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Medicinal Plants, an Important Reserve of Antimycobacterial and Antitubercular Drugs: An Update

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Abstract

Background: Tuberculosis is a global and serious Public Health problem due to the increase of multidrug-resistant and extensively drug-resistant cases; as a result, diverse research groups worldwide are focusing their efforts on finding novel antituberculous agents that can provide greater effectiveness, less toxicity and having a specific mechanism of action, possibly being coadjuvants in the treatments currently prescribed.

Methods: The present review covers the literature published concerning secondary metabolites of those Mexican medicinal plants and secondary metabolites isolated from them showing *in vitro* antimycobacterial activity with MIC <50 µg/mL against sensitive and MDR *M. tuberculosis* strains as well as against NTM strains. The review also includes a special section for those natural compounds or plant extracts with antitubercular activity evaluated an *in vivo* experimental tuberculosis model.

Results: Some pure compounds with MIC<25 µg/mL are: 2-oxo-14-(3´,4´-methylenedioxyphenyl) tetradecane, 2-oxo-16-(3´,4´-methylenedioxyphenyl)hexadecane, 5,6-dehydro-7,8-dihydro methysticin, cepharanone B and piperolactam A (from *Piper sanctum*), suberosin (from *Arracacia tolucensis*) and leubethanol (from *Leucophyllum frutescens*). In addition, (-)-licarin A (from *Aristolochia taliscana*) was active against *M. tuberculosis* H37Rv, 12 MDR *M. tuberculosis* clinical isolates and four non-tuberculous mycobacteria.

On the other hand, the antitubercular activity of (-)-licarin A, ursolic acid and oleanolic acid has been determined in a TB murine experimental *in vivo* model; (-)-licarin A reduces the bacterial lung load and the percentage of pneumonia in animals infected with *M. tuberculosis* H37Rv and MDR *M. tuberculosis*. The mixture of ursolic and oleanolic acids showed a significant reduction of bacterial loads and pneumonia in animals infected with *M. tuberculosis* H37Rv and MDR *M. tuberculosis*.

Conclusion: Since (-)-licarin A, ursolic acid and oleanolic acid have been evaluated as antitubercular compounds, these metabolites are candidates proposed feasible to be proposed for development of anti-tuberculosis drugs.

Keywords: Secondary metabolites; Antimycobacterial activity; Antitubercular activity; Medicinal plants; *Mycobacterium tuberculosis* H37Rv; MDR *M. tuberculosis*

Abbreviations:

AIDS: Acquired Immunodeficiency Syndrome; *C. tepejilote*: *Chamaedora tepejilote*; medium; DOTS: Directly Observed Therapy Short Course; DR: Drug-Resistant; EMB: Etambutol; HIV: Human Immunodeficiency Virus; IFN-γ: Interferon Gamma; INH: Isoniazid; *L. hispida*: *Lantana hispida*; MABA: Microplate Alamar Blue Assay; MDR: Multidrug-Resistant; MIC: Minimal Inhibitory Concentration; *M. avium*: *Mycobacterium avium*; *M. chelonae*: *Mycobacterium chelonae*; *M. fortuitum*: *Mycobacterium fortuitum*; *M. smegmatis*: *Mycobacterium smegmatis*; *M. tuberculosis*: *Mycobacterium tuberculosis*; RIF: Rifampicin; STR: Streptomycin; TB: Tuberculosis; TGF-β1: Transforming Growth Factor-Beta 1; TNF-α: Tumor Necrosis Factor Alpha; XDR: Extensively Drug Resistant; WHO: World Health Organization.

Background

Epidemiology of tuberculosis

Tuberculosis (TB), malaria and intestinal parasitosis are diseases that were thought to be eradicated; unfortunately, nowadays they still comprise an emerging problem for health systems worldwide. TB and the closely associated Human immunodeficiency syndrome (HIV) are the main infectious diseases that are considered medical, economic and social disasters of great magnitude [1].

The World Health Organization (WHO) declared TB to be a health problem in 1993 and, today, it is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis*, of

which 10% are at risk for of developing the active disease. Annually, 10 million new cases appear and about 3.5 million individuals die due to this cause [1-3]. In 2012, 8.6 million people developed TB and 1.3 million died from it due to the disease [4]. Recent records indicate that by the year 2020, there will be one billion infected people, and if effective decisions for the control of TB are not adopted, 150 million individuals might develop the disease and 36 million persons will die from this condition [5,6]. On the other hand, nearly 8% of newly infected cases will develop some form of TB. Patients with the HIV/Acquired immune deficiency syndrome (AIDS) virus are 50-100 times more exposed to develop active TB, and it is estimated that 50-60% of the persons with latent TB and HIV/AIDS will develop the active form of the disease [6]. Diabetes mellitus and rheumatoid arthritis are also some factors that predispose people to develop TB.

