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Natural Compounds from Mexican Medicinal Plants as Potential Drug Leads for Anti-Tuberculosis Drugs

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ABSTRACT

In Mexican Traditional Medicine 187 plant species are used in the treatment of respiratory conditions that may be associated with tuberculosis. In this contribution, we review the ethnobotany, chemistry and pharmacology of 63 species whose extracts have been assayed for antimycobacterial activity *in vitro*. Among these, the most potent is *Aristolochia brevipes* (MIC= 12.5 μg/mL), followed by *Aristolochia taliscana*, *Citrus sinensis*, *Chrysactinia mexicana*, *Persea americana*, and *Olea europaea* (MIC<64 μg/mL). Other potent extracts (inhibition > 95%, 50 μg/mL) include: *Amphipterygium adstringens*, *Larrea divaricata*, and *Phoradendron robinsoni*. Several active compounds have been identified, the most potent are: Licarin A (isolated from *A. taliscana*), and 9-amino-9-methoxy-3,4-dihydro-2H-benzo[h]-chromen-2-one (transformation product of 9-methoxytariacuripyrone isolated from *Aristolochia brevipes*), both with MIC= 3.125 μg/mL, that is 8-fold less potent than the reference drug Rifampicin (MIC= 0.5 μg/mL). Any of the compounds or extracts here reviewed has been studied in clinical trials or with animal models; however, these should be accomplished since several are active against strains resistant to common drugs.

Key words: Tuberculosis, medicinal plants, natural products, *Mycobacterium tuberculosis*.

INTRODUCTION

Tuberculosis (TB) is caused by the slow-growing acid-fast bacillus *Mycobacterium tuberculosis*. Until 1980 it was considered as a controlled disease worldwide; however, this scenario was transformed due to drug resistance, and HIV pandemic; therefore, tuberculosis has become one of the most serious public health challenges of the 21st century. In 1993, TB was declared as a global emergency by the World Health Organization (WHO). In 2013, there were 9 million new cases, and 1.5 million deaths caused by TB, as well as 0.35 million deaths associated to HIV-patients (WHO 2014). *Mycobacterium tuberculosis* (MTb) is a facultative intracellular pathogen that has developed resistance

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to first and second line anti-tuberculosis drugs. Antibiotics resistance and multidrug-resistant TB strain are a serious problem due to negative effects in designing treatment strategies.

The recent rise of TB is associated with the human immunodeficiency virus HIV and the rapid spread of multidrug resistance to TB strains; therefore, new classes of anti-mycobacterial compounds are required (Luna-Herrera et al. 2007). These compounds can be obtained from plants which are an important natural source of novel leads in the field of anti-tuberculosis therapeutics (Cantrell et al. 2001, Copp and Pearce 2007, Gutierrez-Lugo and Bewley 2008).

On recent years, ethnobotanical information has been used as an alternative pathway to discovery of new bioactive compounds. Usefulness of this approach is now recognized (Béjar et al. 2000, Ríos et al. 2012). Mexican higher flora has been estimated to be in the range of 25,000 to 34,000 species, therefore, it is included among the five most import megadiverse countries of the world. A compilation of the Mexican Medicinal Flora lists 3,103 species known at the present time (INI 1994). A similar number can be projected from the reported botanical collection of the IMSSM herbarium (Aguilar-Contreras et al. 1998, Aguilar et al. 1994). These numbers indicate that at least one of each ten plant species has been used medically in the country (Béjar et al. 2000, Ríos et al. 2012).

In the present paper, we compiled the medicinal plants used in Mexican Traditional Medicine against respiratory affections, coldness, pulmonary affections that may be associated with tuberculosis (Aguilar-Contreras et al. 1998, Aguilar et al. 1994, Díaz 1976, INI 1994). Only 62 out of the 187 plants quoted have been evaluated for anti-mycobacterial activity bioassays *in vitro*; 17 of these have been investigated chemically, and active compounds have been identified or proposed. A brief assessment of the *in vitro* anti-TB bioassays is also provided for a comprehensive analysis.

MATERIALS AND METHODS

LITERATURE SEARCH

An extensive literature search was carried out using published books, articles, and electronic sources of information as PubMed (Medline) and BADEPLAM data base**.** The keywords used (in various combinations) in the search were: Plants, Natural, Remedies, Mexican Traditional Medicine, Herbal, Tuberculosis, Mycobacteria, Anti-mycobacterial and Anti-tuberculosis. The search comprised results of *in vitro* antimycobacterial tests of compounds, and extracts. In sake of a comprehensive review, the antimycobacterial methods were briefly described, discussed and exemplified.

