Chemical composition, antileishmanial, and antifungal activities of essential oils from *Cinnamomum cassia* bark, *Schinus molle* dried leaves and their blends

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Abstract: This study reports on the chemical composition and antileishmanial and anticandidal activities of essential oils (EOs) distilled from *Schinus molle* dried leaves (SM-EO), *Cinnamomum cassia branch* bark (CC-EO) and their blends against promastigote forms of *Leishmania* (*Leishmania*) *amazonensis* and nine *Candida* strains. Major constituents of SM-EO were spathulenol (26.93%), β-caryophyllene (19.90%), and caryophyllene oxide (12.69%), whereas cinnamaldehyde (60.11%), cinnamyl acetate (20.90%) and (*E*)-*cis*-2-methoxycinnamic acid (10.37%) were predominant in CC-EO. SM-EO (IC_{50} = 21.45 μg/mL) and CC-EO (IC_{50} = 23.27 μg/mL) displayed good activity against *L. amazonensis*. SM-EO and CC-EO also proved to be good or moderate activity against nine *Candida* strains, with Minimum Inhibitory Concentration (MIC) values ranging from 31.25 to 250 μg/mL. While the three SM-EO and CC-EO blends were not more active than the EOs tested individually, they exhibited remarkably high antileishmanial activity, with IC_{50} values ranging between 3.12 and 7.04 μg/mL, which is very similar to the IC_{50} of amphotericin B (positive control). These results show that SM-EO, CC-EO, and their blends may be considered to participate in the formulation of drugs with antileishmanial and antifungal activities.

Keywords: American pepper tree; candidiasis; spices; *Leishmania amazonensis*; natural active ingredients.

1. Introduction

Leishmaniasis, a parasitic Neglected Tropical Disease (NTD) caused by protozoa of the genus *Leishmania*, leads to approximately 30,000 deaths annually [1]. The manifestations of this disease, whether tegumentary (TL) or visceral (VL), are determined by specific *Leishmania* species responsible for the infection and the mammalian host's immunological and nutritional status. In the Americas, *L. amazonensis* is the primary causative agent of TL [2]. TL can lead to skin lesions that may be either healed on their own or progress to form disfiguring scars, along with damage in nasopharyngeal mucosal tissues [3]. Early diagnosis and treatment of TL are challenging as the disease advances slowly and affects extensive skin areas [4]. Furthermore, the drugs that are currently available for the treatment of leishmaniasis, such as pentavalent antimonials, amphotericin B, pentamidine, miltefosine, and paramomycin, display some drawbacks. These drawbacks encompass toxicity, high cost, and the emergence of parasitic resistance [5].

The genus *Candida* comprises approximately 150 species, with a significant number of these species acting as endosymbionts in humans, particularly affecting immunosuppressed individuals. While *Candida albicans* is responsible for about 80% of infections, there is an increasing incidence of infections caused by non-albicans *Candida* species such as *C. glabrata*, *C. tropicalis*, and *C. krusei* [6]. Current antifungal drugs used to treat *Candida* infections display some significant drawbacks, such as poor oral bioavailability and decreased efficacy due to the emergence of resistant strains [7].

The genus *Cinnamomum* (Lauraceae) is native to Indonesia and comprises about 250 perennial tree species that grow up to 8-9 m in height [8]. *Cinnamomum* species, popularly known as cinnamon, or "sweet wood", have been among the most popular plants in the world since remote antiquity [9]. It has been used by the Chinese since 2500 BC and by Egyptians along with other spices for embalmment. Cinnamon comes from the tree trunk; its dried branches are separated from the bark, which is reddish brown and has a strong fragrance [8]. The dry inner trunk bark of cinnamon is considered their main product because it is a rich source of essential oils (EOs). Cinnamon EOs have been widely sold due to their biological and pharmacological properties, such as anti-inflammatory, antithermitic, nematicidal, larvicidal, insecticidal, antimycotic, and anticancer [10]. The four most important *Cinnamomum* species

in the international market are Ceylon cinnamon (*Cinnamomum verum* Presl, sin. *C. zeylanicum* Bl.); Saigon cinnamon (*C. loureirii* Nees); Chinese cassia or Chinese cinnamon (*C. cassia* Presl.); and Indonesian cinnamon or Padang cassia (*C. burmannii* (C.G. and Th. Nees) Bl.) [9]. *C. cassia*, for instance, has been traditionally used as tooth powder to treat toothache, dental problems, oral microbiota, and bad breath [10].

