Research Article

In Vitro Schistosomicidal Activity of the Alkaloid-Rich Fraction from Ruta graveolens L. (Rutaceae) and Its Characterization by UPLC-QTOF-MS

Lara Soares Aleixo de Carvalho, Lucas Sales Queiroz, Ismael José Alves Junior, Ayla das Chagas Almeida, Elaine Soares Coimbra, Priscila de Faria Pinto, Marcos Paulo Nascimento da Silva, Josué De Moraes, and Ademar A. Da Silva Filho

1Faculty of Pharmacy, Department of Pharmaceutical Sciences, Federal University of Juiz de Fora, Juiz de Fora, MG 36036-900, Brazil
2Department of Parasitology, Microbiology and Immunology, Biological Sciences Institute, Federal University of Juiz de Fora, Juiz de Fora, MG 36036-900, Brazil
3Department of Biochemistry, Biological Sciences Institute, Federal University of Juiz de Fora, Juiz de Fora, MG 36036-900, Brazil
4Núcleo de Pesquisa em Doenças Negligenciadas, Universidade de Guarulhos, Guarulhos, SP 07025-000, Brazil

Correspondence should be addressed to Ademar A. Da Silva Filho; ademar.alves@ufjf.edu.br

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Schistosomiasis is a neglected tropical disease (NTD) caused by Schistosoma parasites, mainly S. mansoni, that is associated with long-term undernutrition, anaemia, organ scarring, and fibrosis, resulting in disabling patient symptoms [1]. About 190 million people are infected worldwide with Schistosoma infections, with more than 70 million of new cases and thousands of deaths annually registered [2]. Only in Brazil, around 8 million people are infected with this chronic debilitating disease [3]. However, the treatment of schistosomiasis is based on only one drug, praziquantel (PZQ), which has a limited effect on already developed liver and spleen lesions [4].

Leishmaniasis, also a NTD, is caused by the protozoan Leishmania and transmitted by infected female phlebotomine sand flies. Leishmaniasis is endemic in more than 95 countries of tropical and subtropical areas, with more than 1 million of...
cases worldwide every year [5]. Although some antileishmanial compounds have been registered as medications, such as amphotericin B, pentamidine, and miltefosine, none of the available drugs can be considered perfect because of their high toxicity, long duration of treatment, and severe adverse reactions, which often lead to treatment abandonment [5]. In this scenario, there is an urgent need for new and better antileishmanial drugs [5, 6].

In this regard, Ruta graveolens (Rutaceae), also known as “rue,” has been used in the folklore medicine for the treatment of several inflammatory diseases, such as rheumatism [7], and also to treat cutaneous leishmaniasis [8, 9] in Brazil. Previous studies showed that R. graveolens exhibits antiparasitic activity against Leishmania amazonensis [9] and contains several biologically active metabolites, such as alkaloids and coumarins [8, 10]. Meanwhile, neither schistosomicidal studies nor antileishmanial activities against Leishmania braziliensis has not yet been described to R. graveolens. Thus, the aim of this study was to evaluate the in vitro schistosomicidal activities of the hydroalcoholic extract and the alkaloid-rich fraction from R. graveolens. Also, the characterization of the alkaloid-rich fraction from R. graveolens by UPLC-ESI-QTOF-MS analysis and its in vitro antileishmanial activity against Leishmania braziliensis were also performed.

2. Materials and Methods

2.1. Plant Material and Extraction. Aerial parts of R. graveolens L. were collected at the Faculty of Pharmacy’s Medicinal Herb Garden, Juiz de Fora city, MG, Brazil, in January, 2017. A voucher specimen (CESJ 70472) was identified and stored at the Herbarium of the Botany Department of the Federal University of Juiz de Fora, MG, Brazil. Plant material (250 g) was dried, powdered, and exhaustively extracted by maceration at room temperature, using EtOH: H2O (8:2 v/v). After filtration, the solvent was removed under reduced pressure to yield 25 g of the crude hydroalcoholic extract of R. graveolens. The crude extract of R. graveolens (Rg) (22 g) was chromatographed over silica gel (70–230 mesh, Merck) using a vacuum liquid chromatography system (VLC, glass columns with 5–10 cm i.d) and hexane-ethyl acetate mixtures in increasing proportions as eluents, furnishing 4 fractions: Rg-FC1 (960 mg), Rg-FC2 (220 mg), Rg-FC3 (670 mg), and Rg-FAE (1700 mg). Based on its schistosomicidal and antileishmanial activities, fraction Rg-FAE was selected for UPLC-ESI-QTOF-MS analysis.

