{-1}$. While MuEO-induced anti-*Leishmania* activity is not mediated by increases in both lysosomal activity and nitric oxide production in macrophages, the results suggest the antiamastigote

*Abbreviations:* $IC_{50}$, Half-maximal inhibitory concentration; $CC_{50}$, Half-maximal cytotoxicity concentration; $HC_{50}$, Half-maximal hemolytic concentration; MIC, Minimum inhibitory concentration; SI, Selective index; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide; DMSO, Dimethyl sulfoxide; FBS, Fetal bovine serum; BOD, Biological oxygen demand; PBS, Phosphate-buffered saline; MuEO, Essential oil of *Mycracrodruon urundeuva*; GC-MS, Gas Chromatography-Mass Spectrometry; NO, Nitric Oxide; ANOVA, Analysis of variance.

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activity is associated with an immunomodulatory activity of macrophages due to an increase of phagocytic capability induced by MuEO. Thus, MuEO presented significant activity against *Leishmania amazonensis*, probably modulating the activation of macrophages, with low cytotoxicity to murine macrophages and human erythrocytes.



## 1. Introduction

Leishmaniasis is a complex of infectious parasitic diseases caused by protozoa from Trypanosomatidae family and *Leishmania* genus. It is considered a public health problem which affects more than 12 million people throughout the world, with 2–3 million new cases in each year. Furthermore, leishmaniasis is included in the group of neglected tropical diseases (NTD) (Feasey et al., 2010; Who, 2014).

The parasites from *Leishmania* genus have a heteroxenic life cycle, which is characterized by two distinct developmental forms: the mobile promastigote form, with outwardly flagellum and present in the gastrointestinal tract of the insect vector, and the amastigote form, with inner flagellum and acting as an obligate intracellular form in mononuclear phagocyte cells. The amastigote forms are responsible for the clinical manifestations of leishmaniasis (De Almeida et al., 2003; Chappuis et al., 2007). Clinical manifestations are diverse, and can vary from the mucocutaneous form, which is characterized by the presence of ulcerative and nodular lesions in the skin, to the visceral form, the most severe and potentially fatal form (David and Craft, 2009). *Leishmania* (*Leishmania*) *amazonensis* is one of the species distributed throughout the New World, specially in Latin America, and is associated with different clinical forms of leishmaniasis. It is the main agent of diffuse cutaneous leishmaniasis, which is commonly refractory to the currently available treatments (Guimarães-Costa et al., 2009).

Currently, the conventional therapy recommended for leishmaniasis has some drawbacks, such as long-term treatment with high doses, severe toxic adverse effects, and increased chemoresistance of the parasite. The pentavalent antimonials have been the first choice treatment since 1945. The second-line drugs, such as amphotericin B, pentamidine and paromomycin, are used in cases of resistance to antimonials. However, they have high cost, and may be even more toxic than the antimonials (Croft and Coombs, 2003; Chappuis et al., 2007). Therefore, there is a globally necessity for the discovery of new antileishmanial drugs.

In the ongoing search for leishmanicidal compounds, many studies have shown that plant-derived products may be promising sources for the development of new drugs (Machado et al., 2012). In this context, essential oils are composed by a wide diversity of small hydrophobic molecules such as monoterpenes, sesquiterpenes, and phenylpropanoids, and have demonstrated *in vitro* and *in vivo* anti-*Leishmania* activity against promastigote and amastigote forms of *Leishmania* spp. The effective action of essential oil from *Eugenia uniflora* L. (Rodrigues et al., 2013b), *Pistacia vera* L. (Mahmoudvand et al., 2016), Cymbopogon *citratus* (D.C.) Staff (Machado et al., 2012), and *Copaifera cearensis* Huber ex Ducke (Santos et al., 2008) against *Leishmania* spp. shows that essential oils can be a promising source of new drugs with anti-*Leishmania* activity.

*Myracrodruon urundeuva* (Engl.) Fr. All., commonly known as “aroeira-do-sertão”, is a medicinal plant species from Anacardidaceae family. It is native from South America, and commonly found in regions with tropical and subtropical climate, including the northeastern region of Brazil (Sá et al., 2009). Phytochemical investigations have shown the presence of flavonoids, polyphenols, chalcones, tannins, and terpenoids (Viana et al., 2003; Calou et al., 2014; Figueredo et al., 2014). In Brazil, *M. urundeuva* is used in traditional medical practices for the treatment of mycoses, candidiasis, bacterial infections, and allergy, as well as an anti-inflammatory agent (Silvino et al., 2014). Interestingly, pharmacological assays have demonstrated its antifungal, anti-inflammatory, antiulcerogenic, antihistamine, antibradicinina and analgesic activities (Barbara Caroline et al., 2015).

Considering the potential pharmacological benefits of *M. urundeuva*, allied to the interest about discovering essential oils with anti-*Leishmania* activity, the aim of this study was to determine the chemical composition of essential oil from the leaves of *M. urundeuva*, and investigate its anti-*Leishmania* activity, as well as its cytotoxicity and underlying mechanisms of action.

## 2. Materials and methods

### 2.1. Chemicals

Dimethyl sulfoxide (DMSO: 99%), anhydrous sodium sulfate, glacial acetic acid, ethanol, formaldehyde, sodium chloride, calcium acetate, zymosan, and neutral red were purchased from Merck Chemical Company (Germany). The *n*-alkane (C8–C24) homologous series, Schneider's medium, RPMI 1640 medium, heat-inactivated fetal bovine serum (FBS), Azul de Alamar® (AbD Serotec, Oxford, UK, MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide), Griess reagent (1% sulfanilamide in $H_3PO_4$ 10% (v/v) in Milli-Q water), and the antibiotics penicillin and streptomycin were purchased from Sigma Chemical (St. Louis, MO, USA). The antibiotic amphotericin B (90%) was purchased from Cristalia (São Paulo, SP, Brazil).

### 2.2. Extraction of the essential oil of Myracrodruon urundeuva (MuEO)

*M. urundeuva* leaves were collected from a mature tree in the flowering stage in Teresina city, Piauí state, located in the Northeast Region of Brazil (5°5′20″S e 42°48′7″W). A voucher specimen (no. 20,026) was deposited at the Graziela Barroso Herbarium from the Federal University of Piauí. The plant material was air-dried for 7 (seven) days, subjected to hydrodistillation using a Clevenger-type apparatus (300 g, 6 h). Afterwards, the essential oil was dried over anhydrous sodium sulfate, filtered, and weighed, and then the oil yield was determined as 0.2% (w/w). MuEO was then stored in a dark flask and refrigerated at 4° C until use. For the *in vitro* tests, MuEO was solubilized in DMSO, diluted in culture medium, and added to the cultures.

### 2.3. Gas chromatography-mass spectrometry analysis of the essential oil

MuEO was analyzed using a SHIMADZU GC-17/MS-QP5050A instrument (Shimadzu, Duisburg, Germany) under the following conditions: DB-5 HT (i.d.; 0.25 mm, 1.0 μm film thickness) fused silica capillary column, with the following temperature program: