Chemical Constituents and Antileishmanial and Antibacterial Activities of Essential Oils from *Scheelea phalerata*  

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**ABSTRACT:** *Scheelea phalerata* Mart. ex Spreng (Arecaceae) is a palm tree found in the Brazilian cerrado. There are no topics related to volatile oils from *S. phalerata* leaves in the literature. This work determines its chemical composition and evaluates the biological activity under two different seasonal conditions (dry and rainy seasons). The dry essential oil yield was 0.034 ± 0.0011% and the rainy essential oil yield was 0.011 ± 0.0033%. Both essential oils presented different qualitative and quantitative compositions (99.4 and 98.5%). The main constituents of the dry essential oil were phytol (36.7%), nonadecane (9.7%), linolenic acid (9.1%), (Z)-hex-3-en-1-ol (4.2%), and squalene (4.0%). The main constituents of the rainy essential oil were phytol (26.1%), palmitic acid (18.7%), hexan-1-ol (15.6%), (Z)-hex-3-en-1-ol (9.7%), and oleic acid (4.0%). The antileishmanial activity against promastigotes of *Leishmania amazonensis* was observed only for the rainy season essential oil (IC$_{50}$ value of 165.05 ± 33.26 μg mL$^{-1}$). A molecular docking study showed that alcohols exert a paramount efficacy and that the action of some essential oil compounds may be similar to that of amphotericin B. Still, only the essential oil from the dry season showed moderate antibacterial activity against *S. sanguinis* (MICs 200–400 μg mL$^{-1}$). The cytotoxicity against Vero cells was identical (>512) for both essential oils. The novel data here for both chemical characterization and biological activity, in particular, evidence that the action of these compounds is similar to that of amphotericin B, providing valuable information to the drug-discovery field.

**INTRODUCTION**

Neglected tropical diseases affect between 700,000 and 1 million people around the world, with 26,000–65,000 deaths annually. Among these tropical diseases, one transmitted by the protozoa of the genus *Leishmania* stands out. This disease affects socially vulnerable human populations in countries such as Afghanistan, Algeria, Brazil, Colombia, Iran, Iraq, and the Syrian Arab Republic. Between 600,000 and 1 million new cases occurred worldwide annually.¹⁻³ This situation may become more critical in the coming years. Therefore, searching for compounds or prototypes for the development of new potentially bioactive molecules may be important for the survival of future generations.

Palm tree species are widely distributed in the southeastern and midwestern regions of Brazil and produce edible fruits rich in lipids, carbohydrates, and fiber.⁴ Among the palm tree species, *Scheelea phalerata* Mart. ex Spreng (Arecaceae), well known as “bacuri”, has significant economic value. The literature shows that the oil extracted from the “bacuri” fruit is rich in carotenoid oleic acid, a monounsaturated fatty acid. This oil is beneficial to human health and is used in dermatological products for babies as an antipyretic agent.
and to relieve lung congestion and arthritis. The oil is also used in the production of biodiesel.5–7 Previous studies have been conducted on the chemical composition of its fixed oils.5,8 However, there are still no reports in the literature of the chemical composition of the essential oils from the leaves of *S. phalerata* and their biological activities.