2.2. UPLC-ESI-QTOF-MS Analysis

2.2.1. LC Conditions. The ultraperformance liquid chromatograph (UPLC) analysis was carried out, using an Acquity UPLC system (Waters Corporation, Milford, MA, USA) equipped with a binary pump, inline degasser, and autosampler coupled to an electrospray ionization quadrupole time-of-flight tandem mass spectrometer (ESI-Q-TOF/MS) (Waters Corporation, USA). Separation was carried out on BEH C18 column (100 mm × 2.1 mm, 1.7 μm, Milford, USA). The mobile phase consisted of LC grade water with 0.1% formic acid (A) and LC grade acetonitrile (B) with the following gradient profiles: 0–2 min, 5% B; 2–14 min, 5–98% B; 14–16 min, 98% B; and 16–20 min, 98–5% B. The flow rate was 0.4 mL·min−1. Before the analysis, samples were dissolved in methanol (10 mg·mL−1), centrifuged at 10,000 rpm, filtered using a 0.22 μm filter, and injected (injection volume of 15 μL).

2.2.2. MS Conditions. Mass spectrometry was performed with a XEVO G2S QTOF mass spectrometer (Waters Corporation, Milford, MA, USA) with ESI operating in the positive ion mode for scanning. The scanning range was m/z 150–1200. The capillary voltage was 2.5 kV, the low collision energy was 6 eV, and the higher collision energy was 15–30 eV. The ion source temperature was 120°C, and the desolvation temperature was 450°C. Nitrogen was used as the source of desolvation gas (800 L·h−1) and cone gas (50 L·h−1). For accurate mass measurements, data were centroided during acquisition, and 200 pg·mL−1 of leucine-enkephalin (m/z 556.2771) (Sigma-Aldrich, Steinheim, Germany), dissolved in acetonitrile/0.1% formic acid (50:50, v/v), was infused continuously as an external reference (LockSpray™) into the ESI source with automatic mass correction enabled. The data were processed using Chromalynx™ application manager with MassLynx™ 4.1 software (Waters Corporation, Milford, MA, USA). Besides the observed MS spectra and data obtained by QTOF-MS analysis, the main tools for compound identification were the interpretation of the observed QTOF-MS spectra in comparison with those found in the literature and several online databases (ChemSpider, MassBank, and Spectral Database for Organic Compounds).

2.3. Schistosomicidal Assays

2.3.1. Parasite. Schistosoma mansoni (BH strain) worms were maintained in Biomphalaria glabrata snails as intermediate hosts and Mesocricetus auratus hamsters as definitive host at the Adolfo Lutz Institute (São Paulo, Brazil), according to standard procedures previously described [11]. At 49 days after infection, adult S. mansoni specimens were recovered from each hamster by perfusion in the Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, So Paulo, Brazil) and supplemented with heparin. All experiments were authorized by the Committee for Ethics in Animal Care of Adolfo Lutz Institute (São Paulo, Brazil), in accordance with nationally and internationally accepted principles for laboratory animal use and care (CEUA #11.794/08). The study was conducted in adherence to the institution’s guidelines for animal husbandry.