Schinus molle L. (Anacardiaceae), commonly known as the "American pepper tree" is native to South America but has become a global species [11]. This aromatic plant can reach heights of 5-15 m and is commonly used in folk medicine as an anti-inflammatory, antispasmodic, antipyretic, and antitumoral [12] and to treat bronchitis, coughs, colds, fever, and tuberculosis [13]. The EOs of *S. molle* have been reported to have antibacterial [11,14-16], antifungal [11,14-17], insecticidal [18,19], and larvicidal [20] properties.

As part of our ongoing project on the biological activities of EOs [21-24], and based on the literature reports on the antifungal and antiparasitic activity of the EOs of *C. cassia* [25-29] and *S. molle* [15,30-32], this study aimed to investigated the anticandidal and antileishmanial activity of the EOs from *S. molle* dried leaves (SM-EO) *C. cassia* bark (CC-EO) and their blends, as well as to determine their chemical composition using gas chromatography-mass spectrometry (GC-MS) and gas chromatography flame ionization detection (GC-FID).

2. Results

2.1. Chemical composition of SM-EO and CC-EO

The essential oil from *S. molle* dried leaves (SM-EO) was obtained as a light-yellowish oil in 1.52±0.36% yield. A total of twenty compounds were identified in SM-EO, with a predominance of oxygenated sesquiterpenes (55.15%) and monoterpenes (17.34%), as shown in Table 1. Spathulenol (26.93%), β-caryophyllene (19.90%), and caryophyllene oxide (12.69%) were the major compounds in SM-EO.

The chemical composition of the essential oil of *C. cassia* (CC-EO) purchased from the supplier doTERRA® is shown in Table 1. Major constituents identified in CC-EO-were the phenylpropanoids (*E*)-cinnamaldehyde (60.11%), cinnamyl acetate (20.90%), and (*E*)-*cis*-2-methoxycinnamic acid (10.37%). This chemical composition is similar to that reported by the company on its official website [33].

2.2. *Antileishmanial activity of SM-EO and CC-EO*

The *in vitro* antileishmanial activity of SM-EO, CC-EO, and blends 1 (SM-EO: CC-EO 3:1 v/v), 2 (SM-EO: CC-EO 1:1 v/v), and 3 (SM-EO:CC-EO 1:3 v/v) against promastigote forms of *L. amazonensis* is shown in Table 2. At concentrations of 25, 50, and 100 µg/mL, CC-EO caused a higher inhibition percentage of *L. amazonensis* promastigote forms compared to SM-EO and blends 1, 2, and 3. On the other hand, at concentrations lower than 12.5 µg/mL, blend 2 was more active than blends 1 and 3, and SM-EO and CC-EO when tested individually. The IC_{50} (i.e., the half-maximal inhibitory concentration) values of blends 1 (IC_{50} = 7.04± 1.20 µg/mL), 2 (IC_{50} = 3.12± 1.60 µg/mL), and 3 (IC_{50} = 23.27± 4.50 µg/mL) were lower compared to those of SM-EO (IC_{50} = 21.45± 4.06 µg/mL), and CC-EO (IC_{50} = 7.04± 1.20 µg/mL).

