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Anti-tubercular activity of eleven aromatic and medicinal plants occurring in Colombia

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Introduction. Human tuberculosis is a contagious-infectious disease mainly caused by Mycobacterium tuberculosis. Although regimens exist for treating tuberculosis, they are far from ideal. Development of effective strategies for treatment of human tuberculosis has posed a challenge, considering the increase in infections associated with the human immunodeficiency virus and immunocompromised patients. Essential oils -volatile, aromatic oil extracts from plants- have been used in traditional treatment of many diseases; however careful investigation of these oils has not been undertaken with respect to treatments of tuberculosis.

Objective. The in vitro antitubercular activity of essential oils from 11 medicinal plants grown in Colombia were assessed for efficacy as new medications (phytomedicines) for treatment of M. tuberculosis H37Rv.

Material and methods. Essential oil extraction and analysis were performed as described Stashenko et al. (2004). Minimal inhibitory concentrations were determined by a colorimetric macrodilution method, following the protocol described by Abate et al. (1998). Isoniazide and rifampin were used as control treatments. Bactericidal and bacteriostatic activity was measured using the method developed by the Clinical and Laboratory Standards Institute consigned in the M26-A protocol.

Results. Essential oils from Achyrocline alata and Swinglea glutinosa were the most active with minimal inhibitory concentrations of 62.5±0.1 and 100±36 µg ml⁻¹, respectively. Carvacrol, thymol, p-cymene, 1,8-cineole, limonene, and β-pinene were the major components, most often identified in the 11 plant extracts of essential oils. Time-kill curve assays demonstrated the bacteriostatic activity of these essential oils.

Conclusions. The essential oils from A. alata and S. glutinosa plants, and the components identified therein, are candidates as potential phytotherapeutic agents for human tuberculosis control.

Key words: Mycobacterium tuberculosis, tuberculosis, anti-infective agents; plants, medicinal; phytotherapy, Colombia
Materiales y métodos. La extracción y el análisis de los aceites esenciales se realizó bajo la metodología desarrollada por Stashenko et al. La obtención de la concentración inhibitoria mínima se llevó a cabo por un método colorimétrico de macrodilución en caldo descrito por Abate y et al.; la isonicaída y la rifampicina se usaron como medicamentos control. La actividad bactericida y bacteriostática se determinaron mediante el protocolo M26-A del Clinical and Laboratory Standards Institute.

Resultados. Los aceites esenciales provenientes de las plantas Achyrocline alata y Swinglea glutinosa fueron los más activos con concentraciones inhibitorias mínimas de 62,5±0,01 y 100±36 µg ml⁻¹, respectivamente. Carvacrol, timol, p-cymene, 1,8-cineole, limoneno, y β-pineno fueron los componentes mayoritarios identificados en los 11 aceites. Los ensayos de curva de letalidad evidenciaron que ambos aceites son bacteriostáticos.

Conclusiones. Los aceites esenciales obtenidos de las plantas A. alata y S. glutinosa, así como sus componentes, son candidatos potenciales como fitoterapéuticos para el control de la tuberculosis.

Palabras clave: Mycobacterium tuberculosis, tuberculosis, agentes antiinfecciosos, plantas medicinales, fitoterapia, Colombia

Human tuberculosis (TB) is a contagious infectious disease mainly caused by Mycobacterium tuberculosis. It is an aerobic pathogenic bacterium that usually establishes its infection in the lungs (1). About one third of the world’s population is currently infected with M. tuberculosis. Fully 10% of those infected will develop clinical disease, particularly those who also have the human immunodeficiency virus (HIV) infection. TB is the leading cause of death worldwide from a single pathogen, claiming more adult lives than diseases AIDS, malaria, diarrhea, leprosy, and all other tropical diseases combined (2). The World Health Organization (WHO) estimates that active cases of tuberculosis afflict seven to eight million people annually, and lead up to three million deaths per year (3).