2.3.2. In Vitro Studies with S. mansoni. Adult schistosomes were washed in the RPMI 1640 medium (Gibco) and supplemented with 200 μg·mL−1 streptomycin, 200 IU/mL...
penicillin (Invitrogen), and 25 mM Hepes. Adult worm pairs (male and female) were incubated in a 24-well culture plate (Techno Plastic Products, TPP, St. Louis, MO, USA), containing the same medium supplemented with 10% heat-inactivated calf serum (Gibco BRL) at 37°C in a 5% CO₂ atmosphere. For the in vitro test with *S. mansoni*, a preliminary screening of the crude extract (Rg) and its fractions Rg-FC1, Rg-FC2, Rg-FC3, and Rg-FAE were evaluated at 100 μg/mL, according to previously described [12]. The most active sample (Rg-FAE) was also evaluated at lower concentrations (3.125 to 50 μg/mL). Samples were added to the culture from a 4000 μg/mL stock solution in RPMI 1640, containing dimethyl sulfoxide (DMSO). The final volume in each well was 2 mL. The control worms were assayed in the RPMI 1640 medium, and RPMI 1640 with 0.5% DMSO as control group and PZQ (2 μM) was used as the reference drug. All experiments were performed in triplicate and were repeated at least two times. Parasites were maintained for 72 h and monitored every 24 h using a light microscope in order to evaluate their general conditions, such as motor activity and mortality rate [13].

### 2.4. Antileishmanial Assays

2.4.1. Parasite Culture. Promastigotes of *L. braziliensis* (MHOM/Br/75/M2903) were cultivated in the BHI medium (Himedia, Mumbai, India) supplemented with 10% inactivated fetal bovine serum (FBS) (Cultilab, So Paulo, Brazil), L-glutamine, penicillin at 100UI/mL, and streptomycin at 100 μg/mL (Cultilab, So Paulo, Brazil) and kept in a BOD incubator at 25°C.

2.4.2. In Vitro Antileishmanial Activities. Promastigotes of *L. braziliensis*, at 2 × 10⁶ cells/mL, were incubated with different concentrations (3.125 to 50.0 μg/mL) of the *R. graveolens* crude extract (Rg) or its alkaloid-rich fraction (Rg-FAE) for 72 h at 25°C, according to previously described [14]. Parasite viability was evaluated by MTT assay, and percentages of the inhibition growth were expressed in comparison with untreated control. For the intracellular amastigote assays, peritoneal macrophages, obtained from BALB/c mice, were added in the RPMI 1640 medium (Cultilab, So Paulo, Brazil) supplemented with 10% FBS at 2 × 10⁶ cells/mL. Adherent macrophages were infected with *L. braziliensis* promastigotes in the stationary growth phase (MOI = 10) and incubated for 4 h in 5% CO₂ at 33°C. After washing, various concentrations (6.25 to 50.0 μg/mL) of the *R. graveolens* crude extract (Rg) or its alkaloid-rich fraction (Rg-FAE) were added for 72 h, according to previously described [14]. The slides were stained with Giemsa, and the number of amastigotes was determined using light microscopy. The results were expressed in percentage of inhibition of the number of amastigotes, compared with untreated control. All procedures were performed in agreement with the Ethical Principles in Animal Research and according to protocols approved by the Ethical Committee for Animal Research (CEUA #012/2015).

2.5. Cytotoxicity Assay. Peritoneal macrophages obtained from BALB/c mice were treated with different concentrations (4.69 at 75.0 μg/mL) of the *R. graveolens* crude extract (Rg) and its alkaloid-rich fraction (Rg-FAE) for 72h, according to previously described [14]. Results were determined by MTT assay, and all procedures were performed in agreement with the Ethical Principles in Animal Research and according to protocols approved by the Ethical Committee for Animal Research (CEUA #013/2015).

2.6. Statistical Analysis. Statistical tests were performed with the Graphpad Prism (version 4.0) software. Significant differences were determined by one-way analysis of variance (ANOVA) and applying Tukey’s test for multiple comparisons with a level of significance set at *P* < 0.05.

3. Results and Discussion

The demand for new therapeutic alternatives against the 20 groups of the so-called NTDs is a worldwide need since the few drugs available are often associated with severe side effects and high toxicity [1, 6, 15]. In this context, plant-derived natural products constitute a quite important starting point for new therapies or for the development of new drugs against NTDs, due to their vast chemical diversity and already known antiparasitic potential [15].

Considering the promising anti-parasitic potential of Rutaceae species, in this work, we have highlighted the antischistosomal activity of an alkaloid-rich fraction from the *R. graveolens* extract. To our knowledge, this is the first report for the schistosomicidal activity of *R. graveolens* against adult worms of *S. mansoni*. Also, we have evaluated the antileishmanial activity of *R. graveolens* against *L. braziliensis*, which has not been documented in the literature.