ANTI –TB *in vitro* BIOASSAYS

DISK AGAR DIFFUSION

The disk diffusion solid agar is the most conventional method used for determining the susceptibility of clinical isolates to reference drugs, for testing extracts, and not previously evaluated chemical compounds; in this evaluation the extent of inhibition zones is correlated to susceptibility (Negi et al. 2010, Primm and Franzblau 2007). This method has a disadvantage that hydrophilic compounds may not diffuse, thus they can be missed due to the fact that mycobacteria cell wall is lipophilic (Connell and Nikaido 1994, Gautam et al. 2007). Results can be obtained after 25 days. Murillo-Alvarez et al. (2001) used this method for the screening anti-TB activity of ethanol (EtOH) extracts of 25 desert plants used in Traditional Medicine of Baja California Sur, Mexico. *Bursera odorata* Brandegee. (Burseraceae) was the most active with 67% inhibition at 100 µg/mL; in addition, this extract showed a MIC < 0.076 µg/mL against the HCT-116 (colorectal carcinoma) cell line.

Macro and Micro agar dilution

This method evaluates quantitatively the activity and minimum inhibitory concentration (MIC)

determination by using known concentrations of extracts or compounds on an agar medium (Negi et al. 2010). In this case, colony forming units (CFU) are used to determine the MICs of plant extracts. Performance of the test takes 3 to 4 weeks. This method was used for the anti-mycobacterial evaluation of methanolic extracts of *Persea americana* Mill. (Lauraceae) and *Gymnosperma glutinosum* Less. (Asteraceae). A hexane fraction of the first extract inhibited at 31.2 µg/mL the reference strains H37Ra, and H37Rv (Gómez-Flores et al. 2008).

RADIORESPIROMETRY

The growth of mycobacterium is determined by Bactec 460 instrument; the system detects release of radioactive CO_2 generated by metabolically active MTb in a selective media loaded with ^{14}C-palmitate. This method is used for the susceptibility testing of clinical isolates (Franzblau et al. 1998), and can provide results in an average of 5 days compared with 3-4 weeks for conventional methods (Negi et al. 2010, Newton et al. 2000). Several authors have used this method for extracts and compounds evaluation (Jiménez et al. 2005, Murillo-Alvarez et al. 2001, Rivero-Cruz et al. 2005). Interesting results were obtained with this method while testing the dichloromethane/methanol (CH_2Cl_2/MeOH) 1:1 extract of *Amphipterygium adstringens* (Schltdl.) ex Standl. (Julianaceae) trunk bark, since H37Rv was inhibited in 95% at 50 µg/mL (Rivero-Cruz et al. 2005).

MICRO BROTH DILUTION

These colorimetric assays are based on the oxidation-reduction of an indicator, generally Rezasurin (MABA, REMA) or MTT (TEMA), which are very suitable for testing drug susceptibility using microtitre plates. Results may be easily obtained using a spectrophotometer or fluorometer, and they provide results in average of 7-10 days (Gómez-Flores et al. 2008, Negi et al. 2010, Palomino et al. 2002). Evaluation of anti-mycobacterial activity of extracts plants have reported using this method, for example: hexane extracts from *Artemisia ludoviciana* Nutt. (Asteraseae), *Chamaedora tepejilote* Liebm. (Arecaceae), *Lantana hispida* Kunth (Verbenaceae), *Junglans regia* L. (Jungladaceae), and *Junglans mollis* Engelm. (Jungladaceae) among other plants (Cruz-Vega et al. 2008, Jiménez-Arellanes et al. 2003).

Microplate alamar blue assay (MABA)

The method was developed by Collins and Franzblau in 1997, and is the most used for testing plant extracts and compounds in this review, because it is easily performed, and not expensive. Alamar blue is a solution of resazurin, and has been proved effective with MTb H37Rv, H37Ra and *M. avium* strain ATCC 25291, it is also a reliable technique with clinical isolates of MTb. This method operates as a simple colorimetric mode with visual reading (blue to pink change indicates viability, MIC is recorded as the lowest compound concentration in the wells which remain blue); quantitative spectrophotometric recordings are performed at 570 y 600 nm (Collins and Franzblau 1997, Franzblau et al. 1998). However, the highest sensitivity readout is achieved with fluorescence, through excitation at 530 and emission at 590 nm. MIC is calculated as the lowest concentration of a compound which reduces absorbance or transmittance by $\geq 90\%$ as compared with untreated cultures (Collins and Franzblau 1997).