Although regimens exist for treating tuberculosis, they are far from ideal. Treatment usually involves a combination of the drugs isoniazid (INH) and rifampin (RMP), which must be administered for at least six months, or a combination of pyrazinamide and ethambutol (EMB) (or streptomycin), which are used only in the first two months of treatment (4). Because adherence to this regimen is extremely difficult, WHO recommends a program of directly observed treatment, short-course (DOTS), in which health care workers watch as each patient ingests the medicine (4). Approximately 21% of the world’s TB patients were treated under DOTS in 1998. Therefore, inconsistent or partial treatment is common and has led to the development and spread of drug-resistant strains. Consequently, shorter, simpler therapeutic and prophylactic regimens must be developed to increase adherence. In addition, new drugs are necessary to combat the increasing number of multi-drug-resistant TB strains (MDR-TB) and extensively drug-resistant (XDR-TB) strains. A greater understanding of the molecular mechanisms of drug action and drug resistance will assist and promote the development of newer compounds (1,4).

Plant-based drugs have been used worldwide in traditional medicines for the treatment of a variety of diseases. Approximately 60% of the world population relies on medicinal plants for its primary healthcare. These medicinal plant species serve as a rich source of many biologically active compounds, although very few plant species have been thoroughly investigated for their medicinal properties (5). Interest in phytomedicine has been renewed during the last decade and now, many medicinal plant species are being screened for pharmacological activities (6). Aromatic plants have been used since ancient times for their preservative and medicinal properties, as well as to impart aroma and flavor to food. Hippocrates,
The pharmaceutical properties of aromatic plants are partially attributed to essential oils. Essential oils are natural, complex, multi-component systems composed mainly of terpenes along with a few non-terpene components (7). The ancient Egyptians used aromatic plants in embalming to stop bacterial growth and prevent decay, an effect largely attributed to their essential oil content. Strong in vitro evidence indicates that essential oils can act as antibacterial agents against a wide spectrum of pathogenic bacterial strains (7). Some terpenes, such as citronellol, nerol and geraniol have shown moderate antimycobacterial activity (8). The aim of the present study is to evaluate the antimycobacterial activity of essential oils derived from 11 species of aromatic and medicinal plants, grown in Colombia. If effective, these substances pose as potential candidates for the development of new medication for treatment of TB.

Material and Methods

Plant material

The voucher numbers, the geographic region of plant collection, vernacular names and botanical names are listed in Table 1. The taxonomic identification of the botanical species was performed by José Luis Fernández at Herbario Nacional de Colombia, Natural Sciences Institute, Faculty of Sciences, Universidad Nacional de Colombia, Bogotá; where desiccated samples of each plant remain as permanent vouchers.

Essential oils extraction

The essential oils were extracted from a 300 g sample of plant leaves and stems by microwave-assisted hydrodistillation (30 min, 250 mL water), using a Clevenger-type distillation apparatus and a Dean-Stark distillation trap in a domestic microwave oven (Kendo, MO-124, 2.5 GHz, 800 W) (9). Sodium sulfate (Merck, Darmstadt, Germany) was added as a drying agent to the decanted essential oil.

Essential oils analysis

An aliquot (50 µL) of each essential oil, along with the internal standard (n-tetradecane, 4 µL) was dissolved in dichloromethane to reach a final volume of 1.0 mL. For chromatographic analysis, 1.0 µL of this solution was injected into an Agilent Technologies 6890 Plus gas chromatograph (Agilent Technologies, Palo Alto, CA, USA.), equipped with an Agilent Technologies 5973N mass selective detector, a split/splitless injector (split ratio 1:50), a 7863 automatic Injector, and a MSChemStation G1701-DA data system. The available spectral libraries included the WILEY 138K, NIST 2002 and QUADLIB 2004. A fused-silica capillary column DB-5MS (J&W Scientific, Folsom, CA, USA) of 60 mm x 0.25 mm I.D. x 0.25 µm d., was employed. The oven temperature was programmed from 45°C (5 min) to 150°C (2 min) at 4 °C min⁻¹, then to 250°C (5 min) at 5 °C min⁻¹, and finally, to 275°C (15 min) at 10°C min⁻¹. The ionization chamber and transfer line temperatures were kept at 230°C and 285°C, respectively. Compound identification was based on comparisons with standard terpenic compounds by (1) chromatographic (Kováts indices) criteria and (2) spectroscopic data and their comparison with known standards and with extant databases.