First, the survival and motor activities of *S. mansoni* adult worms, after *in vitro* incubation with the crude extract of *R. graveolens* (Rg), were analyzed. As shown in Table 1, Rg (100 μg/mL) exhibited noticeable schistosomicidal activity, causing 100% mortality and decrease of motor activity of all adult male and female schistosomes (Table 1).

Schistosomicidal activities have been reported for several extracts from Rutaceae species or their secondary metabolites, mainly for alkaloids and coumarins [16–19]. In this regard, ethnolic extracts of *Zanthoxylum naranjillo* (Rutaceae) showed a significant activity on egg reduction of adult schistosomes [16], while ethnolic extracts of *Citrus reticulata* (Rutaceae) roots showed significant *in vivo* schistosomicidal activity [17]. Also, the alkaloid episopiloturine, isolated from the leaves of *Pilocarpus microphyllos* (Rutaceae), showed an *in vitro* effect on schistosomula and adult worms of *S. mansoni*, with no apparent cytotoxicity on mammalian cells [18]. Other compounds, such as furanocoumarins from the leaves of *Citrus* species (Rutaceae), have also been evaluated for their schistosomicidal activity [19].

After, Rg was chromatographed into four fractions, which were also assayed against schistosomes. In the schistosomicidal assay, when tested at 100 μg/mL, only the
fraction Rg-FAE was active (Table 1), causing 100% mortality and decreasing motor activity after 24 hours of incubation, while fractions Rg-FC1, Rg-FC2, and Rg-FC3 did not show any activity for adult schistosomes, even at the highest concentration tested (100 \mu g/mL) (Table 1). When analyzed at lower concentrations, Rg-FAE showed a pronounced schistosomicidal activity at 50, 25, 12.5, and 6.25 \mu g/mL, causing significant decrease in motor activity and death of all male adult worms (Table 1). In contrast, when adult worms were maintained in the RPMI medium containing 0.5% DMSO, their appearance was similar to when adult worms were maintained in the RPMI medium containing 0.5% DMSO, their appearance was similar to that of male worms, such as N-alkylated diamines and amino alcohols [20–23]. Some compounds showed higher selectivity to male adult worms, such as Rg-FAE, and Rg-FC3, and Rg-FAE against adult worms of S. mansoni incubated for 24 h.

| Groups | Dead worms (%)a | Decrease of motor activity (%)a | Cytotoxicity CC50 (\mu g/mL)b |
|--------|-----------------|-------------------------------|-----------------------------|
|        | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| Controlb | 0 | 0 | 0 | 0 | — | — |
| 0.5% | 0 | 0 | 0 | 0 | — | — |
| DMSO | 100 | 100 | 100 | 100 | — | — |
| PZQc | 100 | 100 | 100 | 100 | — | — |
| Rg-FAE | 100 | 100 | 100 | 100 | — | — |
| Rg-FAE | >75 | — | — | — | — | — |
| Rg-FAE | 100 | 0 | 100 | 100 | — | — |
| Rg-FAE | 50 | 100 | 100 | 100 | — | — |
| Rg-FAE | 25 | 100 | 100 | 100 | — | — |
| Rg-FAE | 12.5 | 100 | 100 | 100 | — | — |
| Rg-FAE | 6.25 | 100 | 100 | 100 | — | — |
| Rg-FAE | 3.125 | 0 | 0 | 0 | — | — |

aPercentages relative to 20 worms investigated; bRPMI 1640; ctested at a concentration of 2 \mu M; dtested at a concentration of 100 \mu g/mL; eCC50 values (50% cytotoxicity concentration) on peritoneal macrophages.

Evidence-Based Complementary and Alternative Medicine

Considering the chemical characterization of the active fraction, qualitative chromatographic profiles of Rg-FAE were obtained by UPLC-ESI-QTOF-MS on the positive mode (Figure 1). The detailed information of each peak is listed on Table 2. Chemical structures of all identified compounds (Figure 2) in the active fraction (Rg-FAE) from R. graveolens were proposed through the interpretation of their mass spectra fragmentation patterns in comparison with those found in the literature and several online databases. A total of 11 alkaloids, along with one furanocoumarin, were identified on the basis of the contrasting cleavage rules, fragmentation ion pattern, and mass spectral data.

