Research Article

Antiprotozoal and Antimycobacterial Activities of Pure Compounds from Aristolochia elegans Rhizomes

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1. Introduction

Aristolochia elegans Mast (Aristolochiaceae) syn. A. littoralis is commonly known as guaco, duck flower, or elephant foot and is a perennial shrub cultivated as an ornamental plant in several parts of the world [1, 2]. The genus Aristolochia comprises ca. 400 species and is distributed in wide areas from tropical to template zones [3]. On the American continent, it is found from the south of the USA, throughout Mexico, the Caribbean, and Central America and as far as Argentina [4, 5]. A. elegans has been employed as an expectorant, an antitussive, an antiasthmatic, an analgesic, an antihistamine, and a detoxicant agent [3]. Moreover, A. elegans is utilized as an antitussive against snake bites and toothache, as a purgative, an insecticide, and as an antispasmodic [6]. In Mexican traditional medicine, this plant is used as antimicrobial, antitumoral, anti diarrheal, antipyretic, emmenagog agent, and anti-snake venom and for the treatment of scorpion poisoning [6, 7]. Alkaloids, lignans, neolignans, monoterpenoids, diterpenoids, sesquiterpenoids, tetralones, isoquinolines, porphyrins, biphenyl ethers, aristolactolactams, and aristolochic acid dimers have been isolated from the organic extracts or essential oil of leaves, stems, and roots of this species [2–5]. The hexane (Hex) and methanol (MeOH) extracts of A. elegans have proven to be moderately active against the venom of Centruroides limpidus limpidus,
and the mixture of hexanic extracts from *A. elegans* and *Bouvardia ternifolia* has improved their inhibitory effects up to 70% [6]. On the other hand, *A. elegans* ethanolic (EtOH) extract exhibited antimitotic and antiviral activities [3, 8]. In a preliminary study, we focused on the analysis of the activity of the Hex and MeOH extract (at 100 μg mL⁻¹) from the leaves, seeds, and rhizomes of *A. elegans* against *M. tuberculosis* H37Rv by radiorespirometric Bactec 460 assay. The Hex extract from leaves and seeds reduced the mycobacterium growth by less than 70%; however, with the Hex extract from the rhizome, a 99% inhibition of *M. tuberculosis* H37Rv growth was reached (data no published). Based on these data, we decided to investigate the antimycobacterial activity of the major compounds found in the Hex extract of *A. elegans*-rhizome.

In this paper, the isolation of (8R,8′R,9R)-cubebin, fargesin, and eupomatenoid-1 from the active Hex extract of *A. elegans* rhizome is described and their antimycobacterial activity against four mono resistant and two MDR *M. tuberculosis* strains is demonstrated. In addition, the activity of the isolated compounds was tested against the anaerobic protozoa: *Entamoeba histolytica* and *Giardia lamblia*.

### 2. Methods

#### 2.1. General Experimental Procedures.

The chemical characterization of the isolated compounds was determined by 1H-NMR (Bruker-Avance F, 300 MHz) and 13C-NMR (Variant Unity, 75.4 MHz) using Tetramethylsilane as an internal standard in CDCl₃. Electron impact-mass spectra (EI-MS) were obtained on a Jeol AX-505 HA mass spectrometer at 70 eV. Melting points (m.p.) were determined with a Fisher-Johns apparatus and are uncorrected. Open Column Chromatography (CC) was carried out by using silica gel 60 F 254 precoated aluminum plates (0.2 mm, Merck) and 60 GF254 (70–230 mesh, Merck) as a stationary phase, and the spots were visualized by spraying it with a 10% solution of ethanolic (EtOH) *M. tuberculosis* H37Rv (530 g) was macerated (3 h) with 5 L Hex at room temperature. The extract obtained was filtered and vacuum concentrated to yield 37 g of the crude extract. The Hex extract (35 g) was subjected to CC in silica gel (150 g) and was eluted with Hex:CHCl₃ (100 – 0) and CHCl₃:MeOH (100 – 0), and 171 fractions of 150 mL each were obtained. Primary fractions (F1–F15) were combined according to a TLC analysis as follows: F1 (69 mg); F2 (10 mg); F3 (18 mg); F4 (92 mg); F5 (69 mg); F6 (149 mg); F7 (115 mg); F8 (434 mg); F9 (258 mg); F10 (322 mg); F11 (1,816 mg); F12 (1,218 mg); F13 (669 mg); F14 (14,109 mg); F15 (5,870 mg).