This method has been applied in the investigation of laurel oil expressed from the ripe fruit of *Laurus novocanariensis* Webb & Berthel (Lauraceae) of the Madeira Archipelago, and used for centuries in traditional medicine for the treatment of influenza and other respiratory symptoms. The sesquiterpene lactones, costunolide (Fig. 1) and dehydrocostuslactone were the compounds responsible for the activity against *Mycobacterium tuberculosis* H37Rv with MICs of 6.25 and 12.5 mg/L,

respectively. Anti-mycobacterial activity against drug-resistant *M. tuberculosis* clinical isolates was better for the mixture than pure compounds (Luna-Herrera et al. 2007).

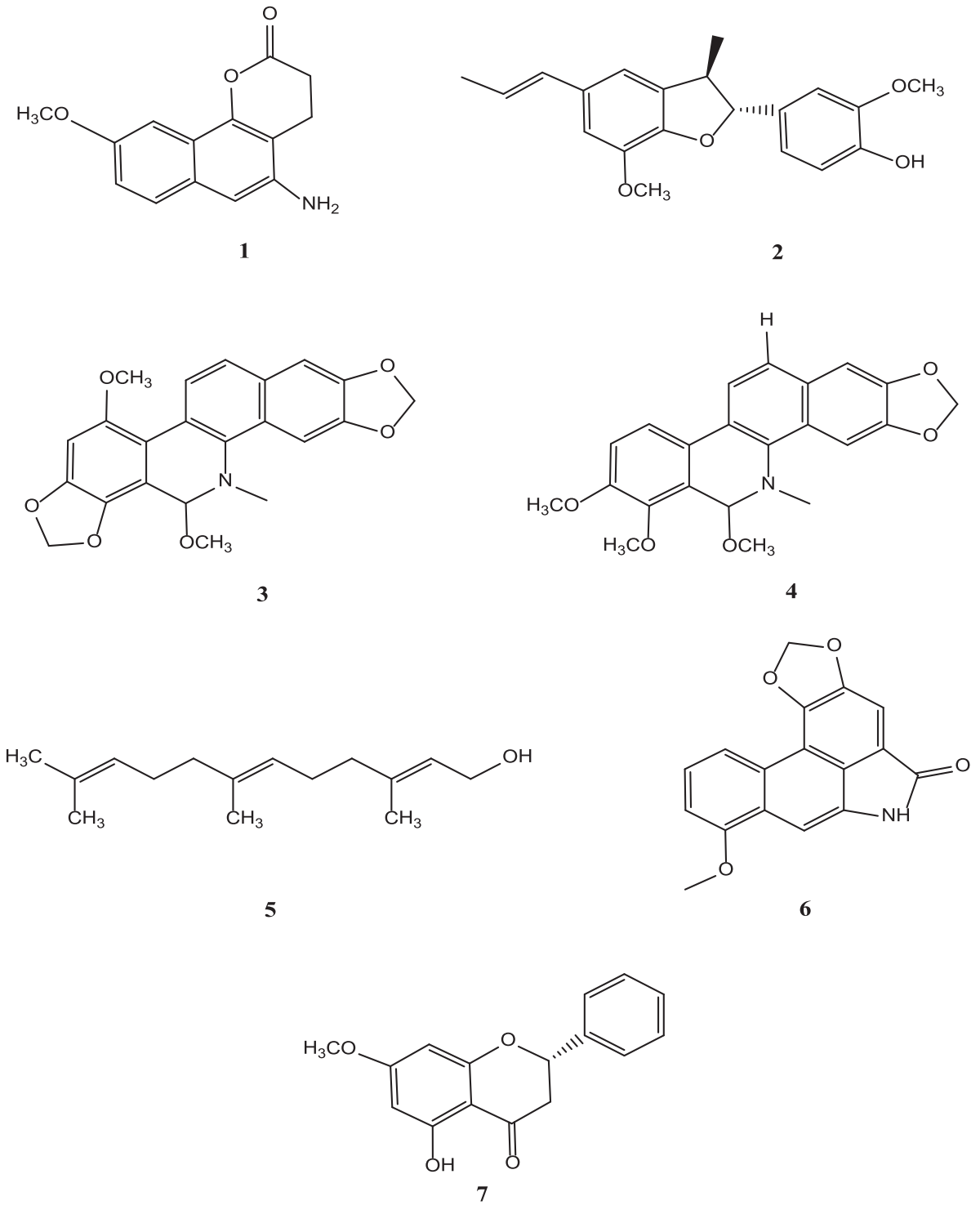

Figure 1 - Anti-mycobacterial compounds isolated from Mexican Medicinal Plants.