Mass data analysis showed that compounds 1 and 2 are quinoline alkaloids, presenting the same m/z fragmentation pattern in the positive ion mode (m/z 198, 188, 184, 172, and 132). Peak 1 (m/z 286.0753) was suggested as 4-hydroxy-2-decylquinoline (compound 1, Figure 2) and peak 2 (m/z 300.0867) as 4-hydroxy-2-undecylquinoline (compound 2, Figure 2) by comparing their mass spectra data with the literature [25].

Peaks 3 (4.81 min) and 4 (5.36 min) were isomers, showing the same molecular formula (C_{14}H_{21}NO_3), but displaying different MS/MS patterns. It was observed that the parent ion-radical (m/z 280.0962) undergoes a loss of CH_3, producing ion fragments at m/z of 265.0717 [M-CH_3]^+. Peak 3 also showed a loss of CO, giving the m/z of 237.0768 [M-CH_3-CHO]^+. As previously reported [26], the loss of CO, from the molecular ion-radical, may lead to the formation of the indole scaffold peak. Finally, a loss of formaldehyde may take the mass fragment of m/z 207.0654 [M-CH_2O]^+. Mass fragmentation data for peak 3 are in agreement with the proposed structure of graveoline (compound 3, Figure 2) [27, 28]. Similarly, peak 4 showed a loss of an OCH_3 methoxyl group, producing fragments at m/z of 250.0862 [M-OCH_3]^+, suggesting that compound 4 may be graveolinine (Figure 2) [27, 28].

Peak 5 (t_R = 5.72) showed an [M+H]^+ ion at m/z 260.0886 and fragment ions at m/z 245.0661 [M-CH_3]^+ and 230.0430 [M-CH_3]^+ in MS^2 mode, suggesting consecutive losses of 15 u, which may be due to the loss of methyl groups from methoxyl groups. In addition, fragment ions were observed at m/z 216 [M-CH_3-CHO]^+ and 199 [M-CH_3-H_2O-CO]^+. Molecular ion and fragmentation patterns are similar to those reported from literature [28, 29], indicating that compound 5 is skimmianine (Figure 2). Similarly, peak 6 (t_R = 6.70) was identified as arborinine (Figure 2) based on its positive molecular ion at [M+H]^+ of m/z 286.1064, as well as by MS/MS studies and fragmentation pattern of previous reports [27]. In addition, peak 7 (t_R = 7.93) showed a molecular ion [M+H]^+ at m/z 315.1586 and an ion fragment [M-(CH_3)_2COH]^+ at m/z 255. Based on its fragmentation pattern along with previous literature data [30], this compound was identified as furanocoumarin chalepin (compound 7, Figure 2).
Figure 1: Typical UPLC-ESI-QTOF-MS chromatogram of R. graveolens fraction- (Rg-FAE-) positive mode.

Table 2: Chemical characterization of Rg-FAE by UPLC-ESI-QTOF-MS.