Fraction F5–F10 was submitted to preparative TLC employing Hex:CHCl₃ 70 : 30 as an elution system; after this procedure, 53.5 mg of eupomatenoid-1 (1) was obtained with Rf = 0.13. On the other hand, primary fraction F14 (13 g) was subjected to repeated CC, utilizing silica gel (75 g) with solvent gradients of Hex:CHCl₃ (100 to 0) and CHCl₃:MeOH (100 to 0). This procedure yielded 13 secondary fractions (FA-FM) of 150 mL each as follows: FA (9 mg); FB (11 mg); FC (69 mg); FD (10 mg); FE (304 mg); FF (819 mg); FG (1,351 mg); FH (794 mg); FI (3,239 mg); FJ (384 mg); FK (2,599 mg); FL (1,489 mg); FM (2,029 mg).

From secondary fractions FG and FH (2 g), fargesin (2) (607 mg) was isolated after successive CC and the recrystallization procedure with Hex. From secondary fraction FI (3 g), a mixture of fargesin and (8R,8′R,9R)-cubebin (2 and 3) was obtained and after successive CC and preparative TLC, 835.9 mg of 3 and 507.7 mg of 2 were purified.

Eupomatenoid-1 (1) was obtained as white crystalline needles with an m.p. of 157–158°C (lit, 154–156°C), soluble in CHCl₃, with a retention time (Rt) = 13.09 min at 220 and 280 nm, and using a Hex: CHCl₃ 1:1 system, it yielded a Retention factor (Rf) = 0.13. IR (KBr): 2,937, 2,849, 1,725, 1,604, 1,493, 1,448, 1,250, 1,142, and 1,041 cm⁻¹. 1H-MS: m/z (rel. int) 322 (100), 295 (10), 291 (10), 202 (15), 121 (6), 77 (5), and 46 (15). 1H-NMR (300 MHz, CDCl₃): 7.03 (1H, d, J = 1.5 Hz, H-4), 6.82 (1H, d, J = 1.5 Hz, H-6), 7.1 (1H, d, J = 2 Hz, H-2′), 7.25–7.32 (1H, d, J = 8.2 Hz, H-5′), 6.98 (1H, dd, J = 8.2 and 0.6 Hz, H-6′), 6.0 (2H, s, OCH₂O), 4.03 (3H, s, OCH₃), 2.40 (3H, s, CH₃), 6.5 (1H, dd, J = 15.6 and 1.5 Hz, Ha), 6.15–6.27 (1H, dq, J = 15.6 and 6.6 Hz, Hb), and 1.91 (3H, dd, J = 6.6 and 1.5 Hz, H-γ). 13C NMR (75.4 MHz, CDCl₃): 151.14 (C-2), 110.5 (C-3), 133.0 (C-3a), 133.6 (C-5), 109.2 (C-4), 104.4 (C-6), 177.8 (C-7), 142.1 (C-7a), 123.7 (C-1′), 109.4 (C-2′), 147.4 (C-3′), 147.9 (C-4′), 114.4 (C-5′), 120.6 (C-6′), 101.2 (OCH₂O), 56.2 (OCH₃), 9.6 (3-CH₃), 131.4 (C-α), 124.4 (C-β), and 18.4 (C-γ).