| Table 1. Chemical Constituents of the Essential Oils from Leaves of *Scheelea phalerata*  
| --- | --- | --- | --- | --- |
| compound | area (%) | AI calculated | method of identification** |
| **dry season** | **rainy season** | **AI reference** | **dry season** | **rainy season** |
| (Z)-Hex-3-en-1-ol | 4.2 | 9.7 | 850 | 858 | 853 | b,c,d |
| (Z)-Hex-2-en-1-ol | 1.1 | 859 | 869 | a,b,c |
| hexan-1-ol | 1.8 | 15.6 | 867 | 871 | 866 | a,b,c |
| nonanal | 0.6 | 1100 | 1103 | b,c |
| 4-hydroxy-2-methylacetophenone | 1.8 | 1309 | 1321 | b,d |
| E/β-ionone | 0.8 | 1485 | 1487 | a,c |
| tridecan-2-one | 2.0 | 1.0 | 1495 | 1498 | 1493 | b,c |
| (E, E)-α-farnesene | 0.8 | 1495 | 1507 | b,c |
| (E)-nerolidol | 1.7 | 1569 | 1568 | a,b,d |
| tetradecan-1-ol | 0.8 | 1675 | 1674 | b,d |
| heptadecane | 0.6 | 1700 | 1700 | b,c |
| pentadecan-2-one | 2.9 | 1694 | 1700 | b,c |
| pentadecan-1-ol | 3.2 | 1700 | 1711 | b,d |
| pentadecanal | 2.7 | 1717 | 1715 | b,d |
| hexadecanal | 2.8 | 1819 | 1817 | b,c |
| hexahydrofarnesyl acetone | 2.2 | 1.1 | 1846 | 1845 | 1841 | b |
| N.I. | 1.6 | | | 1894 |
| hexadecan-1-ol | | 2.0 | 1879 | 1881 | b,d |
| N.I. | | 1.0 | | 1890 |
| N.I. | | 0.8 | | 1897 |
| hexadecanoic acid (palmitic acid) | 1.9 | 18.7 | 1970 | 1968 | 1971 | a,b,d |
| ethyl hexadecanoate (ethyl palmitate) | | 0.8 | 1983 | 1900 | b,d |
| pinamaridene | 2.0 | 1941 | 1971 | b,c |
| nonadecane | 9.7 | 1970 | 1994 | a,b |
| (E,E)-geranyl linalool | 0.6 | 2026 | 2028 | b,c |
| phytol | 36.7 | 26.1 | 2115 | 2116 | 2113 | a,b,d |
| (9Z,12Z)-octadeca-9,12-dienoic acid (linoleic acid) | 1.3 | 1.3 | 2132 | 2138 | b,c,d |
| (9Z)-octadec-9-enoic acid (oleic acid) | 4.0 | 2141 | 2145 | b,c,d |
| octadeca-9,12,15-trienoic acid (linolenic acid) | 9.1 | 1.1 | 2171 | 2170 | 2165 | b,d |
| N.I. | 1.3 | | | 2191 |
| tricosane | 1.7 | 1.9 | 2300 | 2296 | 2292 | b,c |
| N.I. | | 1.4 | | 2374 |
| tetracosane | 1.5 | | 2400 | 2395 | b,c |
| pentacosane | 1.6 | 2.1 | 2500 | 2494 | 2491 | b,c |
| N.I. | | 1.7 | | 2547 |
| hexacosane | 1.2 | | 2600 | 2593 | b,c |
| heptacosane | 1.8 | 1.4 | 2700 | 2756 | 2689 | b,c |
| octacosane | 1.5 | | 2800 | 2886 | b,c |
| squalene | 4.0 | | 2955 | b |
| total (%) | 99.4 | 98.5 | | | **chemical class distribution (%)*** |
| alcohols | 7.1 (3) | 31.3 (5) | | |
| aldehydes | 5.5 (2) | 0.6 (1) | | |
| fatty acids | 11.0 (2) | 25.1 (4) | | |
| ketones | 8.9 (4) | 2.9 (3) | | |
| diterpene | 38.7 (2) | 26.7 (2) | | |
| sesquiterpene | 1.7 (1) | 0.8 (1) | | |
| triterpene | 4.0 (1) | | | |
| long-chain alkanes | 19.6 (8) | 5.4 (3) | | |
| ester | 0.8 (1) | | | |
| N.I. | 2.9 (2) | 4.9 (4) | | | **Note:** N.I., not identified; AI, arithmetic index on the DB-5 capillary column; *relative area: GC/FID yield (%); **method of identification: *mass spectrum comparison with mass spectral database (Shim, Wiley, and Nist Libraries); similarity index with mass spectral database; *NIST (ref 19); *Adams (ref 20); *The Pherobase: http://www.pherobase.com/database/kovats/; ***numbers in parentheses refer to the compounds identified for each function.**
The literature reports some bioactivity of the Arecaceae family plant against *Leishmania amazonensis*. The oils from the Palmae are also used for such purposes, that is, *Cocos nucifera* (Arecaceae), which showed activity against *Leishmania amazonensis*. Additionally, the oils of *Cocos nucifera* (coconut pulp) presented antimicrobial activity against *A. niger*, *S. typhi*, and *S. aureus*, showing the potential of the compounds found in this family with notable biological activities. Promastigote forms of *Leishmania amazonensis* were treated with different concentrations of the aqueous extract from *Syagrus coronata* (Arecaceae). The toxicity of the polymeric procyanidin-rich crude aqueous extract toward *Leishmania amazonensis* with concomitant macrophage activation and no allergic reactions in vivo demonstrated the potential use of *S. coronata* (licuezeiro), another Palmae tree, as a leishmanicidal drug.