| Peak | Proposed compounds | RT (min) | m/z experimental [M+H]^+ | Main fragments via MS/MS | Molecular formula | References |
|------|-------------------|----------|---------------------------|--------------------------|------------------|------------|
| 1    | 4-hydroxy-2-decylquinoline | 3.72 | 286.0753 | 198.0591, 188.0791, 184.0822, 172.0781, 132.0473 | C_{19}H_{27}NO | [25] |
| 2    | 4-hydroxy-2-undecylquinoline | 4.66 | 300.0867 | 198.0937, 188.0735, 184.0739, 172.0781 | C_{20}H_{29}NO | [25] |
| 3    | Graveoline | 4.81 | 280.0962 | 265.0717, 237.0768, 207.0654 | C_{17}H_{13}NO_{3} | [26, 27] |
| 4    | Graveolinine | 5.36 | 280.0962 | 265.0717, 250.0862, 222.0900 | C_{17}H_{13}NO_{3} | [26, 27] |
| 5    | Skimmianine | 5.72 | 260.0886 | 245.0661, 230.0430, 216.0645, 199.0618 | C_{14}H_{13}NO_{4} | [28, 29] |
| 6    | Arborinine | 6.70 | 286.1064 | 271.0844, 253.0732, 244.1687, 225.0770, 197.0848, 182.0599 | C_{16}H_{15}NO_{4} | [27] |
| 7    | Chalepin | 7.93 | 315.1586 | 273.1148, 259.1003, 255.1037, 241.0889, 223.0753, 213.0933, 201.0573 | C_{19}H_{22}O_{4} | [30] |
| 8    | 1-methyl-2-nonyl-4(1H)-quinolone | 8.77 | 286.2171 | 186.0907, 173.0827 | C_{19}H_{27}NO | [31] |
| 9    | 1-methyl-2-decyl-4(1H)-quinolone | 9.23 | 300.2355 | 186.0907, 173.0827 | C_{20}H_{29}NO | [31] |
| 10   | 1-methyl-2-undecyl-4(1H)-quinolone | 10.04 | 314.2481 | 186.0907, 173.0827 | C_{21}H_{31}NO | [31] |
| 11   | 1-methyl-2-dodecyl-4(1H)-quinolone | 10.46 | 328.2617 | 186.0907, 173.0827 | C_{22}H_{33}NO | [31] |
| 12   | Dihydroevocarpine | 11.23 | 342.2783 | 186.0907, 173.0827 | C_{23}H_{35}NO | [31] |

Figure 2: Chemical structures of compounds identified in Rg-FAE by UPLC-ESI-QTOF-MS analysis.
According to literature, the McAlfferty rearrangement occurs in quinolone alkaloids, leading to the formation of stable conjugate systems with ion fragments at m/z 186 and m/z 173 [32]. Therefore, m/z 186 and 173 ion fragments were used as diagnostic ion fragments to identify the quinolone alkaloids 8, 9, 10, 11, and 12 (Figure 2), which differ only in the number of carbons of the side chain. Then, comparing the mass spectra data with the literature [31], peaks 8 (m/z 286.2171), 9 (m/z 300.2355), 10 (m/z 314.2481), 11 (m/z 328.2617), and 12 (m/z 342.2783) were identified, respectively, as quinolone alkaloids 1-methyl-2-nonyl-4(1H)-quinolone, 1-methyl-2-decyl-4(1H)-quinolone, 1-methyl-2-undecyl-4(1H)-quinolone, 1-methyl-2-dodecyl-4(1H)-quinolone, and dihydroevocarpine, respectively. All of these quinolone alkaloids (8, 9, 10, 11, and 12) were previously identified in R. graveolens [33].

In addition, the effect of the crude extract Rg was evaluated against L. braziliensis. However, no significant antileishmanial results were found for Rg (IC\textsubscript{50} > 50 \mu g/mL) against L. braziliensis promastigotes (data not shown). In contrast, previous antileishmanial study with a crude extract of R. graveolens against L. amazonensis showed an inhibition of 74.4% in the number of promastigotes at 100 \mu g/mL [9]. On the contrary, the alkaloid-rich fraction Rg-FAE exhibited pronounced activity against L. braziliensis promastigotes in the antileishmanial assay, inhibiting the parasites growth in all concentrations, displaying an IC\textsubscript{50} value of 5.90 ± 0.44 \mu g/mL, which was better than the reference drug miltefosine (IC\textsubscript{50} value of 12.09 ± 0.017 \mu g/mL). However, Rg-FAE showed low activity against intracellular amastigotes of L. braziliensis, diminishing the number of intracellular amastigotes by 26.58% at the maximum concentration used (50 \mu g/mL), while miltefosine showed an IC\textsubscript{50} value of 2.95 ± 0.44 \mu g/mL. This difference in sensibility between both stages of parasite could be due to biochemical targets, the rate of division, exposure, and inactivation into the parasitophorous vacuole or drug metabolism [34]. Although the antileishmanial effects of Rg-FAE cannot be considered as promising as well as the schistosomicidal activity, our data contribute with the ethnopharmacological use of a traditional medicinal plant from the Brazilian flora, such as R. graveolens, for the treatment of Leishmaniasis.