Antimycobacterial activity

The essential oil antimycobacterial activity was evaluated according to the macrodilution protocol, described by Abate et al. (1998) (10). The M. tuberculosis H37Rv strain (ATCC 27294) was cultured at 37°C in Lowestein-Jensenn medium until log phase growth; then a cell suspension was prepared at a concentration of about 2x10⁶ UFC mL⁻¹ and further diluted 1:20 in Middlebrook 7H9 (Becton Dickinson and Co., Sparks MD, USA) medium. The later was supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase) (Becton Dickinson and Co., Sparks MD, USA) and 0.001% Tween 80 (Sigma, New Jersey, USA). One mL of the bacterial suspension was added to each tube (capped, glass) together with the diluted essential oils diluted. The final concentrations of the essential oils ranged from 31.25 to 500 µg mL⁻¹ and adjusted to a final 2 mL-volume. After a 7-day incubation, 100 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg mL⁻¹) (Sigma, New Jersey, USA) with 20% Tween 80 (Sigma, New Jersey,
Table 1. Plant information, main compounds and minimum inhibitory concentration (MIC, µg ml\(^{-1}\)) against M. tuberculosis H37Rv, of the essential oils studied.

<table>
<thead>
<tr>
<th>Sample N°</th>
<th>Botanical name*</th>
<th>Collection site</th>
<th>Vernacular name and Voucher number</th>
<th>Part of the plant used for essential oil extraction and oil yield</th>
<th>Essential oil main compounds</th>
<th>MIC(^±) ± s(^\circ), µg ml(^{-1}) ((n^\circ = 3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Lippia origanoides</em> (Fam. Verbenaceae)</td>
<td>Pedregal, Nariño</td>
<td>Orégano de monte*, COL.520285, “Thymol chemotype”</td>
<td>Leaves and stems; 3.1%</td>
<td>1. Thymol (54.5%) 2. p-Cymene (10.0%) 3. γ-Terpine (5.0%) 4. Thymyl acetate (4.8%) 5. β-Myrcene (2.8%) 6. trans-β-Caryophyllene (2.4%) 7. Methyl thymyl ether (1.9%) 8. Carvocrol (1.7%) 9. α-Terpine (1.6%) 10. α-Humulene (1.5%)</td>
<td>125 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td><em>Cananga odorata</em> Fam. Annonaceae</td>
<td>Bucaramanga, Santander</td>
<td>“Ylang-ylang”, introduced to Colombia, presumably from Philippines</td>
<td>Fresh, fully mature yellow flowers; 1.2%</td>
<td>1. Linalool (20.7%) 2. Benzyl acetate (20.6%) 3. Benzyl benzoate (14.2%) 4. Germacrene D (10.2%) 5. p-Methyl anisole (6.8%) 6. Cinnamyl acetate (5.1%) 7. Geranyl acetate (2.9%) 8. α-Humulene (2.8%) 9. (E,E)-Franesene (2.4%) 10. Benzyl salicylate (2.3%) (15, 16)</td>
<td>300 ± 217</td>
</tr>
<tr>
<td>3</td>
<td><em>Swinglea glutinosa</em> (Fam. Rutaceae)</td>
<td>Bucaramanga, Santander</td>
<td>“Limón africano”, introduced to Colombia, COL.521530</td>
<td>Fruit peel; 0.7%</td>
<td>1. β-Pinene (49.6%) 2. α-Pinene (12%) 3. Sabinene (11.0%) 4. Bicyclosesquiphellandrene (8.1%) 5. Limonene (4.4%) 6. 1,8-Cineol (3.0%) 7. Terpinen-4-ol (2.7%) 8. trans-β-Caryophyllene (1.5%) 9. γ-Terpine (1.4%) 10. α-Terpineol (1.2%) (18)</td>
<td>100 ± 36</td>
</tr>
<tr>
<td>4</td>
<td><em>Hyptis mutabilis</em> (Fam. Lamiaceae)</td>
<td>Villavicencio, Meta</td>
<td>“Mastranto”, COL.512275</td>
<td>Leaves and stems; 0.3%</td>
<td>1. Fenchone (17.1%) 2. 1,8-Cineol (12.8%) 3. trans-β-Caryophyllene (10.9%) 4. Bicyclogermacrene (8.7%) 5. Germacrene D (6.2%) 6. Limonene (4.8%) 7. α-Pinene (3.8%) 8. β-Pinene (3.7%) 9. β-Boubonene (3.4%) 10. Spathulenol (3.0%)</td>
<td>125 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td><em>Piper aurtum</em> (Fam. Piperaceae)</td>
<td>Cali, Valle del Cauca</td>
<td>“Anisillo”, COL.512209</td>
<td>Leaves; 2.3%</td>
<td>1. Safrol (91.3%) 2. Myristine (4.8%) 3. Methyl eugenol (4.8%) 4. α-Terpineol (0.6%) 5. γ-Terpine (0.5%) 6. p-Cymene (0.2%) 7. trans-β-Caryophyllene (0.3%) 8. Eugenol (0.2%) 9. Elemicine (0.2%) 10. Spathulenol (0.2%)</td>
<td>400 ± 220</td>
</tr>
<tr>
<td>6</td>
<td><em>Lippia origanoides</em> (Fam. Verbenaceae)</td>
<td>Los Santos, Santander</td>
<td>Orégano de monte*, COL.516294, Caryophyllene chemotype</td>
<td>Leaves and stems; 1.5%</td>
<td>1. trans-β-Caryophyllene (11.3%) 2. p-Cymene (11.2%) 3. α-Phellandrene (9.9%) 4. Limonene (7.2%) 5. 1,8-Cineol (6.5%) 6. α-Humulene (6.0%) 7. Borneol (3.1%)</td>
<td>400 ± 220</td>
</tr>
</tbody>
</table>
Anti-tubercular activity of Colombian medicinal plants