2.3. *Anti-Candida activity of SM-EO and CC-EO*

The antifungal activity of SM-EO, CC-EO, and their three blends against nine *Candida* species were evaluated in terms of their Minimal Inhibitory Concentration (MIC) values. MIC values ranged between 31.2 and 125 µg/mL, as shown in Table 3. SM-EO displayed the lowest MIC against *C. albicans* (MIC = 62.5 µg/mL), *C. glabrata* (MIC = 62.5 µg/mL), *C. krusei* (MIC = 31.2 µg/mL), *C. orthopilosis* (MIC = 31.2 µg/mL), and *C. parapsilosis* (MIC = 62.5 µg/mL). On the other hand, the lowest MIC values against *C. rugosa*, *C. tropicalis*, and *C. metapsilosis* were obtained for blends 1, 2, and 3 (MIC = 31.2 µg/mL), respectively. Amphotericin B, which was used the

positive control against the tested *Candida* strains, displayed MIC values ranging from 0.25 to 1.0 μg/mL.

3. Discussion

3.1. Chemical composition of SM-EO and CC-EO

In a study on the seasonality effect on the yield and chemical composition of the EOs from four *S. molle* accessions in Brazil Southeastern, Pereira and co-workers reported that the highest S. *molle* EO yields (3.26%) were obtained in Autumn and Spring, whereas the lowest EO yield (1.30%) was obtained in Summer [34]. Herein, SM-EO was obtained in 1.52±0.36% yield from a specimen collected in Brazil Southeastern at the end of Summer, which is very similar to the average EO yield reported by Pereira and co-workers for specimens collected in the Summer (1.53%) [34].

In literature, several studies report the extraction and chemical composition of essential oils from *S. molle* dried leaves collected in different regions of Brazil [15,31,32,34-40]. Monoterpenes and sesquiterpenes are the main constituents in the EO from *S. molle* leaves, with the predominance of myrcene [15,32,34], α-pinene [31,32,35], β-pinene [31,32,35], limonene [15,31,37], sabinene [31,34], α-phellandrene [15,39], β-phellandrene [39], β-caryophyllene [37,40], bicyclogermacrene [34,37], caryophyllene oxide [36,40], spathulenol [35,36,40], cubenol [35,36], and elemol [15,39]. Some studies have also demonstrated that the chemical composition of the EO from *S. molle* leaves is affected by the season [34,38,41] and region of collection [41], the extraction method [42], and the extraction time [38]. SM-EO was found to be rich in sesquiterpenes (76.8%), especially spathulenol (26.93%), β-caryophyllene (19.90%), and caryophyllene oxide (12.69%).

This chemical composition is very similar to that of the EO obtained from a *S. molle* chemotype from Brazil's Midwest region, as reported by Silva and co-workers [40].

The phenylpropanoids (*E*)-cinnamaldehyde, *cis*-2-methoxycinnamic acid, and cinnamyl acetate were the main compounds identified in CC-EO. (*E*)-cinnamaldehyde is been considered a marker of cinnamon EOs, and its occurrence as the major compound in the EO from *C. cassia* bark has been commonly reported [25,26,28,43-53], followed by (*E*)-o-methoxy-cinnamaldehyde [25,28,43,45,47,48,50-53], and cinnamyl acetate [25,26,44,48,50,53]. On the other hand, the identification of (*E*)-*cis*-2-methoxycinnamic acid as a major compound in the EO from *C. cassia* bark is rare [54,55], whereas the presence of benzaldehyde with a relative area higher than 5% is uncommon. Although benzaldehyde is one of the most common constituents in the EO of *C. cassia* bark [25,26,28,43-49,53,54], its relative area commonly ranges from 0.1 to 1.5%.

3.2. *Antileishmanial activity of SM-EO, CC-EO, and their blends*

According to the literature, IC_{50} values lower than 10 μg/mL, between 11 and 50 μg/mL, between 51 and 100 μg/mL, and higher than 100 μg/mL denote EOs that are highly active, active, moderately active, and inactive, respectively [56]. Based on these criteria, SM-EO (IC_{50} = 21.45±4.06 μg/mL) and CC-EO (IC_{50} = 23.27± 4.50 μg/mL) can be considered active against *L. amazonensis* promastigote forms *in vitro.*