Considering the potential of bioactivity already confirmed for some species of the palm tree family (Arecaceae) and the need to search for new molecules that act against microorganisms, the purpose of this work was to determine the chemical constitution of the essential oils from the leaves of *Scheelea phalerata* in different seasonal periods (dry and rainy conditions) and to evaluate, in vitro, their antileishmanial, antibacterial, and cytotoxic activities. Additionally, molecular docking analysis aimed to clarify the behavior of the major components that present optimal biological activity, which is an unprecedented verification for the volatiles of this species.

**RESULTS AND DISCUSSION**

**Yield and Chemical Composition of the Essential Oils.** The yields of the leaf essential oils were 0.034 ± 0.001 (% w/w ± SD) in the dry season and 0.011 ± 0.003 (% w/w ± SD) in the rainy season. Both essential oils presented different qualitative and quantitative compositions (99.4 and 98.5%, respectively). Significant differences were observed in the yields between essential oils with respect to the period of collection, showing that the synthesis of essential oils for this species is influenced, probably, by humidity among other factors.

The essential oil collected during the dry season revealed the presence of 25 compounds (Table 1). The main constituents of the dry essential oil were phytol (36.7%), nonadecane (9.7%), linolenic acid (9.1%), (Z)-hex-3-en-1-ol (4.2%), and squalene (4.0%). For the essential oil collected during the rainy season, 24 compounds were found. The main constituents for the rainy essential oil were phytol (26.1%), palmitic acid (18.7%), hexan-1-ol (15.6%), (Z)-hex-3-en-1-ol (9.7%), and oleic acid (4.0%). A change in the biosynthesis of compounds in greater abundance was observed probably due to the environmental conditions at the seasonal periods studied; only phytol was maintained as the most abundant bioactive compound for both essential oils. This compound is biosynthesized by both mevalonic acid and 2-C-methylerythritol-4-phosphate pathways, and although ubiquitous in plants as a constituent of chlorophyll, it rarely occurs naturally in its free form. Many pharmacological activities of phytol and its derivatives are well known, including antimicrobial, cytotoxic, antitumorous, antimatagenic, antitumorogenic, anti-inflammatory, anxiolytic, antidepressant, immunomodulator, hair-growth-facilitating, hair-loss defense, and antidiarrheic properties. The chemical class distribution of the volatile constituents of *S. phalerata* in the dry and rainy seasons is summarized in Table 1. It was observed that the terpenoid compounds were biosynthesized in a major proportion in the rainy season (44.4 versus 27.5% in dry season), probably due to luminosity-dependence factors inside glandular trichomes, where specific terpenoid compounds are biosynthesized.

Diterpenes were highlighted in both seasonal periods, due to the great representability of phytol, and also fatty acids, with 11.0 and 25.1% of compounds of this class in the dry and rainy seasons, respectively. Classes of compounds differentially synthesized as a consequence of the seasonal period were alkanes (19.6%) in the dry season and alcohols (31.3%) in the rainy season. Alkanes are also present in waxes and act as a protective barrier for the leaves. These compounds may have a particularly important role during the dry season, avoiding loss of water due to their nonpolarity. The higher proportion of alcohols in the rainy season may be due to the fact that the humidity favors a greater proliferation of microorganisms, and the plant requires compounds that act as antimicrobial agents. Alcohols are a class of compounds that generally possess bactericidal activity, for example, acting as protein-denaturing agents.