Resazurin microtiter assay, REMA

A solution is prepared with resazurin salt, a used as previously described for MABA. This method is simple and inexpensive for detection of drug resistance. A tensoactive agent, usually Tween 80 can be added while preparing MABA solution, allowing a better diffusion into *Mycobacteria*. Recordings can be performed using a spectrophotometer, or fluorometer (O'Brien et al. 2000, Palomino et al. 2002). Recently thirty-six plant extracts from the Brazilian Atlantic forest were tested for their anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv and *M. kansasii*, using the method REMA (Fernandez et al. 2008).

Tetrazolium microplate assay, TEMA

Tetrazolium dyes are used for testing drug susceptibility with mycobacteria. The colored end product of MTT (Formazan) is insoluble, but inclusion of 10% ethanol during the 24hr incubation is sufficient to solubilize the color without a cell lysis step. TEMA is reported to be less expensive than MABA (Primm and Franzblau 2007); however, it is seldom used.

ANTI-TUBERCULOSIS ACTIVITY OF MEXICAN PLANTS

In this review, 187 plant species were found to be used in the treatment of respiratory diseases, including tuberculosis and related diseases caused by *Mycobacterium*. These plants are applied as herbal infusions or decoctions, generally of the aerial parts, but also trunk barks, flowers, fruits and roots. (Table SI – Supplementary Material).

Only 62 of these plant species (33%) have been tested *in vitro* for anti-mycobacterial activity. The organic extracts, but also few aqueous extracts, have been tested. The most used assay is MABA, followed by BACTEC 460 system. Commonly the tested reference strain is H37Rv from the ATTC; however, clinical isolates and multidrug-resistant strains have also been evaluated (Table SI).

Several authors have proposed a MIC < 200 μg/mL for an extract to be considered as promising, and to deserve further research, such as identification of active compounds, *in vivo* tests with animal models, etc. (Jiménez-Arellanes et al. 2003). However, MIC threshold has also been proposed as < 100 μg/mL (Cantrell et al. 2001). In any case, the most active species with MIC < 64 μg/mL includes: *Aristolochia taliscana, Aristolochia brevipes, Citrus sinensis, Chrysactinia mexicana, Persea Americana* and *Olea europaea.* Not all the studies calculate MIC, and their results are expressed in percentage of inhibition. So, we considered that the most active extracts are those with an inhibitory activity >95% when tested at 50 μg/mL, and includes *Amphipterygium adstringens, Larrea divaricata,* and *Phoradendron robinsoni.* These species and others with the similar activity are discussed in the following lines.

***Aristolochia taliscana* Hook. & Arn. (Aristolochiaceae).** According to ethnobotanical data, *A. taliscana,* and some other species of this genus, have been widely used in Mexican traditional medicine to treat cough (Díaz 1976). This species is commonly known in Mexico as "*guaco"* or "*raíz de guaco"*. This plant is known since the sixteenth century, and some applications can be related to tuberculosis symptoms, for instance against "cold fevers", and for "cleaning the chest". The Spanish physician and naturalist Francisco Hernández (1946) in his book *History of the Plants of the New Spain (1570-1577)* wrote: "the roots are almost hot in the third degree, and cure the tumors, calm the pain and banishes the cold fevers, reinforces the heart, stomach, and mind, cleans the chest and stomach, relieves dropsy evacuating their cause". A preliminary biological evaluation of hexane extract from *A. taliscana* roots showed that it possessed high anti-mycobacterial effect *in vitro* against MTb

H37Rv and *M. avium* (MICs= 50 µg/mL). Three neolignans were isolated, Licarin A, Licarin B and Eupomatenoid-7. Licarin A was the most active compound, with MICs of 3.12-12.5 µg/mL against the *M. tuberculosis* strains: H37Rv, four mono-resistant H37Rv variants and 12 clinical isolates, as well as against five non-tuberculosis mycobacteria strains. The three neolignans were assayed on murine macrophage J774A.1 cell line; in the case of licarin A the IC_{50} was 6.25 µg/mL, therefore exhibited citotoxicity in the range of pharmacological concentrations. However, the acute toxicity of the crude extract and Licarin A in mice was >1706 mg/kg, indicating it has low toxicity *in vivo*. The authors concluded that licarin A represents a potentially active anti-TB agent to treat MDR *M. tuberculosis* and non-tuberculosis *Mycobacterium* strains (León-Díaz et al. 2010).