Moreover, considering their safety, Rg and Rg-FAE were also evaluated on cytotoxicity assay against murine macrophages. No significant toxic effects were observed for Rg (CC\textsubscript{50} > 75 \mu g/mL) or Rg-FAE (CC\textsubscript{50} value > 75 \mu g/mL) to mammalian cells (Table 1) at concentrations that effectively kills worms of S. mansoni and promastigotes of L. braziliensis, giving support to its potential in identifying lead compounds for the development of novel antiparasitic drugs.

R. graveolens is an important medicinal plant that has been used as anthelmintic and to treat several diseases, such as leishmaniasis [9, 10]. Alkaloids and coumarins, present in this plant species, have showed antileishmanial, antimalarial, and trypanocidal activities [35]. Among the compounds identified in Rg-FAE, several alkaloids, along with the identified furanocumarin, could be related to the antiparasitic activity of this fraction.

Regarding the antiparasitic activity of Rg-FAE and its chemical composition, it was shown that 2-substituted quinoline alkaloids are highly active in vitro and in vivo against Leishmania sp. [36]. Also, some quinoline and quinoline alkaloids have showed some activity against larval [37] and adult worms [38], schistosomes. Since R. graveolens possesses a wide pharmacological potential and may have low toxicity [10], additional investigations are necessary to determine the antiparasitic potential of this species, especially of its active alkaloid-rich fraction Rg-FAE in treating schistosomiasis and leishmaniasis.

4. Conclusions

The present study has demonstrated, for the first time, that the R. graveolens extract and its alkaloid-rich fraction are active against adult worms of S. mansoni in vitro, with no cytotoxicity on mammalian cells. Eleven alkaloids, together with a furanocumarin, were identified by UPLC-ESI-QTOF-MS analysis as constituents of the active fraction Rg-FAE. Our findings open the route to further antiparasitic studies with the active fraction and its isolated compounds, especially alkaloids.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

[1] World Health Organization (WHO), “Investing to overcome the global impact of neglected tropical diseases,” Third WHO Report on Neglected Diseases, World Health Organization (WHO), Geneva, Switzerland, 2015.

[2] GBD Collaborators, “Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016,” The Lancet, vol. 390, no. 10100, pp. 1211–1259, 2017.

[3] Ministério da Saúde (BR) and Secretaria de Vigilância em Saúde, Coordenação-Geral de Desenvolvimento da Epidemiologia em Serviços. Guia de Vigilância em Saúde, Ministério da Saúde, Brasília, Brazil, 2017.

[4] E. M. Lago, R. P. Xavier, T. R. Teixeira, L. M. Silva, A. A. da Silva Filho, and J. de Moraes, “Antischistosomal agents: state
of art and perspectives,” *Future Medicinal Chemistry*, vol. 10, no. 1, pp. 89–120, 2018.

[5] J. P. B. De Menezes, C. E. S. Guedes, A. L. d. O. A. Petersen, D. B. M. Fraga, and P. S. T. Veras, “Advances in development of new treatment for leishmaniasis,” *BioMed Research International*, vol. 2015, Article ID 815023, 11 pages, 2015.

[6] A. S. Nagle, S. Khare, A. B. Kumar et al., “Recent developments in drug discovery for leishmaniasis and human African trypanosomiasis,” *Chemical Reviews*, vol. 114, no. 22, pp. 11305–11347, 2014.

[7] M. Ratheesh and A. Helen, “Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan-induced paw edema in rats,” *African Journal of Biotechnology*, vol. 6, no. 10, pp. 1209–1211, 2007.

[8] S. K. Raghav, B. Gupta, A. Shrivastava, and H. R. Das, “Inhibition of lipopoly saccharide-inducible nitric oxide synthase and IL-1β through suppression of NF-κB activation by 3-(1′,1′-dimethyl-allyl)-6-hydroxy-7-methoxy-coumarin isolated from *Ruta graveolens* L.,” *European Journal of Pharmacology*, vol. 560, no. 1, pp. 69–80, 2007.

[9] A. C. De Queiroz, T. L. Dias, C. B. Da Matta et al., “Anti-leishmanial activity of medicinal plants used in endemic areas in northeastern Brazil,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 478290, 9 pages, 2014.