**Antileishmanial and Cytotoxic Activities.** To determine biological activities for the essential oils of the leaves from *S. phalerata*, the antileishmanial effects against *Leishmania amazonensis* were evaluated (Table 2). Additionally, the toxicity of these essential oils was verified by the cytotoxic test performed using Vero cells.

| Sample                | IC₅₀ (µg mL⁻¹) | CC₅₀ (µg mL⁻¹) | Selectivity index (SI) |
|-----------------------|----------------|----------------|------------------------|
| *Leishmania amazonensis* (promastigote stage) | **Table 2. Antileishmanial Activity, Cytotoxic Activity, and Selectivity Index of the Leaf Essential Oils from *S. phalerata*** |
| Dry essential oil     | >512           | >512           | 3.10                   |
| Rainy essential oil   | 165.05 ± 33.26 | >512           | 29.8                   |
| Amphoterin B⁺         | 0.29 ± 0.01    | 8.65⁺         | 29.8                   |
| Cisplatin             | 7.01 ± 0.66    |               |                        |

*Positive control. ⁺Nigussie et al.; IC₅₀, inhibitory concentration; CC₅₀, cytotoxic concentration.

Only the essential oil of the rainy season presented inhibitory activity against *Leishmania amazonensis*, suggesting that the compounds synthesized in higher concentrations in this seasonal period may be related to the result found. Additionally, the selectivity index (SI) for the rainy season essential oil was positive (3.10), indicating specificity for the parasite in detriment to the normal cell line evaluated (Table 2).

The rainy essential oil of *S. phalerata* is constituted of long-chain fatty acids (25.1%) such as oleic acid (4.0%), palmitic acid (18.7%), and oleic acid (4.0%), all of which have already been associated with antileishmanial activity. There are no studies regarding the evaluation of the isolated phytol compounds against *Leishmania* species. However, this major compound identified in the essential oil of *S. phalerata* is also present in the hexane-rich fraction of another species (*Lacistema pubescens*) that presented action against *Leishmania amazonensis* promastigote and amastigote forms. Therefore, it appears that phytol is an important molecule in
antileishmanial activity, but the individual action of this compound seems insufficient, since it is also present in the essential oil of the dry season in high concentrations, and yet did not present any biological activity. Another hypothesis is that other compounds present in the mixture may disturb the phytol action by forming complexes, rendering this molecule inactive.

Still, the essential oil from the dry season presented very low antileishmanial activity ($IC_{50} > 512 \mu g mL^{-1}$). It is possible that the low concentration of fatty acids in its composition interferes with the biological activity.

**Preliminary Evidence of an Action Similar to that of Amphotericin B in terms of Antileishmanial Activity.**

*Leishmania* parasites have a type of sterol in their membranes, ergosterol, which is a lipid target of amphotericin B, a drug used for the treatment of leishmaniasis. Amphotericin B is a macrocyclic molecule derived from a natural product that has a biological effect primarily through binding to ergosterol and permeating the membrane via channel formation that represents a second complementary mechanism, further increasing the drug potency. The amphotericin B molecule is composed of 37 carbon atoms forming a closed macrocyclic ring by lactonization. It has a chain of unsubstituted conjugated double bonds and, in the opposite portion, a polyhydroxylated chain with seven free hydroxyl groups. At one end of the amphotericin B molecule is present a mycosamine residue (lactone) with a free amine group, forming a side chain. It is already established that the mycosamine portion is critical for the amphotericin B binding capacity and, thus, for forming possible hydrogen bonds to ergosterol (Figure 1). This interaction allows further hydrophobic interactions and penetration of the drug into the membrane. Once in the membrane, amphotericin B molecules interact, forming a pore and potentiating their biological activity.