Aristolochia brevipes Benth. (Aristolochiaceae). Ethnobotanical data for this species, also known as "guaco" in Mexico, indicate the use of the rhizomes to treat arthritis, diarrhea, to cleanse wounds, and for cough with blood, and also in the treatment of snake bites. The dichloromethane extract (rhizome) possesses strong *in vitro* activity against *Mycobacterium tuberculosis* H37Rv (MIC= 12.5 µg/mL). Eight compounds were isolated, being the most active aristolactam I, with MIC values ranging between 12.5 and 25 µg/mL, other compounds, including alkaloids, nitro compounds, and triterpenes, showed MIC > 20 µg/mL (Navarro-Garcia et al. 2011).

Citrus sinensis **(L.) Osbeck (Rutaceae).** This well-known Asian plant (orange), is named in México as "naranja"; it is used in Traditional Mexican Medicine as antiscorbutic, astringent, against colds, coughs and sore throats. The warm juice of the edible fruits used for the treatment of cough (INI 1994). In the eighteenth century in the *Book of the Jew* it was wroten: "its fruit eaten with salt is very good for phlegms" (Osado et al. 1983). The hexane extract of the fruit peel has been tested against isoniazid and ethambutol resistant *M. tuberculosis* strains *in vitro*, the MICs were 25 and 50 µg/mL. The hexane extract of *C. aurantifolia* fruit peel showed identical results with these strains (Camacho-Corona et al. 2008).

Chrysactinia mexicana **A. Gray (Asteraceae).** It is used as a treatment of respiratory affections and "coldness" of the spleen, and tonic in the traditional medicine from Northeast of Mexico, the local name is "*Hierba de San Nicolás"*. This plant was reported to have anti-mycobacterial activity, but the effect was not quantified (Cantrell et al. 2001). Afterwards, the "ethyl" (*sic*) extract from the roots of *C. mexicana* was tested showing a MIC= 62.5 µg/mL against a drug-resistant strain of *M. tuberculosis*. Phytochemical analysis allowed to isolate and identify several monoterpenes, but no biological activity was reported for these. The antifungal activity of the essential oil obtained from the leaves of this plant has also been reported (Molina-Salinas et al. 2007).

Persea americana **Mill. (Lauraceae).** The name of the edible fruit "avocado" derives from the Spanish popular name "*aguacate",* which is a corruption of the word "*aua´catl"* (testicles) in native Náhuatl language. The Mexican variety has aromatic leaves, resembling the odor of *Pimpinella anisum.* The decoction of the leaves is commonly used in Mexico to treat diverse diseases including cough, bronchitis, chronic catarrh, bronchial cough, and pertussis. At the beginning of the 18th century, Juan de Esteyneffer (1978), a Jesuit missionary, in his book *Florilegio Medicinal* (1712) wrote about its medicinal applications clearly related to tuberculosis symptoms, as follows: "a decoction of the leaves breaks up clumps of blood and prevents blood spitting." The leaves of *P. americana* have been reported to possess anti-inflammatory and

antifungal activities. The hexane extract of the leaves showed activity against strain H37Ra (MIC= 125μg/mL) and H37Rv (MIC= 62 μg/mL) (Gómez-Flores et al. 2008).

***Olea europaea* L. (Oleaceae).** The leaves of this common European plant have been reported for treating tuberculosis and other respiratory diseases in Mexican Traditional Medicine. However, we could not find the original source of the ethnobotanical information. In any case, the hexane extract of leaves from *O. europaea* was reported to exhibit a MIC= 25μg/mL against the isoniazid-resistant variant of H37Rv (Camacho-Corona et al. 2008); therefore it result the most potent of the extracts here reviewed.