Data on the antileishmanial activity of *S. molle* are still scarce. To mention, Delgado-Altamirano and co-workers reported that the aqueous and dichloromethane:methanol 1:1 extracts from *S. molle* leaves and branches collected in Mexico displayed IC_{50} values of 15.4±5.5 and 29.4±6.0 μg/mL, respectively, against *L. amazonensis* promastigotes. These extracts were also

active against intracellular amastigotes of *L. amazonensis*, with IC_{50} of 25.9±4.9 and 21.8±4.5 μg/mL, respectively [57]. However, to the best of our knowledge, the antileishmanial activity of *S. molle* EOs has not been previously investigated to date. Our results indicated that SM-EO is active against *L. amazonensis* promastigotes *in vitro*, with an IC_{50} value (IC_{50} = 21.45±4.06 μg/mL) very similar to those reported by Delgado-Altamirano and co-workers for their aqueous and dichlorometane: methanol *S. molle* extracts [57]. This activity may be associated with the sesquiterpenes spathulenol [58], caryophyllene oxide [58], and β-caryophyllene [59], which are major in SM-EO and had their antileishmanial activity against *L. amazonensis* reported in the literature [58,59].

Conversely, the antileishmanial activity of extracts and EOs from *C. cassia* has been tested against different *Leishmania* species. Afrin and co-workers reported that the dichloromethane extract from *C. cassia* bark collected in India was active against promastigote forms of *L. donovani*, with an IC_{50} of 33.60 μg/mL [60]. Le and co-workers isolated the EO from *C. cassia* fresh leaves collected in Vietnam and assessed its antileishmanial activity against *L. mexicana* promastigote forms. The authors obtained a high activity for the tested EO, with an IC_{50} value of 8.49 μg/mL [29]. Our results revealed that SM-EO is active against *L. amazonensis* promastigote forms, displaying an IC_{50} value of 23.27 μg/mL. This activity may be due to the high concentrations of (*E*)-cinnamaldehyde (60.11%) in SM-EO. The antileishmanial activity of cinnamaldehyde against *L. amazonensis* promastigote and amastigote forms has been reported [61].

Based on recent studies reporting that binary combinations of EOs may be more active than the EOs tested individually [62,63], we also tested the antileishmanial activity of SM-EO and CC-EO combinations (i.e., blends). Three blends were prepared and tested for the

antileishmanial activity against *L. amazonensis* promastigotes: SM-EO:CC-EO 3:1 (v/v), SM-EO:CC-EO 1:1 (v/v), and SM-EO:CC-EO 1:3 (v/v), which were referred as blends 1, 2, and 3, respectively. All three blends were highly active, displaying IC_{50} of 7.04 μg/mL, 3.12 μg/mL, and 4.17 μg/mL, respectively. These results revealed an interesting synergism between SM-EO and CC-EO constituents, which makes IC_{50} decrease 3-7 times compared to the IC_{50} of SM-EO and CC-EO tested individually. The antileishmanial activity of blend 2 is noteworthy because its IC_{50} is very similar to that of amphotericin B (IC_{50} < 3.12 μg/mL), which was used as the positive control.

3.3. *Antifungal activity of SM-EO, CC-EO, and their blends*

In literature, based-MIC criteria to classify the antifungal activity are not uniform. For example, Barbosa and co-workers considered that natural products with MIC values equal to or below 500 μg/mL are potent inhibitors of microbial activity [64]. On the other hand, Souza and co-workers established that compounds with MIC ≤ 1000 μg/mL display relevant antifungal activity, whereas MIC ≤ 250 μg/mL denotes highly interesting antifungal activity [65]. Based on these criteria, and inspired by the Oliveira and co-workers' review [66], in this study, the antifungal activity will be classified as follows: MIC ≤ 10 μg/mL, 10 < MIC ≤ 100 μg/mL, 100 < MIC ≤ 500 μg/mL, and 500 < MIC ≤ 1000 μg/mL denotes high, good, moderate, and weak activities, whereas MIC > 1000 μg/mL indicate inactivity.