Molecular docking is a computational technique that predicts the interaction between small molecules and proteins or other macromolecules. Hence, in this work, preliminary studies with molecular docking of the alcohols and hydrophobic molecules present in the essential oils from *S. phalerata* against ergosterol showed different energy values (Figure 2). The binding energy ($E_b$; blue circles) of alcohols are slightly lower in comparison with those of hydrophobic molecules, with average values of $-1.52$ and $-0.86$ kcal/mol, respectively. On the other hand, the intermolecular energy ($E_i$; red circles) showed a notable difference between these molecules, with hydrophobic molecules presenting an average value of $-5.18$ kcal/mol, which is 1.6-fold lower than that of the alcohol molecules ($-3.17$ kcal/mol). These results suggest that alcohol molecules present stronger types of interactions, although their intermolecular interactions are weaker in comparison with those of hydrophobic molecules. Therefore, these molecules may interact in multiple ways: alcohols may form a hydrogen bond with the polar/head portion of the ergosterol while the hydrophobic molecules bind to the hydrophobic/body portion through hydrophobic interactions.

Alcohols are known modulators of membrane properties. For example, alcohols can change the permeability and diffusion of a membrane, thus affecting microorganisms. In the context of the membrane environment, alcohol molecules may be essential to the initial recognition of ergosterol.
molecules present in the membrane, acting similar to the mycosamine portion of amphotericin B and increasing the permeability and allowing further penetration of the hydrophobic molecules. In this sense, the essential oils studied in the present work may act simultaneously to express their biological activity in Leishmania membranes. It is interesting to note that the same two steps are observed in the amphotericin B drug, suggesting that essential oils studied in the present work may act in a way similar to that of the established drug, corroborating the applicability of the technique to our problem. Thus, molecular docking was important to give us insights into how the essential oil from Scheelea phalerata can present antileishmanial activity and how these molecules may act synergically, being comparable to amphotericin B.

**Antibacterial Activity.** The results of the antibacterial activities found for essential oils from the leaves of *S. phalerata* collected during the dry and rainy seasons are shown in Table 3. The MIC results showed that better antibacterial activity was found for the essential oil from the dry season.

### Table 3. Antibacterial Activity of the Leaf Essential Oils from *S. phalerata*α

| bacteria                  | dry essential oil (μg mL⁻¹) | rainy essential oil (μg mL⁻¹) | CD |
|---------------------------|-----------------------------|-----------------------------|----|
| aerobic                   |                             |                             |    |
| *Streptococcus mutans*b   | 400                         | >400                        | 0.92 |
| *Streptococcus mitis*b    | 400                         | >400                        | 3.68 |
| *Streptococcus sanguinis*b| 200                         | >400                        | 1.84 |
| *Actinobacillus*           |                             |                             |    |
| *actinomycetemcomitans*c  | 400                         | >400                        | 7.84 |
| anaerobic                 |                             |                             |    |
| *Porphyromonas gingivalis*c| 400                         | >400                        | 3.68 |
| *Fusobacterium nucleatum*c| 400                         | >400                        | 1.84 |
| *Actinomyces naeslundii*c  | 400                         | >400                        | 1.84 |

α, CD, chlorhexidine dihydrochloride (positive control). b, Gram-positive bacteria. c, Gram-negative bacteria.

This essential oil showed moderate activity against all bacteria evaluated, based on the classification of Holetz et al., with MICs ranging from 200 to 400 μg mL⁻¹. The essential oil from the rainy season showed no antibacterial activity until the concentration of 400 μg mL⁻¹. Particularly for essential oils, MIC values equal to or below 2000 μg mL⁻¹ can be considered noteworthy for the antimicrobial activity.

Some diterpenes have already been investigated for activity against oral microorganisms and exhibited strong and promising antibacterial effects. Additionally, some studies indicate the phytol compound as an important antimicrobial agent.