7. *Achyrocline alata* (Fam. Asteraceae) Nariño, Potosí, “Viravira” Leaves and stems; 0.3%

- Thymol (24.0%) (15)
- Thymyl acetate (2.3%) (15)
- Viridiflorene (2.3%)
- Caryophyllene oxide (2.2%) (15)
- α-Bisabolol (1.7%)
- α-Eudesmol (1.6%)
- α-Humulene (2.4%)
- trans-β-Caryophyllene (13.3%)
- 3-p-Cymene (3.2%)
- α-Pinene (2.6%)
- α-Humulene (2.4%)
- Thymyl acetate (2.3%)
- Viridiflorene (2.3%)
- Caryophyllene oxide (2.2%)
- α-Bisabolol (1.7%)
- α-Eudesmol (1.6%)
- α-Humulene (2.4%)
- trans-β-Caryophyllene (13.3%)
- 3-p-Cymene (3.2%)
- α-Pinene (2.6%)
- α-Humulene (2.4%)
- Thymyl acetate (2.3%)
- Viridiflorene (2.3%)
- Caryophyllene oxide (2.2%)

8. *Lippia origanoides* Piedecuesta, (Fam. Verbenaceae) Santander, “Orégano de monte”, Leaves and stems; 4.4%

- Carvacrol (46.2%)
- 3-Thymol (9.9%)
- γ-Terpinene (9.5%)
- α-Terpinene (2.7%)
- β-Mycene (2.5%)
- trans-β-Caryophyllene (2.0%) 160 ± 72
- α-Thujene (1.5%)
- α-Humulene (1.2%)
- Terpinen-4-ol (1.1%) (15)

9. *Lippia alba* Venadillo, (Fam. Verbenaceae) Tolima, “Pronto alivio”, Leaves and stems; 2.5%

- Carvone (50.3%)
- Limonene (30.2%)
- Piperitone (6.1%)
- 4-Bicyclosesquiphellandrene (3.5%)
- β-Bourbonene (1%)
- Piperitone (3.1%)
- Dihydrocarvone (0.8%)
- 6-Methyl-5-hepten-2-one (2.1%)
- cis-Verbenol (1.9%)
- trans-Verbenol (1.5%)
- α-Humulene (1.4%) (17)

10. *Piper bogotense* Ipiales, (Fam. Piperaceae) Nariño, “Matico” Leaves; 0.2%

- Sesquisabinene hydrate (14.2%) 130 ± 95
- α-Phellandrene (13.7%) 130 ± 95
- α-Pinene (8.7%)
- Limonene (5.3%)
- Linalool (4.7%)
- 6-p-Cymene (4.4%)
- β-Mycene (0.8%)
- δ-Cadinene (3.4%)
- α-Bisabolol (3.5%)
- trans-β-Caryophyllene (3.1%)

11. *Lippia alba* Bucaramanga, (Fam. Verbenaceae) Santander, “Pronto alivio”, Leaves and stems; 1.6%

- Geranial (31.5%)
- Neral (23.8%)
- Geraniol (7.9%)
- trans-β-Caryophyllene (5.8%)
- Geranyl acetate (3.6%)
- Limonene (2.5%)
- 6-Methyl-6-hepten-2-one (2.1%)
- cis-Verbenol (1.9%)
- trans-Verbenol (1.5%)
- α-Humulene (1.4%) (17)