Although the antifungal activity of the EOs of *S. molle* against a wide diversity of fungi and yeasts has been investigated [15-17,42,67-71], data on their activity against *Candida* species are still scarce [32,72]. To mention, Prado and co-workers assessed the anti-*Candida* activity of the

EO of *S. molle* fresh leaves from a specimen collected at Brazil Southeastern against six *Candida* species (*C. albicans* ATCC 36801, *C. guilliermondii* ATCC 22017, *C. krusei* ATCC 6258, *C. orthopsilosis* ATCC 96141, *C. metapsilosis* ATCC 96142, *C. parapsilosis* ATCC 90018, and *C. neoformans* ATCC 90012). The authors reported poor activity of the EO against all the *Candida* species tested, with MIC values of 5000 μg/mL or higher against most of the *Candida* species and MIC = 2500 μg/mL against *C. albicans* [67]. SM-EO displays good activity against most of the *Candida* species, with MIC values lower than 100 μg/mL, and moderate activity against *C. tropicalis* and *C. metapsilosis* (MIC = 125 μg/mL). These MIC values are considerably lower than those reported by Prado and co-workers [67]. Taking into account that some *Candida* ATCC strains used in this study are the same as those of Prado and co-workers, differences between the MIC values are likely due to different chemical composition of SM-EO and the EO of *S. molle* tested by Prado and co-workers [67]. Indeed, the monoterpenes α-pinene (18.72%), β-pinene (25.23%), and myrcene (11.54%) are the major compounds in the EO reported by the authors, whereas the sesquiterpenes spathulenol (26.93%), β-caryophyllene (19.90%), and caryophyllene oxide (12.69%) are predominant in SM-EO. Thus, spathulenol, whose anticandidal activity is well-known [73,74], can be considered as one of the responsible for the antifungal activity of SM-EO. Differences between the anticandidal activity of EOs from *S. molle* fruits obtained from specimens collected in Yemen [75] and Zimbawe [72] against C. *albicans* have been also reported.

Many studies have addressed the anticandidal activity of the EO from *C. cassia* bark. Although most of these studies are focused on *C. albicans* [50-52,76-78], the activity against other *Candida* species like *C. glabrata* [51,77,78], *C. krusei* [78], *C. apicola* [79], and *C. auris* [53] has also been reported. The synergism between amphotericin B and the EO from *C. cassia* bark against

C. albicans ATCC 90029 has been also investigated [50]. In this study, CC-EO displayed moderate activity against most of the *Candida* species tested, with MIC values of CC-EO ranging from 125 to 250 μg/mL. However, comparison of the anticandidal activity of CC-EO with previous studies reporting the anticandidal activity of EOs from *C. cassia* bark is complex because of discrepancies in the methodologies, including dilutions of the EO based on v/v [50,51,78,80] rather than m/v and the use of differences in the *Candida* strains [52].

Gucwa and co-workers investigated the mode of action of the EO from *C. cassia* barks against *C. albicans* and *C. glabrata* and found out that this EO influenced potassium ion influx; however, alterations in the cellular morphology and damaged both cell wall and plasma membrane in treated *Candida* cells due to (*E*)-cinnamaldehyde [81] and other lipophilic compounds **[7]** could not be proved [77]. Recently, Gu and Workers investigated the mechanism of antifungal action of the EOs from *C. cassia* barks from China against *C. albicans* and found out that (*E*)-cinnamaldehyde and (*E*)-cinnamoyl acetate showed excellent binding with specific targets of AKR1B1, PPARG, BCHE, CYP19A1, CYP2C19, QPCT, and CYP51A1 [76].

In general, combinations of SM-EO and CC-EO (i.e., blends 1, 2, and 3) did not lead to a significant increase in the anticandidal activity compared to the EOs tested isolated, except in the case of blend 1 against *C. orthopsilosis* (MIC = 31.2 μg/mL), blend 2 against *C. tropicalis* (MIC=31.2 μg/mL), and blend 3 against *C. metapsilosis* and *C. parapsilosis* ATCC 90018 (MIC = 31.2 μg/mL).