[10] M. Malik, D. F. C. Moraes, F. M. M. Do Amaral, and M. N. S. Ribeiro, “*Ruta graveolens*: phytochemistry, pharmacology, and biotechnology,” in *Transgenesis and Secondary Metabolism*, pp. 177–204, Springer, Cham, Switzerland, 2017.

[11] J. De Moraes, C. Nascimento, L. F. Yamaguchi, M. J. Kato, and E. Nakano, “*Schistosoma mansoni*: in vitro schistosomicidal activity and tegumental alterations induced by piplartine on schistosomula,” *Experimental Parasiology*, vol. 132, no. 2, pp. 222–227, 2012.

[12] V. S. Carrara, S. C. H. Vieira, R. G. De Paula et al., “In vitro schistosomicidal effects of aqueous and dichloromethane fractions from leaves and stems of *Piper* species and the schistosomicidal effects of aqueous and dichloromethane fractions from leaves and stems of *Piper* species and the isolation of an active amide from *P. amalago* L. (Piperaceae),” *Journal of Helminthology*, vol. 88, no. 3, pp. 321–326, 2014.

[13] L. M. S. De Almeida, L. S. A. De Carvalho, M. C. Gazolla et al., “Flavonoids and sesquiterpene lactones from *Artemisia absinthium* and *Tanacetum parthenium* against *Schistosoma mansoni* worms,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, Article ID 9521349, 9 pages, 2016.

[14] L. M. R. Antinarelli, I. d. O. Souza, N. Glanzmann et al., “Aminoquinoline compounds: effect of 7-chloro-4-quinolinylhydrazo derivatives against *Leishmania amazonensis*,” *Experimental Parasitology*, vol. 171, pp. 10–16, 2016.

[15] P. M. Cheuka, G. Moyoka, P. Mutai, and K. Chibale, “The role of natural products in drug discovery and development against neglected tropical diseases,” *Molecules*, vol. 22, no. 1, p. E58, 2017.

[16] C. G. Braguine, E. S. Costa, L. G. Magalhães et al., “Schistosomicidal evaluation of *Zanthoxylum naranjillo* and its isolated compounds against *Schistosoma mansoni* adult worms,” *Zeitschrift fur Naturforschung. C, Journal of Bioscience*, vol. 64, no. 11-12, pp. 793–797, 2009.

[17] M. A. Hammel and M. H. Hetta, “Efficacy of *Citrus reticulata* and Mirazid in treatment of *Schistosoma mansoni*,” *Memórias do Instituto Oswaldo Cruz*, vol. 100, no. 7, pp. 771–778, 2005.

[18] L. M. Veras, M. A. Guimaraes, Y. D. Campbell et al., “Activity of epipsiploiturine against *Schistosoma mansoni*,” *Current Medicinal Chemistry*, vol. 19, no. 13, pp. 2051–2058, 2012.
[34] P. Escobar, S. Matu, C. Marques, and S. L. Croft, "Sensitivities of Leishmania species to hexadecylphosphocholine (miltefosine), ET-18-OCH3 (edelfosine) and amphotericin B," *Acta Tropica*, vol. 81, no. 2, pp. 151–157, 2002.

[35] E. S. K. Mwangi, J. M. Keriko, A. K. Machocho et al., "Antiprotozoal activity and cytotoxicity of metabolites from leaves of *Teclea trichocarpa*," *Journal of Medicinal Plants Research*, vol. 4, no. 9, pp. 726–731, 2010.

[36] K. A. Reynolds, W. A. Loughlin, and D. J. Young, "Quinolines as chemotherapeutic agents for leishmaniasis," *Mini-Reviews in Medicinal Chemistry*, vol. 13, no. 5, pp. 730–743, 2013.

[37] S. Perrett and P. Whitfield, “Atanine (3-dimethylallyl-4-methoxy-2-quinolone), an alkaloid with anthelmintic activity from the Chinese medicinal Plant, *Evodia rutacearpa,*” *Planta Medica*, vol. 61, no. 3, pp. 276–278, 1995.

[38] S. El Bardicy, I. El Sayed, F. Yousif et al., "Schistosomicidal and molluscicidal activities of aminoalkylamino substituted neo- and norneocryptolepine derivatives," *Pharmaceutical Biology*, vol. 50, no. 2, pp. 134–140, 2012.