A correlation between the antibacterial effect and phytol compound was observed in in silico model studies, where the activity of the oils collected in different seasons was proportional to their concentration. In our work, and despite the considerable presence of phytol in the *S. phalerata* rainy essential oil, this compound did not show activity against the bacteria until 400 μg mL⁻¹. This fact may be related to the contribution of the other major compounds, which are probably working in unison.

Nonadecane and linolenic acid are also major compounds present in the essential oil collected during the dry season. The antibacterial activities of linolenic acid are well known, and long-chain alkanes have already been confirmed as antimicrobial agents. Lipophilic constituents present in the essential oils inhibit microbial growth. The interaction of these compounds with the lipid part of the cell membrane makes the microbial cell more permeable, resulting in ion loss, enzymatic interruption, and bacterial death. Additionally, essential oils are capable of inhibiting the synthesis of RNA, DNA, proteins, and polysaccharides within the bacterial cell. Further investigations with respect to the compounds identified in *S. phalerata* should be conducted to better understand the actions against susceptible bacteria.

### CONCLUSIONS

This work reports for the first time the chemical composition of the leaf essential oils from Scheelea phalerata and their action on *Leishmania amazonensis* promastigotes and antibacterial activity. The essential oils from *S. phalerata* presented different constitutions as a consequence of the seasonal effects, except for the phytol compound, which was biosynthesized at high concentrations independent of the collection period. The differences between the constituents reflected in the antileishmanial and antibacterial activities. Hence, the rainy season essential oil presented the best antileishmanial result (165.05 ± 33.26 μg mL⁻¹) and the dry season essential oil showed the best antibacterial activity (200–400 μg mL⁻¹) compared with the rainy season essential oil (>400 μg mL⁻¹) against all bacteria tested.

Based on molecular docking analyses, alcohol compounds played a fundamental role in the action of this oil against *L. amazonensis*, since they likely caused a disturbance in the cell membrane through an interaction with ergosterol, allowing the action of the other major compounds. This mechanism was observed in the amphotericin B drug, whose intermediary equivalent to the action of mycosamine would be the alcohol molecules, suggesting that the essential oils from *S. phalerata* may act in a way similar to that of a drug that is already well-established in the treatment of leishmaniasis.

### EXPERIMENTAL SECTION

**Plant Material.** The leaves of *S. phalerata* were harvested in Araguari, Brazil, at coordinates 18°45′48.9″ S and 48°14′51.3″ W, and 1013 m of altitude, during two different collection periods to observe seasonal effects: dry season (September, 2015) and rainy season (January, 2016). A voucher specimen of the plant material was deposited on the Herbarium of the Federal University of Uberlândia (HUB), under the registration number 71040, after authentication by the taxonomists of the HUB. This plant was registered on the National System of Genetic Resource Management and Associated Traditional Knowledge for Research (SYSGEN) under the code AAA3202.

**Essential Oil Extraction.** Scheelea phalerata fresh leaves from each season were air-dried under the shade for 1 week, cleaned, and cut into small pieces. The volatile oils were obtained in a Clevenger-type apparatus using 400 g of sample and 2.0 L of distilled water for 4 h. Then, the essential oils were extracted with 5.0 mL of dichloromethane. After dichloromethane evaporation, the organic fraction was dried.
over sodium sulfate, filtered, and kept frozen (−10 °C) until analysis. The percent yield was calculated relative to the dried mass of the initial sample; this procedure was performed in triplicate.