4. Materials and Methods

4.1. Plant material and EO extraction

Schinus molle L. leaves were collected in Rio Verde, Goiás (GO) state, Brazil (17°47'25''S and 50°57'54''W) in February 2023. They were identified by the biologist Aternoskaires R.S. da Silva and a voucher specimen of *S. molle* (HJ5028SM) was deposited at the Herbarium Jataiense Professor Germano Guarim Neto. Leaves were then taken to the Laboratory of Natural Product Chemistry at IF Goiano - Campus Rio Verde, located in Rio Verde, GO. Leaves were weighed and dehydrated in an air circulation oven at 40 °C for 24 h.

Samples of *S. molle* dried leaves (3 x 300 g) were added to 1-L round-bottom flasks containing 500 mL distilled water and submitted to hydrodistillation on a Clevenger-type apparatus for 3 h. After manual collection of the EO samples, traces of remaining water in the EOs were removed with anhydrous sodium sulfate, which was followed by filtration. The isolated oil (SM-EO) was stored under refrigeration up to the analysis and assays. The SM-EO yield was calculated based on the dried leaves (w/w).

EO from *C. cassia* bark (CC-EO) was purchased from doTERRA® in May 2023 (lot number 60203421).

Blends of SM-EO and CC-EO were obtained by mixing the EOs as follows: Blend 1: SM-EO:CC-EO 3:1 (v/v); Blend 2: SM-EO:CC-EO 1:1 (v/v); Blend 3: SM-EO:CC-EO 1:3 (v/v).

4.2. GC-MS and GC-FID analyses

Gas chromatography flame ionization detection (GC-FID) analyses were performed by a Shimadzu GC2010 plus gas chromatograph equipped with an AOC-20s autosampler and fitted with flame ionization detector (FID) and a data-handling processor. An Rtx-5 (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30-m x 0.25-mm i.d.; 0.25-μm film thickness) was employed. Operation conditions were as follows: column temperature programmed to rise from

60 to 240 °C at 3 °C/min and then hold at 240 °C for 5 min; carrier gas = He (99.999 %), at 1.0 mL/min; injection mode; injection volume, 0.1 μL (split ratio of 1:10); and injector and detector temperatures = 240 and 280 °C, respectively. Relative concentrations of the EOs components were obtained by peak area normalization (%) and expressed as the mean of three replicate analyses.

GC-MS analyses were carried out by a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler. The column was an RTX-5MS (Restek Co., Bellefonte, PA, USA) fused silica capillary one (30 m x 0.25 mm i.d. x 0.25 μm film thickness). Electron ionization mode occurred at 70 eV. Helium (99.999 %) was employed as the carrier gas at a constant flow of 1.0 mL/min. The injection volume was 0.1 μL (split ratio of 1:10). Injector and ion-source temperatures were set at 240 and 280 °C, respectively. The oven temperature program was the same as the one used for GC. Mass spectra were taken at a scan interval of 0.5 s, in the mass range from 40 to 600 Da. Identification of SM-EO and CC-EO constituents was based on their retention indices on an RTX-5MS capillary column under the same operating conditions as those used in the GC-FID analyses, related to a homologous series of n-alkanes (C_8-C_{40}). Structures were computer-matched with the Wiley 7, NIST 08, and FFNSC 1.2 spectra libraries, and their fragmentation patterns were compared with literature data [82].

4.3. *Antileishmanial assays*

SM-EO, CC-EO, and blends 1, 2, and 3 were evaluated against promastigote forms of *Leishmania* (*Leishmania*) *amazonensis* (IFLA/BR/67/PH8), based on the methodology described by Oliveira and co-workers [83]. Therefore, promastigote forms maintained in RPMI 1640 medium

supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotic (Penicillin 10,000 IU/mL and Streptomycin 10,000 mg/mL) (Cultilab) were transferred to a 96-well plate (1 x 10^6) containing RPMI (Roswell Park Memorial Institute)1640 medium (Gibco) supplemented and incubated at different concentrations of essential oils and blends (6.25 to 200 μg/mL) previously solubilized in dimethylsulfoxide (DMSO). Cultures were incubated in a Biochemical Oxygen Demand (BOD) incubator at 24 °C for 24 h. The antileishmanial activity was determined by counting the total number of live promastigotes in a Neubauer chamber, considering flagellar motility under an optical microscope. As the negative control, promastigote forms were maintained in RPMI 1640 medium containing 0.1% DMSO and, as the positive control, promastigote forms were incubated with Amphotericin B at concentrations ranging from 0.0027 to 1.56 μM.