Fargesin (2) was obtained as a white powder with an m.p. of 136–139°C (lit, 137–139°C and 133-134°C), soluble in CHCl₃, with an Rt = 13.52 min. at 220 and 280 nm, and showing Rt = 0.56 with a Hex:EtOAc 1:1 system. IR (KBr): 2,960, 2,870, 2,841, 1,606, 1,592, 1,512, 1,492, and 1,240 cm⁻¹. 1H-MS: m/z (rel. int) 370 [M⁺ (100)], 339 (12), 2006. The plant was botanically identified by Abigail Aguilar, M.Sc., and a voucher specimen was deposited at the Herbarium of the Instituto Mexicano del Seguro Social, Mexico (IMSSM) with code number 16080.

#### 2.3. Extraction and Isolation. Powdered air-dried rhizome (530 g) was macerated (3 x 48 h) with 5 L Hex at room temperature. The extract obtained was filtered and vacuum concentrated to yield 37 g of the crude extract. The Hex extract (35 g) was subjected to CC in silica gel (150 g) and was eluted with Hex:CHCl₃ (100 – 0) and CHCl₃:MeOH (100 – 0), and 171 fractions of 150 mL each were obtained. Primary fractions (F1–F15) were combined according to a TLC analysis as follows: F1 (69 mg); F2 (10 mg); F3 (18 mg); F4 (92 mg); F5 (69 mg); F6 (149 mg); F7 (115 mg); F8 (434 mg); F9 (258 mg); F10 (322 mg); F11 (1,816 mg); F12 (1,218 mg); F13 (669 mg); F14 (14,109 mg); F15 (5,870 mg).

Fargesin (2) was obtained as a white powder with an m.p. of 136–139°C (lit, 137–139°C and 133-134°C), soluble in CHCl₃, with an Rt = 13.52 min. at 220 and 280 nm, and showing Rt = 0.56 with a Hex:EtOAc 1:1 system. IR (KBr): 2,960, 2,870, 2,841, 1,606, 1,592, 1,512, 1,492, and 1,240 cm⁻¹. 1H-MS: m/z (rel. int) 370 [M⁺ (100)], 339 (12),
with an m.p. of 127-128 °C, 14.85 min. at 280 nm, and an 1H-NMR (300 MHz, CDCl 3): 6.76–6.9 (6H, 119.3 (C-6′), 134.1 (C-1), 135.1 (C-1′), 101.0 (OCH 2O), 133.6 (C-1), 135.1 (C-1′), 2.6. Antiprotozoal Activity. E. histolytica were included as a positive control. 3. Results

3.1. Chemical Characterization of the Purified Compounds. In this study, we describe the isolation of eupomatenoid-1 (1), fargesin (2), and (8R,8R,9R)-cubebin (3) (Figure 1) from the Hex extract of A. elegans rhizomes by chemical fractionation on CC. Their structures were elucidated according to 1H-NMR, 13C-NMR, and MS data and were in agreement with those previously described in the literature. In the HPLC analysis, the eupomatenoid-1 showed an Rf = 13.09 min. using acetonitrile/formic acid 98:2 system, while fargesin and (8R,8R,9R)-cubebin showed Rf = 13.52 and 14.85 min., respectively, when MeOH was employed; all compounds were detected at 220 and 280 nm.

3.2. Antimycobacterial and Antiprotozoal Evaluation. The antimycobacterial activity of the Hex extract and purified compounds determined by the MABA is depicted in Table 1. Although Hex extract and eupomatenoid-1 were inactive against M. tuberculosis H37Rv (MIC > 100 μg mL−1), fargesin and (8R,8R,9R)-cubebin exhibited good activity against this strain (MIC = 50 μg mL−1). It is noteworthy that the Hex extract and compound 3 were active against the two MDR M. tuberculosis clinical isolates: CIBIN/UMF15:99, and SIN 4 (MIC = 50 μg mL−1), while compound 2 inhibited only the growth of SIN4 (MIC = 50 μg mL−1). In addition, compound 2 was the most active against the monoresistant variants of M. tuberculosis H37Rv (MIC = 12.5–25 μg mL−1) with the exception of the ethambutol-resistant strain (MIC > 50 μg mL−1). Compounds 1 and 3 were moderately active against all monoresistant strains of M. tuberculosis H37Rv tested (MIC = 100 μg mL−1).