**Essential Oil Characterization.** The essential oil chemical identification was carried out by gas chromatography–mass spectrometry (GC17A/QPS010, Shimadzu, Japan), equipped with a DB-5 capillary column (5% phenyl, 95% polydimethylsiloxane; J&W, 30 m × 0.25 mm × 0.25 μm film thickness). The carrier gas was helium, at a flow rate of 1.0 mL/min. The injector and detector temperatures were 220 and 240 °C, respectively. The injector volume was 1.0 μL, and the split ratio was 1:20. The oven temperature was programmed from 60 to 240 °C at a ramping rate of 3 °C/min. The electron impact energy was set at 70 eV, and fragments from 40 to 650 Da were collected. The identification of the chemical constituents was carried out by a comparison with software libraries (Shim; Wiley139; Nist08; Nist27).39 The identification was also based on a comparison of the mass spectral fragmentation patterns, and the AI obtained was compared with Al's of the National Institute of Standards and Technology (NIST) and Wiley 275 library spectra.19,20 The quantification of each essential oil was obtained by gas chromatography flame ionization (GC-FID) using a Shimadzu GC2014 spectrometer and DB-5 capillary column under the same conditions as for GC/MS, but nitrogen was used as the carrier gas. Results represent average values of three experiments for each seasonal essential oil.

**Antileishmanial Activity.** To test for the effectiveness against *Leishmania amazonensis*, the *S. phalerata* essential oils from the two different seasons were solubilized in methanol (Merck KgA, Germany) and diluted with Dulbecco’s modified Eagle’s medium (DMEM, Sigma Aldrich) in a stock solution at 1280 μg mL⁻¹. The cell viability test was carried out using *Leishmania amazonensis* in the promastigote stage (PH8 strain). The analysis was performed using 96-well plates. Stock solutions of the essential oil were added to the microplate, and serial dilutions were made, with concentrations of 512, 256, 128, 64, 32, 16, and 8 μg mL⁻¹, but not exceeding a value of 3% for methanol concentration. Then, a solution containing 1 × 10⁶ parasites in 20 μL of the medium was pipetted into each well and the plates were incubated for 48 h at 25 °C. Positive, negative, and control samples were prepared on each plate, with amphotericin B (AmBisome, Merck KgA, Germany) for American Visceral Leishmaniasis (AVL) used as the positive control. Subsequently, a solution of 3 mM resazurin (New Road Gillingham, UK) diluted in phosphate-buffered solution (PBS, Sigma Aldrich) was added to each well as a revealing solution and incubated for 24 h at 25 °C. At the end of this period, the absorbance (594 nm) was read using a microplate spectrophotometer (Spectra Max 190, Molecular Devices). All assays were performed in five replicates; the concentration that causes 50% growth inhibition of promastigote forms (IC₅₀) was calculated using a dose–response graph (Microsoft Excell 365 vs 2016) with a linear regression for each test.40

**Antibacterial Activity.** The tested strains were obtained from the American Type Culture Collection (ATCC; Rockville). The following microorganisms were used in the evaluation of antibacterial activity: *Streptococcus mitis* (ATCC 49456), *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC10556), *Aggregatibacter actinomycetemcomitans* (ATCC 43717), *Actinomyces naeslundii* (ATCC 19039), *Porphyromonas gingivalis* (ATCC 33277), and *Fusobacterium nucleatum* (ATCC 25586). The antibacterial activity of *S. phalerata* was determined in triplicate by the verification of the minimum inhibitory concentration (MIC) using the microdilution broth method in 96-well microplates.41 Each essential oil was dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich) at a concentration of 8000 μg mL⁻¹, followed by dilution in tryptic soy broth (TSB; Merck, Germany) for aerobic microorganisms and in Schaedler broth (BBL, Microbiology Systems, Cockeysville) supplemented with hemin (5.0 mg mL⁻¹) and vitamin K1 (1.0 mg mL⁻¹) for anaerobic microorganisms. The tested concentrations ranged from 400 to 25 μg mL⁻¹. The final DMSO content was 4% (v/v⁻¹); this solution was used as a negative control. The inoculum was adjusted for each organism to yield a cell concentration of 5 × 10⁵ colony forming units (CFU) per milliliter. The microplates with the anaerobic microorganisms were incubated aerobically, at 37 °C, for 24 h. The anaerobic microorganisms were incubated for 48–72 h in an anaerobic chamber (Don Whitley Scientific Bradford, UK), in a 5–10% H₂, 10% CO₂, 80–85% N₂ atmosphere, at 37 °C. After that, resazurin (Acros Organics NV, Belgium) in an aqueous solution (0.01% w/v⁻¹) was added to the microplates to indicate microorganism viability. Chlorhexidine dihydrochloride (Sigma Aldrich) was used as a positive control. The negative control (DMSO) was tested with concentrations ranging from 1 to 10% (v/v⁻¹) and did not influence bacterial growth. Sterility tests were performed for the TSB and Schaedler broths, control culture (inoculum), positive control, oils, and DMSO.