4.4. *In Vitro Anti-Candida Assays*

Candida species reference strains, namely *C. albicans* ATCC 90028, *C. glabrata* ATCC 2001, *C. krusei* ATCC 6258, *C. rugosa* ATCC 10571, C. *tropicalis* ATCC 13903, *C. orthopsilosis* ATCC 96141, *C. metapsilosis* ATCC 96143, *C. parapsilosis* ATCC 22019, and *C. parapsilosis* ATCC 90018 and were used for evaluating anti-*Candida* activity of EOs. Strains were maintained at -80 °C in sterile distilled water and 50% glycerol and subcultured in Sabouraud Dextrose Agar (SDA, Difco, Detroit, MI, USA) and CHROMagar Candida medium (Becton, Dickinson and Company, Sparks, MD, USA) at 37 °C for 24 h to ensure purity and viability. *In vitro* antifungal susceptibility assays were performed by the broth microdilution method in agreement with protocol M27-S4 issued by the Clinical and Laboratory Standards Institute (CLSI) [84]. Sterile 96-well microtiter plates (Corning Inc., Corning, NY, USA) were used. The final inoculum size was 2.5

× 10^3 cells/mL. Amphotericin B (Amp B), SM-EO, CC-EO, and blends ranging from 0.03 to 16 μg/mL and from 3.90 to 2.000 μg/mL, respectively, were used. AmpB and EOs were solubilized in dimethyl sulfoxide (DMSO, 2%) and diluted in RPMI 1640 (Sigma) medium with 0.2% glucose. *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258 strains, and AmpB were used as quality controls [84]. AmpB was used as the positive control because of its broad-spectrum antifungal [85]. Minimum Inhibitory Concentration (MIC) values were determined by the fluorometric indicator resazurin at 0.01% (w/v) [86]. MIC was defined as the lowest antifungal/EO concentration that maintained the blue hue. Wells in which microorganisms grew got pink.

5. Conclusions

The EOs from *S. molle* dried leaves (SM-EO) and *C. cassia* bark (CC-EO) displayed good antileishmanial activity against *L. amazonensis* promastigote forms. These EOs also showed good or moderate activity against the panel of *Candida* standard strains. While the three blends of SM-EO and CC-EO did not affect the anticandidal activity significantly, they were very active against *L. amazonensis* promastigotes, with IC_{50} values lower than 10 μg/mL. Noteworthy, the IC_{50} of blend 2 (SM-EO:CC-EO 1:1 v/v) was similar to that of amphotericin B, which was used as the positive control. These results revealed that combinations between SM-EO and CC-EO boost the antileishmanial activity compared to the EOs tested individually and motivate further studies on the antiparasitic activity of these blends. To understand the synergism between SM-EO and CC-EO deeper, studies are being carried out to evaluate the synergistic relationships between (*E*)-cinnamaldehyde, β-caryophyllene, caryophyllene oxide, and spathulenol.

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Table 1: Chemical composition of the essential oils of *S. molle* (SM-EO) and *C. cassia* (CC-EO), as determined by GC-FID and GC-MS.