The antiprotozoal activity of the Hex extract and of pure compounds 1–3 was tested against the anaerobic protozoa E. histolytica and G. lamblia (Table 1). It was observed that the Hex extract was active against these two parasites, exhibiting IC50 = 0.235 and 0.315 μg mL−1, respectively. On the other hand, compound 1 was the most active compound.
The presence of the lignans and neolignans in *A. elegans* rhizome. In this work, the analytical conditions that can be employed for detecting these compounds are also described.

Compound 1 has previously been isolated from *Eupomatiaria laurina*, *A. taliscana*, and *Caryodaphnopsis bavensis*, and a related compound, such as eupomatenoid-7, has been found in *A. taliscana* [13–17]. Compound 2 has been isolated from *Horsfieldia iryaghedhi* (*Myristica horsfieldia*), *Piper sarmentosum*, *Magnolia biondii*, *Stauranthus perforatus*, and *Aristolochia malmeana* [18–23]. Compound 3 has been isolated from related species such as *A. legasiana*, *A. malmeana*, *A. odoratissima*, and *A. pubescens* [21, 22, 24]. In fact, structurally similar compounds such as aristelegin A-C have been reported for the roots and stems of *A. elegans* [5].

Of the three pure compounds, fargesin (2) was the most active against the mycobacterium strains tested (MIC < 50 μg mL⁻¹); compound 3 showed activity against *M. tuberculosis* H37Rv and two MDR strains of *M. tuberculosis*. Eupomatenoid-1 (1) was slightly active against *M. tuberculosis* H37Rv, its monoresistant variants and two MDR *M. tuberculosis* clinical isolates, in comparison with eupomatenoid-7, a compound structurally similar to eupomatenoid-1, that we have previously demonstrated to be more active against the same strains with MIC values < 25 μg mL⁻¹ [16]. These data suggest that the methylenedioxy group in the eupomatenoid-1 molecule exerts a negative influence on its antimycobacterial activity, since eupomatenoid-7 does not possess this group and was more active against several mycobacterium strains; nevertheless, further structure-activity studies are needed to confirm this hypothesis.

It is noteworthy that fargesin was active against *M. tuberculosis* H37Rv, its monoresistant strains, and to a lesser degree against the MDR SIN4 isolate (MIC < 50 μg mL⁻¹); on the other hand some related compounds such as (+)-sesamin and horsfieldin (isolated from *Piper sarmentosum*) were inactive against the *M. tuberculosis* H37Rv strain (MIC > 200 μg mL⁻¹) [25]. The bacteriostatic activity of (8R,8'R,9R)-cubebin has been reported against *Streptococcus mitis*, *Enterococcus faecalis*, *Ostrinia nubilalis*, and *Anticarsia gemmatalis* [21, 24–27]. Interestingly, in this study it has been demonstrated that compound 3 was active against the two MDR *M. tuberculosis* clinical isolates tested showing a MIC value of 50 μg mL⁻¹. Our data suggest that compounds 2 and 3 are two of the possible compounds responsible for the antimycobacterial activity exerted by the Hex extract of *A. elegans*-rhizome.

Current tuberculosis chemotherapy is prolonged (24 months), poorly effective, expensive, and is accompanied by severe side effects. Besides, the presence of MDR *M. tuberculosis* cases is rapidly increasing. MDR accounts for 5.3% of all TB cases reported around the world [28, 29], underlining the importance of using new alternatives in the treatment of tuberculosis. In this regard, medicinal plants have proven to be an important source of antimycobacterial compounds [28, 30–32]. In fact, it was demonstrated that purified compounds 2 and 3 showed significant activity against monoresistant and MDR *M. tuberculosis* strains.