INH Isoniazid -- -- -- -- 0.19±0.07
RIF Rifampin -- -- -- -- 0.3±0.21

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* – Vernacular name in Colombia

** Minimum Inhibitory Concentration (µg ml⁻¹)

--- Standard error of the mean

--- Number of assays
USA) was added to the glass tubes. A violet color indicated bacterial growth. The tubes were evaluated for color change on day 8. For standard tests, the MIC values of rifampin and isoniazid (Sigma, New Jersey, USA) were determined each time. The acceptable minimum inhibitory concentration (MIC) ranges of these drugs were 0.50-0.03 µg ml⁻¹, respectively. The MIC of each oil corresponded to the lowest concentration at which the bacteria tested did not show growth. Susceptibility testing was performed 3 times. The results were expressed as the mean of the three tests. Results were expressed as geometric mean (GM) ± standard error of the mean (s) of the MICs.

**Antimycobacterial time kill curves**

Time-kill curves were used to measure the bactericidal activity of essential oils with lowest MICs values. Bactericidal activity was measured in glass tubes, each containing 2 mL of Middlebrook 7H9 (Becton Dickinson and Co., Sparks MD, USA) medium. The medium was supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase) (Becton Dickinson and Co., Sparks MD, USA) and 0.001% Tween 80 (Sigma, New Jersey, USA) at concentrations 0, 1-, 2-, and 5-fold above the respective MIC. The final concentration of mycobacteria was approximately 10⁶ CFU ml⁻¹. Samples were taken every two days until the sixth day and were serially diluted in sterile distilled water to avoid significant carryover, and then plated in Lowenstein-Jensenn tubes with screw caps. The tubes were incubated at 37°C in an Incubator Shaker Model G25 (New Brunswick Scientific Co, New Jersey, USA). Isoniazid and rifampin were used as control drugs. The time-kill curve assay was done according to the recommendations of the Clinical and Laboratory Standards Institute (11).

**Data analysis**

MICs results are expressed as geometric mean (GM) ± standard error of the mean (S.E.M). The results of kill-kinetic determinations are shown graphically by plotting log₁₀ CFUs against time and expressed as mean ± standard error of the mean (S.E.M). A bactericidal effect can be seen by a 3 log₁₀ (99.9% killing) decrease in CFU at the time specified.

**Results**

The results of the essential oil antimycobacterial screening and the composition of each oil are presented in table 1. All essential oils showed activity against Mycobacterium tuberculosis H37Rv. Only the Achyrocline alata essential oil exhibited a pronounced antimycobacterial activity below 100 µg ml⁻¹ (62.5 µg ml⁻¹). Other essential oils presented a moderate activity (MIC between 100 and 200 µg ml⁻¹). The major components of essential oils evaluated are listed in table 1. Carvacrol, thymol, p-cymene, 1,8-cineole, limonene, and β-pinene were the most commonly identified, the major components of A. alata were thymol, trans-β-caryophyllene, p-cymene and α-phellandrene. Figure 1 shows the interaction between essential oils MIC and M. tuberculosis strain H37Rv. As shown, the A. alata essential oil possessed the lowest MIC in comparison with the others.

The time kill curves of A. alata essential oils, Swinglea glutinosa essential oils and the control drugs isoniazid and rifampin are shown in the figures 2 to 5. In comparison with the bactericidal activity of isoniazid and rifampin at concentrations equivalent 0.5-fold above the respective MIC, the essential oils were bacteriostatic.

**Discussion**

Historically, plants have been used worldwide in traditional medicines for the treatment of diseases. Today, approximately two-thirds to three-quarters of the world’s population are estimated to rely on medicinal plants as their primary source of medicines (12). The current study represents the first phase of ongoing research to identify new, safe and effective agents for the treatment of TB. Lippia origanoides and Lippia alba plants of the Verbenaceae family already have many ethnomedicinal applications. Both species possess several chemotypes (13,14). Cananga odorata (ylang-ylang) trees are an introduced species and have been widely distributed as ornamentals in Colombian cities. Ylang-ylang essential oil pharmacological effects have
Figure 1. Interaction between essential oils MIC with *M. tuberculosis* H37Rv. Values are reported as mean ± S.E.M. *Achyrocline alata* (sample 7) and *Swinglea glutinosa* (sample 3) produced MICs below 100 µg/mL. Isoniazide (INH) and rifampin (RIF) were used as controls.