Compounds	**Retention index (RI)**		**% RA**	
	RI_{exp}	**RI_{lit}**	**SM-EM**	**CC-EO**
α-Thujene	930	928	1.64±0.32	
β-Pinene	973	972	1.58±0.25	
Benzaldehyde	980	982		8.62±0.61
Linalool	1096	1098	1.04±0.38	
Nopinone	1140	1137	1.43±0.84	
trans-Pinocarveol	1142	1140	2.72±0.91	
trans-Verbenol	1145	1144	1.90±0.56	
Pinocarvone	1161	1160	2.04±0.47	
p-Cymen-8-ol	1179	1179	2.43±0.95	
(*E*)-Cinnamaldehyde	1189	1189		60.11±1.22
Myrtenal	1192	1193	3.58±0.44	
Verbenone	1205	1197	2.20±0.33	
β-Caryophyllene	1419	1419	19.90±1.23	
γ-Cadinene	1523	1524	1.66±0.61	
(*E*)-*cis*-2-Methoxycinnamic acid	1546	1546		10.37±0.9
Spathulenol	1578	1576	26.93±1.84	
Caryophyllene oxide	1583	1581	12.69±1.12	
Globulol	1584	1583	0.58±0.02	
(E)-Cinnamyl acetate	1588	1590		20.90±1.01
Viridiflorol	1592	1592	1.04±0.07	
1,10-di-*epi*-cubenol	1613	1613	2.27±0.34	
Cubenol	1643	1642	8.59±0.88	
α-Cadinol	1654	1653	2.39±0.75	
Khusinol	1674	1674	0.66±0.13	
Monoterpene hydrocarbons			3.22	
Oxygenated monoterpenes			17.34	
Sesquiterpene hydrocarbons			21.65	
Oxygenated sesquiterpenes			55.15	
Phenylpropanoids				91.5
Others				8.5
Not identified			2.64	

[a] **RI**: Retention Index; [b] **RA**: relative area**.**

Table 2. *In vitro* leishmanial activity of SM-EO, CC-EO and their blends against *Leishmania amazonensis* promastigote forms.*

	% of inhibition of flagellar mobility ± SD (24 h)						IC_{50} (µg/mL)
	100	**50**	**25**	**12.5**	**6.25**	**3.12**	
SM-EO	100.00±0	91.92±4.51	55.56±4.17	24.49±6.51	3.54±5.51	0±0	21.45±4.06
CC-EO	100.00±0	100.00±0	93.44±4.62	0±0	0±0	0±0	23.27± 4.50
Blend 1	100.00±0	83.84±1.33	80.23±1.14	56.46±2.47	49.87±1.24	32.70±2.47	7.04± 1.20
Blend 2	100.00±0	90.68±1.33	80.04±2.09	74.90±0.19	57.98±1.71	55.51±2.19	3.12± 1.60
Blend 3	99.49±0.11	99.68±0.11	74.40±0.11	66.29±0.48	58.75±2.47	47.53±0.76	4.17± 0.25

*Positive control: Amphotericin B (IC_{50} < 3.12 µg/mL). **SM-EO**: essential oil from *S. molle* dried leaves; **CC-EO**: essential oil from *C. cassia* bark.

Table 3. Minimal Inhibitory Concentration (µg/mL) values of SM-EO, CC-EO, and their blends against *Candida* species.

	SM-EO	**CC-EO**	**Blend 1**	**Blend 2**	**Blend 3**	**Amp B***
C. albicans ATCC 90028	62.5	125	62.5	100	100	1.00
C. glabrata ATCC 2001	62.5	62.5	62.5	100	62.5	0.25
C. krusei ATCC 6258	31.2	125	100	100	62.5	0.25
C. tropicalis ATCC 13903	125	250	100	31.2	100	0.35
C. rugosa ATCC 10571	62.5	250	31.2	100	100	0.25
C. orthopsilosis ATCC 96141	31.2	125	31.2	100	62.5	0.50
C. metapsilosis ATCC 96143	125	250	100	100	31.2	0.25
C. parapsilosis ATCC 22019	62.5	125	100	62.5	100	1.00
C. parapsilosis ATCC 90018	62.5	125	100	62.5	31.2	1.00

*Amphotericin B (positive control); **SM-EO**: essential oil from *S. molle* dried leaves; **CC-EO:** essential oil from *C. cassia* bark; **Blend 1**: mixture of SM-EO:CC-EO 3:1 (v/v); **Blend 2**: mixture of SM-EO:CC-EO 1:1 (v/v); **Blend 3**: SM-EO:CC-EO 1:3 (v/v).