A murine model of tuberculosis previously developed by Hernández-Pando et al. [33] could be further used to

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**Figure 1:** Chemical structures of isolated compounds from *A. elegans* hexanic extract.

against *E. histolytica* and *G. lamblia*, achieving IC₅₀ values of 0.624 and 0.545 μg mL⁻¹, respectively. Compounds 2 and 3 demonstrated moderate antiprotozoal activity with IC₅₀ < 275.00 μg mL⁻¹ against both parasites. Because of its important antiprotozoal activity, eupomatenoid-1 was evaluated against *T. vaginalis*, showing an IC₅₀ = 0.840 μg mL⁻¹.

### 4. Discussion

The presence of the lignans and neolignans in *A. elegans* has been described [2, 5]; however, in this study the presence of eupomatenoid-1 (neolignan), fargesin, and (8R,8’R,9R)-cubebin (lignans) has been described for the first time in *A. elegans* rhizome.
demonstrate of its activity of eupomatenoid-1 needs to be supported by a active than eupomatenoid-1, respectively. The antiprotozoal more potent than the Hex extract and 2.5 and 10 times be mentioned that metronidazole was just 1.4 and 4 times activity of both E. histolytica and G. lamblia, although it is not always e

clear understanding of its action mechanisms. The antiprotozoal activity of neolignans and lignans has recently been evaluated, the acute and subacute tox-
icty of active compounds in a mouse model. Further in vivo studies may well support the antimitcrobial and antipro-
tozaol activities of A. elegans-rhizome purified compounds. The antiprotozoal activity of neolignans and lignans has scarcely been described in the literature, and our results en-
courage further studies on this issue.

Conflict of Interests

The authors declare that they have no competing interest. All authors read and approved the final paper.

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References

[1] C. Cano and F. Marroquín, *Taxonomía de Plantas Superiores*, Editorial Trillas, México, 1994.

[2] T. S. Wu, Y. L. Tsai, P. L. Wu, F. W. Lin, and J. K. Lin, "Constituents from the leaves of *Aristolochia elegans*," *Journal of Natural Products*, vol. 63, no. 5, pp. 692–693, 2000.

[3] L. S. Shi, P. C. Kuo, Y. L. Tsai, A. G. Damu, and T. S. Wu, "The alkaloids and other constituents from the root and stem of *Aristolochia elegans*," *Bioorganic and Medicinal Chemistry*, vol. 12, no. 2, pp. 439–446, 2004.

[4] R. Vila, R. Mundina, L. Muschietti et al., "Volatile constituents of leaves, roots and stems from *Aristolochia elegans*," *Phytochemistry*, vol. 46, no. 6, pp. 1127–1129, 1997.

[5] T. S. Wu, Y. L. Tsai, A. G. Damu, P. C. Kuo, and P. L. Wu, "Constituents from the root and stem of *Aristolochia elegans*," *Journal of Natural Products*, vol. 65, no. 11, pp. 1522–1525, 2002.

[6] J. E. Jiménez-Ferrer, Y. Y. Pérez-Terán, R. Román-Ramos, and J. Tortoriello, "Antitoxin activity of plants used in Mexican traditional medicine against scorpion poisoning," *Phytomedicine*, vol. 12, no. 1-2, pp. 116–122, 2005.

[7] A. Argüeta, L. Cano, and M. Rodarte, *Atlas de las Plantas de la Medicina Tradicional Mexicana*, vol. 2-3, Editorial Instituto Nacional Indigenista, México, 1st edition, 1994.

[8] T. S. Wu, A. G. Damu, C. R. Su, and P. C. Kuo, "Terpenoids of *Aristolochia* and their biological activities," *Natural Product Reports*, vol. 21, no. 5, pp. 594–624, 2004.

[9] B. U. Jaki, S. G. Franzblau, L. R. Chadwick et al., "Purity-activity relationships of natural products: the case of anti-TB active ursolic acid," *Journal of Natural Products*, vol. 71, no. 10, pp. 1742–1748, 2008.