***Amphipterygium adstringens* (Schltdl.) ex Standl. (Julianaceae)**. The bark of the trunk is used in a traditional Mexican Medicine to treat respiratory problems, such as, cough, swollen tonsils, colds, tuberculosis and lung diseases. Its popular names are: "cuachalalate", "cuachinala", words which derive from the Náhuatl word "cuachalalatl" ("árbol de las chachalacas" = tree of the *Ortalis* sp. bird). The bark is boiled until the water gets colored, afterwards it is sweetened to reduce its astringent taste, the decoction is drunk four times a day, as long as necessary, or instead of common water ("agua de uso") (INI 1994). The bark of *A. adstringens* contains triterpenoids with anti-inflammatory, hypocholesterolemic, and antiulcer activity. The CH_2Cl_2/MeOH (1:1) extract of the bark inhibited in 95% the growth of *M. tuberculosis* at a concentration of 50 μg/mL. The extract was subjected to chromatography, and some compounds were isolated, and evaluated with H37Rv strain. Two triterpenes were the most active (14b, 24E)-3-oxolanosta-7,24-dien-26-oic acid (MIC= 64μg/mL), and (14b, 24E)-3-hidroxylanosta-7,24-dien-26-oic acid (MIC= 32μg/mL) (Rivero-Cruz et al. 2005).

It has been proposed that *A. adstringens* really comprise a set of several related species, which can be botanically distinguished as: *A. molle, A. simplicifolium, A. glaucum,* and *A. amplifolia* (Cuevas-Figueroa 2005). The leaf and bark extracts of CH_2Cl_2-MeOH (1:1) of these species were found to be active (IC_{50}) in the range 1.37-2.35 μ/mL, showing low cytotoxicity to human macrophaghes THP1, but did not inhibited meaningfully (< 40%) the reverse transcriptase of human immunodeficiency virus (HIV-RT) (Gómez-Cansino et al. 2015).

***Larrea divaricata* Cav. (Zygophyllaceae).** The popular name is "gobernadora", and in the traditional medicine it is used to treat colds and coughs. The decoction of the roots, branches or bark is drunk instead of water as "agua de uso" (INI 1994, Marquez and Lara 1996). *Larrea divaricata* is a perennial bush with dark green stems and yellowish green leaves. Its leaves and stems are also used in a traditional medicine as an antiseptic, blood purifier, diuretic and anti-inflammatory agent (INI 1994). The CH_2Cl_2/MeOH (1:1) extract inhibited in 100% the growth of *M. tuberculosis* at 50 μg/mL. The phytochemical analysis afforded several flavonoids, lignans, and chromenes. Several compounds were tested with H37Rv strain and found active. The MIC was 50 μg/mL for the following compounds: 5,7-dihydroxy-3-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one, β,γ-dimethyl-α,δ-bis (3,4-dihydroxyphenyl) butane, and 5,6,7-trihydroxy-3-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (Rivero-Cruz et al. 2005).

***Phoradendron robinsoni* Trel. (Santalaceae).** Its common name is "muerdago" and it is used in a traditional medicine to reduce blood pressure levels, against coughs, and to strengthen the immune system. The infusion of the whole plant is drunk as everyday drinkable water in the treatment of lung and kidney problems (Marquez and Lara 1996). *P.*

robinsonni is a hemiparasitic and dioecius plant with inconspicuous flowers. It has not been chemically investigated; however, *P. coryae* and *P. tomentosum* have yielded several flavonoids. The extract of CH_2Cl_2/MeOH (1:1) showed 99% inhibition of the growth of *M. tuberculosis* at 50 µg/mL. A compound, 5-hydroxy-2-(4′-hydroxyphenyl)-7-methoxy-2,3-dihydro-4H-chromen-4-one, was isolated and tested with H37Rv strain, its MIC was 128 µg/mL (Rivero-Cruz et al. 2005).

Calophyllum brasiliense Cambess. (Clusiaceae). A tree from the tropical rain forest distributed from Brazil to Mexico. There are no ethnobotanical data related to its use for treatment of respiratory diseases or tuberculosis; however, the CH_2Cl_2-MeOH extract (1.1) from the leaves has shown to be highly active against *M. tuberculosis* and HIV-RT *in vitro* with an IC_{50} = 3.02 and 26.24 µg/mL (respectively). An HPLC analysis of this extract indicates it contains calanolide A, B, and soulatrolide (Gómez-Cansino et al. 2015). Interestingly, several active compounds against *M. tuberculosis* and HIV have been isolated from *Calophyllum* species, including calanolide A, B, and soulatrolide (Xu et al. 2004) and mammea A/AA. Other Clusiaceae species, such as *Vismia mexicana*, and *V. baccifera*, showed also good activity against *M. tuberculosis* and HIV, but their active principles have not been identified yet (Gómez-Cansino et al. 2015).