**Cytotoxic Activity.** Samples of essential oils of *S. phalerata* were dissolved in methanol (Merck KgA, Germany), diluted in DMEM (Sigma Aldrich), and supplemented until a solution of 1280 μg mL⁻¹ was achieved. The cell viability test was performed with ATCC Vero cells (CCL 81; kidney fibroblasts from the African green monkey). The microplate dilution method was used to evaluate cytotoxicity. For each test, a solution containing 1 × 10⁶ cells in supplemented DMEM was prepared. Then, 100 μL of this solution was added to each well, and the plate was incubated for 6 h at 37 °C with a humidified atmosphere and 5% CO₂, causing adhesion to the well. Once attached, the culture medium was removed and solutions of the samples were added, reaching concentrations of 1024, 512, 256, 128, 64, 32, 16, and 8 μg mL⁻¹ through serial dilutions from the stock solution. The final concentration of methanol in each well did not exceed 3%. Growth, negative (100% lysed cells), and solvent controls (methanol) as well as samples were prepared. The microplates were incubated for 48 h at 37 °C with a humidified atmosphere and 5% CO₂. After that time, a revealing solution of 3 mM resazurin in PBS (Sigma Aldrich) was added to each well, and the plate was incubated again for 24 h under the same conditions. The absorbance was read at 594 nm using a microplate spectrophotometer (Spectra Max 190, Molecular Devices). The assays were performed in five replicates, and the results of absorbance for each concentration tested were calculated according to the growth curve. The cytotoxic concentration at which 50% of the cells are viable (CC₅₀) was calculated using a dose–response graph with linear regression for each test. Cytotoxicity to cisplatin (Merck, KgA, Germany) was also calculated as a toxicity control for this cell type. To check
the specificity, a relationship between CC50 from the Vero cell line and IC50 from L. amazonensis was established by the selectivity index (SI) and calculated according to the equation proposed by Bero et al.42

Molecular Docking. The interaction of the molecules found in the essential oil from S. phalerata against ergosterol was analyzed through molecular docking using Autodock v.4.2 under the AutoDockTolls v.1.5.6 workspace.35 The molecules from the essential oil were considered to be flexible ligands, while ergosterol was defined as the receptor. However, all torsions were considered once Autodock accepted flexible receptors. This configuration allowed us to perform molecular docking with both flexible receptors and ligand molecules. All molecules were built using the server Automated Topology Builder (ATB) v.3.0, considering hydrogen atoms.36 In the AutoDockTools workspace, Gasteiger charges were computed for each molecule. Then, an Autogrid was applied to determine the searching area for docking between the molecules using a grid box centered on the ergosterol structure, with a size of 80–60–50 Å and a spacing of 0.375 Å. Furthermore, Autodock was performed to generate the docking solutions using the genetic algorithm (GA) in a standard configuration, except for the population size and the number of evaluations, with the parameters set to 200 and 25 × 105, respectively. Each Autodock run generated 10 solutions, which were analyzed carefully according to two energy values: (i) binding energy (Eb), or the affinity of the complex (receptor–ligand) considering the type and number of interactions, and (ii) intermolecular energy (Ei), or the energy of the complex after conformational change.

Statistical Analysis. The essential oil yields were expressed as the mean ± standard deviation (SD), and the statistical analysis (p ≤ 0.05) of this parameter was performed by one-way analysis of variance (ANOVA) followed by a Holm–Sidak test, using SigmaPlot 11.0 software.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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