[10] A. Jiménez-Arellanes, M. Meckes, J. Torres, and J. Luna-Herrera, "Antimycobacterial triterpenoids from *Lantana hispanica* (Verbenaceae)," *Journal of Ethnopharmacology*, vol. 111, no. 2, pp. 202–205, 2007.

[11] R. Cedillo-Rivera, B. Chávez, A. González-Robles, A. Tapia, and L. Yépez-Mula, "In vitro effect of nitazoxanide against *Entamoeba histolytica*, *Giardia intestinalis* and *Trichomonas vaginalis* trophozoites," *Journal of Eukaryotic Microbiology*, vol. 49, no. 3, pp. 201–208, 2002.

[12] R. Argüello-García, M. Cruz-Soto, L. Romero-Montoya, and G. Ortega-Pierres, "Variability and variation in drug susceptibility among *Giardia duodenalis* isolates and clones exposed to 5-nitroimidazoles and benzimidazoles in vitro," *Journal of Antimicrobial Chemotherapy*, vol. 54, no. 4, pp. 711–721, 2004.

[13] F. Abe, S. Nagafuji, T. Yamauchi et al., "Trypanocidal constituents in plants 1. Evaluation of some Mexican plants for their trypanocidal activity and active constituents in Guaco, roots of *Aristolochia taldscana*," *Biological and Pharmaceutical Bulletin*, vol. 25, no. 9, pp. 1188–1191, 2002.

[14] N. H. Anh, H. Ripperger, A. Porzel, T. van Sung, and G. Adam, "Neolignans from *Caryodaphnopsis baviensis*," *Phytochemistry*, vol. 46, no. 3, pp. 569–571, 1997.

[15] R. G. Enríquez, M. A. Chávez, and W. F. Reynolds, "Phytochemical investigations of plants of the genus *Aristolochia*. 1. Isolation and NMR spectral characterization of eupomatenoid derivatives," *Journal of Natural Products*, vol. 47, no. 5, pp. 896–899, 1984.

[16] R. León-Díaz, M. Meckes, S. Saíd-Fernández et al., "Antimycobacterial neolignans isolated from *Aristolochia taldscana*," *Memorias de Instituto Oswaldo Cruz*, vol. 105, no. 1, pp. 45–51, 2010.

[17] R. W. Read and W. C. Taylor, "Constituents of *Eupomatia* species. V. The isolation of eupomatenoid-13 (a new neolignan), (±)-trans-dehydrodiisoeugenol, and other extractives from the bark of *Eupomatia laurina*," *Australian Journal of Chemistry*, vol. 32, no. 10, pp. 2317–2321, 1979.

[18] A. L. Anaya, M. Macías-Rubalcava, R. Cruz-Ortega et al., "Allelochemicals from *Staurantherus perforatus*, a Rutaceae tree of the Yucatan Peninsula, Mexico," *Phytochemistry*, vol. 66, no. 4, pp. 487–494, 2005.

[19] A. A. L. Gunatilaka, A. M. Y. de Silva, S. Sotheeswaran, and L. M. V. Tillekeratne, "Horsfieldia, a lignan and other constituents from *Horsfieldia iryagchedhi*," *Phytochemistry*, vol. 21, no. 11, pp. 2719–2723, 1982.

[20] H. Kakisawa, Y. P. Chen, and H. Y. Hsü, "Lignans in flower buds of *Magnolia fargesii*," *Phytochemistry*, vol. 11, no. 7, pp. 2289–2293, 1972.

[21] G. B. Messiano, L. Vieira, M. B. Machado, L. M. X. Lopes, S. A. de Bortoli, and J. Zuckerman-Schpector, "Evaluation of insecticidal activity of diterpenes and lignans from *Aristolochia malmeana* against *Anticarsia gemmatalis*," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 8, pp. 2655–2659, 2008.