ANTI-TUBERCULOSIS NATURAL PRODUCTS FROM MEXICAN PLANTS

Several anti-tuberculosis natural compounds have been isolated from plants distributed in Mexico; however, not all of them have been selected from on ethnomedical grounds, but rather from a bio-prospective viewpoint. This section is a selection of active natural compounds so far found to be active against *M. tuberculosis*.

The compound 9-amino-9-methoxy-3,4-dihydro-2H-benzo[h]-chromen-2-one (**1**) (Figure 1) showed a MIC= 3.125 µg/mL when tested against the strain H37Rv. Interestingly this compound is a transformation product obtained by enzymatic reduction with *Saccharomyces cerevisiae* of 9-methoxytariacuripyrone isolated from *Aristolochia brevipes* (Alvarez-Fitz et al. 2012). Despite its activity, this compound has not been further studied.

Another potent compound Licarin A (**2**) (Figure 1) was isolated from the roots of *Aristolochia. taliscana* and showed a MIC= 25 µg/mL when tested with H37Rv; interestingly this compound was more potent when tested with a number of *M. tuberculosis* strains, showing a MIC= 3.12-12.5 µg/mL against four mono-resistant H37Rv variants and 12 clinical MDR isolates with multidrug resistance (León-Díaz et al. 2010); however, this compound was cytotoxic when evaluated on murine macrophage J774A with an IC_{50}= 6.25 µg/mL; it induced an apoptotic effect by means of caspase-3 activation (Park et al. 2004). On the other hand, Lee et al. (2004) reported that licarin A is a potent inhibitor of phospholipase Cγ1 with an IC_{50}= 15.8±1.3 µM, it also exerts antiproliferative effects against three human cancer cell lines [A-569 (lung), MCF7 (breast) and HCT-15 (colon)] (Lee et al. 2004, Park et al. 2004).

The alkaloids 6-methoxydihydrochelirubine (**3**) and 6-methoxydihydrocheleritrine (**4**) (Figure 1) isolated from *Bocconia arborea* tested with MTb H37Rv showed a MIC= 12.5 µg/mL. These metabolites showed good activity against three multi-drug-resistant *M. tuberculosis* clinical isolates. The 6-methoxydihydrochelirubine (**3**) showed MICs= 25-50 µg/mL, while 6-methoxydihydrocheleritrine (**4**) had a MIC= 12.5 µg/mL, these results suggest that 6-methoxydihydrochelirubine could be a useful template for the development of new antituberculosis drugs (Camacho-Corona et al. 2009).

The monoterpene farnesol (**5**) (Figure 1) isolated from the leaves of *Chamaedora tepejilote* inhibited the strain H37Rv 99% at a concentration

of 100µg/mL and showed a MIC= 8 µg/mL (Jiménez et al. 2005). This compound has been previously reported by Rajab et al. (1998). This plant is reported in Mexican Traditional Medicine to treat cough and pneumonia, but not specifically tuberculosis (INI 1994).

The aristolactam I **(6**) isolated from *A. brevipes* was the most active compound, among several others isolated from this species. The tests were performed against monoresistant and multidrug resistant clinical isolates of *Mycobacterium* strains, showing values on the range 12.5 to 25 µg/mL (Navarro-Garcia et al. 2011).

The flavanone pinostrobin (**7**) (Figure 1) isolated from *Teloxis graveolens* was evaluated against the strain H37Rv and showed a MIC 12.5 µg/mL. In assessing the structure-activity relationships of several related compounds, it was suggested that its highest activity could be explained by its increased liphophilicity, which may facilitate passage across mycobacteria cell membrane (Camacho-Corona et al. 2009).

DISCUSSION

In the present review it was found that only 63 out of 187 plant species used in Mexico for treatment of respiratory affections, including tuberculosis, have been tested for antimycobacterial activity *in vitro*. The most active extracts with MIC < 64 µg/mL includes: *Aristolochia brevipes*, *A. taliscana, Citrus cinensis*, *Chrysactinia mexicana*, *Persea americana* and *Olea europaea*. Chemical studies have only been performed for *Aristolochia brevipes,* and *A. taliscana*. Three neolignans were isolated from the later species. Licarin A was the most active compound, with minimum inhibitory concentrations of 3.12-12.5 µg/mL against a number of *M. tuberculosis* strains H37Rv, including multidrug clinical isolates, and non-tuberculosis mycobacteria strains. This compound tested *in vitro* on murine macrophages showed an of IC_{50}= 6.25 µg/mL, indicating cytotoxicity in the range of active concentrations. However, the acute toxicity of the crude extract and Licarin A in mice was >1706 mg/kg, indicating it has low toxicity *in vivo* (León-Díaz et al. 2010). It is clear that other studies must be performed in order to determine if this species, and its leader compound may have some potential in therapeutics.