Figure 2. Time kill curve of isoniazide against *M. tuberculosis* H37Rv. Values are reported as mean ± S.E.M. Concentrations at 0.5-, 1-, and 2-fold levels of the respective MIC indicate the bactericidal activity in comparison with the control.

Figure 3. Time kill curve of rifampin against *M. tuberculosis* H37Rv. Values are reported as mean ± S.E.M. Concentrations at 0.5-, 1-, and 2-fold above the respective MIC present bactericidal activity in comparison with the control.
been thoroughly investigated (15-17). Swinglea glutinosa (Rutaceae family) is a shrub planted in cities and countryside as a natural fence. Its extract is used in agriculture in natural preparations of biocides (18,19). Piper auritum and Piper bogotense of the family Piperaceae are widely distributed in Colombia and serve a variety of applications in folk medicine. Hyptis mutabilis (family Asteraceae) and Achyrocline alata (family Lamiaceae) play similar roles (20, 21).

The activity of essential oil from Achyrocline alata is probably due to the high concentration of terpenoids (22), which have been evaluated for their in vitro antimycobacterial activity (8,23). More specifically, thymol has an antimycobacterial activity with a MIC of 100 µg mL⁻¹ (24). Other terpenes as carvacrol and α-pinene have shown a MIC against M. tuberculosis H37Rv of 64 and 128 µg mL⁻¹ respectively (25). In addition, the antitubercular activity of citronellol, nerol and

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**Figure 4.** Time kill curve of *S. glutinosa* against *M. tuberculosis* H37Rv. Values are reported as mean ± S.E.M. Concentrations at 0.5-, 1-, and 2-fold levels of the respective MIC indicate the bacteriostatic activity in comparison with the control.

**Figure 5.** Time kill curve of *A. alata* against *M. tuberculosis* H37Rv. Values are reported as mean ± S.E.M. Concentrations at 0.5-, 1-, and 2-fold levels of the respective MIC indicate the bacteriostatic activity in comparison with the control.
geraniol has been evaluated with MICs between 64-128 µg ml$^{-1}$ (8).

In many essential oils, the antimicrobial activity is due to the presence of isoprenes such as monoterpenes, sesquiterpenes or related alcohols and phenols (26). The lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of essential oil components (26) which have high antimicrobial and antifungal activities (26). Possibly, the antibacterial activity of terpenes is due to a perturbation of the microorganism lipid fraction of the plasma membrane, and results in alterations of membrane permeability and in leakage of intracellular materials (27). This effect may be a consequence of the interaction between the major and minority components of essential oil. It was demonstrated that the physical properties significantly influenced the actions of the individual components, increasing or reducing antimicrobial efficacy (26).

The essential oil concentrations decrease substantially in broth and agar media, when incubated under open conditions. This decrease is caused primarily by evaporation and adherence on plastic surfaces (28). For these reasons, broth dilution assays must be carried out under sealed conditions to prevent evaporative loss, and glass materials must be used in place of plastics to prevent loss by absorption. In the current study, the antmycobacterial assays performed by a macrodilution method in glass tubes corrected these problems and consequently reproducible results were obtained. In addition, the screening method permitted the detection of resistance by $M.\ tuberculosis$ to INH, RMP and even for EMB (10,29).

Some authors use Tween 80 in the broth medium to enhance oil solubility (30). In the current study, MIC of Tween 80 against $M.\ tuberculosis$ H37Rv determined in the antmycobacterial assay (0.99%) was much higher than the concentration used for solubilizing oil (0.001%). Therefore, the addition of Tween 80 to the culture medium did not alter the MIC values obtained.

The essential oils and their volatile components may provide an important source of new antmycobacterial agents. Evaluations in checkerboard assays of the individual components and their interactions of $A.\ alata$ and $S.\ glutinosa$ essential oils are necessary in order to determinate the active principles and toxicity of these complex mixes. Performing the macrodilution method with capped glass tubes is an alternative tool for obtaining more reproducible MIC values by controlling the essential oil high volatility.

**Conflict of interest**

The authors are in agreement with the results published in this article and claim no conflicts of interest with the same.

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