[22] A. Usubillaga, N. Khouiri, S. Cedillo-Vaz, and E. Yibirin, "Antisnake venom effect of *Aristolochia odoratissima* L. aqueous extract on mice," *Acta Horticulturae*, vol. 3, no. 677, pp. 85–89, 2006.

[23] Y. Shen, E. C. K. Pang, C. C. L. Xue, Z. Z. Zhao, J. G. Lin, and C. G. Li, "Inhibitions of mast cell-derived histamine release by different *Flos Magnoliae* species in rat peritoneal mast cells," *Phytochemistry*, vol. 15, no. 10, pp. 808–814, 2008.

[24] I. C. de Pascoli, I. R. Nascimento, and L. M. X. Lopes, "Configurational analysis of cubebin and bicubebin from *Aristolochia lagasiana* and *Aristolochia pubescens*," *Phytochemistry*, vol. 67, no. 7, pp. 735–742, 2006.

[25] P. Tuntiwachwuttiwut, P. Phansa, Y. Pootaeng-on, and W. C. Taylor, "Chemical constituents of the roots of *Piper sarmentosum*," *Chemical and Pharmaceutical Bulletin*, vol. 54, no. 2, pp. 149–151, 2006.

[26] C. B. Bernard, J. T. Arnason, B. J. R. Philogène, J. Lam, and T. Waddell, "Effect of lignans and other secondary metabolites of the asteraceae on the mono-oxygenase activity of the European corn borer," *Phytochemistry*, vol. 28, no. 5, pp. 1373–1377, 1989.

[27] M. L. Silva, H. S. Coimbra, A. C. Pereira et al., "Evaluation of Piper cubeba extract, (-)-cubebin and its semi-synthetic derivatives against oral pathogens," *Phytotherapy Research*, vol. 21, no. 5, pp. 420–422, 2007.

[28] J. C. Palomino, D. F. Ramos, and P. A. da Silva, "New anti-tuberculosis drugs: strategies, sources and new molecules," *Current Medicinal Chemistry*, vol. 16, no. 15, pp. 1898–1904, 2009.

[29] E. M. Zager and R. McNerney, "Multidrug-resistant tuberculosis," *BMC Infectious Diseases*, vol. 8, p. 10, 2008.

[30] C. L. Cantrell, S. G. Franzblau, and N. H. Fischer, "Antimycobacterial plant terpenoids," *Planta Medica*, vol. 67, no. 8, pp. 685–694, 2001.

[31] G. F. Pauli, R. J. Case, T. Inui et al., "New perspectives on natural products in TB drug research," *Life Sciences*, vol. 78, no. 5, pp. 485–494, 2005.

[32] B. R. Copp and A. N. Pearce, "Natural product growth inhibitors of *Mycobacterium tuberculosis*," *Natural Product Reports*, vol. 24, no. 2, pp. 278–297, 2007.

[33] R. Hernández-Pando, D. Aguilar-León, H. Orozco et al., "16α-Bromoepiandrosterone restores T helper cell type 1 activity and accelerates chemotherapy-induced bacterial clearance in..."
a model of progressive pulmonary tuberculosis,” *Journal of Infectious Diseases*, vol. 191, no. 2, pp. 299–306, 2005.

[34] M. Lalle, “Giardiasis in the post genomic era: treatment, drug resistance and novel therapeutic perspectives,” *Infectious Disorders—Drug Targets*, vol. 10, no. 4, pp. 283–294, 2010.

[35] E. Barbosa, F. Calzada, and R. Campos, “Antigiardial activity of methanolic extracts from *Helianthemum glomeratum* Lag. and *Rubus corifolius* Focke in suckling mice CD-1,” *Journal of Ethnopharmacology*, vol. 108, no. 3, pp. 395–397, 2006.

[36] E. Barbosa, F. Calzada, and R. Campos, “*In vivo* antigiardial activity of three flavonoids isolated of some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea,” *Journal of Ethnopharmacology*, vol. 109, no. 3, pp. 552–554, 2007.