Other active extracts, with an inhibition >95% when tested at 50 µg/mL, were obtained from *A. adstringens, L. divaricata,* and *P. robinsoni.* Nevertheless, the compounds so far isolated from these extracts and tested, did not exhibited remarkable anti-TB activity *in vitro* (MIC ≥ 32 µg/mL), suggesting that the most active compounds have not been isolated yet. It is also likely that the high activity of these extracts arise from a synergic phenomenon.

The ability of plants to synthesize secondary metabolites has been of importance in drug development (Cowan 1999). Natural products or their semisynthetic derivatives, especially from fungi, have provided in recent years drug leads in tuberculosis chemotherapy (Newton et al. 2000, Shu 1998). Examples of such compounds, which are used as first choice in the treatment of tuberculosis, include streptomycin (0.095 µg/mL) from *Streptomyces griseus* (Copp 2003), and rifampicin (MIC= 0.25 µg/mL), a semisynthetic drug derived from rifamycin a product of *Amycolatopsis mediterranei,* a bacteria found in natural environments (Tribuddharat and Fennewald 1999). Rifampicin and isoniazid (MIC= 0.5 µg/mL) are the most used drugs for the treatment of tuberculosis.

Anti-TB second choice drugs (used in cases of resistance to first line drugs) derived from natural products include: capreomycin (MIC > 5.0 µg/mL) isolated from *S. capreolus* (Shu 1998) and kanamycin (MIC= 0.63 µg/mL) obtained from *Streptomyces griseus* (Copp 2003). In the present review, Licarin A, an 9-amino-9-methoxy-3,4-

dihidro-2H-benzo[h]-chromen-2-one, had a MIC= 3.12 µg/mL, therefore in the range of capreomycin, an approved drug with identical MIC. In general, the results of this review agree with those of Newton et al. (2000), who stated that a number of anti-mycobacterial compounds have been isolated from plants used in traditional medicine, but only a few are comparable in potency to those isolated from microorganisms, as well as, approved drugs. However, some of the plant compounds here reviewed are active against strains resistant to common drugs; therefore they may provide leads for the development of second line anti-TB drugs. Since plant remedies used in traditional medicine are complex mixtures of compounds that could have different therapeutic targets, continuing studies on the supported efficacy these plants in animal models, and even in clinical trials are needed.

CONCLUSIONS

Only 33% of the medicinal plants used in Mexican Traditional Medicine in the treatment of respiratory affections including tuberculosis and related diseases or caused by *Mycobacterium* have been studied *in vitro* regarding to their anti-TB properties. Currently, no studies with these 62 plants have been conducted in animal models, or clinical trials. Acute toxicity of a few extracts and isolated compounds have also been performed. The most potent plant extract and compounds, are: *Aristolochia brevipes*, and Licarin A (*Aristolochia taliscana*) with MIC= 12.5 and 3.12 µg/mL, respectively. The biotransformation with *Saccharomyces cereviceae* of 9-methoxytariacuripyrone, a natural compound isolated from *A. brevipes*, yielded 9-amino-9-methoxy-3, 4-dihidro-2H-benzo[h]-cromen-2-one which also showed a MIC= 3.12µg/mL. It is necessary further to evaluate the intracellular activity and cytotoxicity of the most active extracts and compounds; however, the potential for finding new therapeutically leads exists, especially against strains resistant to common drugs (Encarnación-Dimayuga et al 2006, Esquivel-Ferriño et al. 2012, Gould 2009, Gurinder and Daljit 2010, Jiménez-Arellanes et al. 2007, 2012, Levy and Marshall 2004, Molina-Salinas et al 2011, Sandoval-Montemayor et al. 2012, Rajandeep et al. 2011).

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SUPPLEMENTARY MATERIAL

Table SI - Medicinal Mexican plants and natural products which have been assessed for antimycobacterial activity *in vitro*.