Global estimates point out that in 2010, 650,000 new cases of Multidrug-resistant (MDR)-TB were registered, of which around 440,000 cases were acquired; 150,000 individuals died due to this cause and 3.6% of all new TB cases are now MDR. By 2012 and 2013, the WHO estimated around 450,000 and 310,000 MDR cases, respectively, recording 170, 000 deaths related to drug resistant (DR) TB, and from 77,000 patients, only 17% of MDR-TB cases started treatment [4,7]. The Global tuberculosis report 2012 of the WHO estimated that 4% of new cases of TB and 20% of retreated cases are MDR [8]. In addition, WHO estimates that up to half a million new cases of MDR-TB occur worldwide each year [7]. Recent reports described that approximately 30% of MDR cases present treatment failures and 50 million persons worldwide are infected with DR *M. tuberculosis* [1,8,9]. On the other hand, less than 25% MDR cases are currently detected and 3.6% of new TB cases and 20% of retreatment cases are DR [4]. In 2007, 84.5% of MDR cases in the U. S. were found among foreign-born persons, Mexico being the principal country-of-origin [10]. MDR cases require two years of treatment with second-line drugs, which are more toxic and more expensive than first-line drugs [9]. To treat these patients, the administration of expensive injectable drugs for 8 to 20 months is mandatory, being the cost of the treatment in USA of approximately 4000 dls/patient [8].

In 2005, eXtensively drug-resistant (XDR) cases of TB were detected and identified as cases of patients who do not respond to any of the antitubercular drugs employed, they are resistant to at least Isoniazid (INH), Rifampicin (RIF), one fluoroquinolone and any of the second-line injectable drugs (amikacin, kanamycin, or capreomicin). The presence of XDR-TB is due to poor diagnosis, inadequate treatment and the abandonment of the patient´s treatment. Side effects also appear because the second-line drugs are more toxic and the treatment must be undergone for a longer duration period (up to 36 months), it is a complex regime, ineffective, poorly tolerated and expensive [8]. XDR cases have been detected throughout the world (92 countries) including Mexico and its frequency with respect to MDR was 3% for the U.S. [4,11]. Recent articles describe that approximately 50,000 XDR cases occur annually; their prevalence in the world, although unknown, is on the rise [12].

A recent study reports that of 424 MDR cases with complete DR susceptibility, 18 (4.2%) were XDR and 77 (18%) were pre–XDR. The proportion of pre–XDR increased from 7% in 1993 to 32% in 2005. Günther, described that 9.6% of MDR cases are XDR. Among XDR cases, 83% comprised foreign-born patients (from six different countries), and 43% of the cases were diagnosed in patients within 6 months after their arrival in the U.S. Mexico was the most common country-of-origin and five XDR cases were acquired during therapy in California. In this study, the author also indicates that the percentage of MDR isolates tested for susceptibility to fluoroquinolones increased from 50% in 1993 to 80% in 2004 and the proportion of MDR that were evaluated for flouroquinolones and injectable drugs increased from 45% (1995) to 71% (2006) [4,11].

According to data of the Ministry of Health (Secretaría de Salud, SS), Mexico presents 25 cases of TB per 100,000 inhabitants; however, WHO estimates that this average does not reflect the totality of cases. For example, in 2002 and 2004, this organization recorded 33,757 and 32,239 cases per year, respectively, while the SS reported an average of 16,000 cases per year during the same period [13-15]. The average age of infected Mexican patients is 36 years, 69% of whom are males. Data reflect the urgent need for the appropriate treatment and follow-up of persons who are mainly at economically productive ages. From 2000-2010, an average of 18,200 cases was registered with an incidence of 16.8 cases per 100,000 inhabitants; for 2011-2013, an average of 16,500 cases of pulmonary TB was registered [16-18]. The Mexican states with the greatest number of cases are Veracruz, Chiapas, Baja California, Nuevo León, Guerrero and Tamaulipas [18].

Of the TB cases that occurred between the years 1997-2002 in the country, 21% was DR and 4.6% was MDR. Among previously treated TB cases, it was found that 65% were DR and 48% was MDR [9,19]. Recently, MDR-TB has been reported for Chihuahua, Chiapas, Sinaloa and Nuevo León with incidences of 7.8%, 13.6%, 17.9% and 17.3%, respectively [9,20-22]. XDR cases have been described but scare information is available. A recent report conducted in Monterrey, capital of the Mexican state of Nuevo León indicated 139 cases of pulmonary TB; 24 cases were resistant to INH and RIF and 11 cases were resistant to five drugs. The proportion of MDR among previously treated cases was 25.18% (35/139). Moreover, one MDR isolate was resistant to three second-line drugs. The author concludes that Monterrey has a high prevalence of MDR among previously treated cases and that XDR cases are slow to appear or that they already exist but have not yet been detected [22].

Antitubercular pharmacological therapy

Current treatment for sensitive TB is based on a multitherapy consisting of the mixture of five first-line drugs INH, RIF, Ethambutol (EMB), Streptomycin (STR) and/or Pyrazinamide (PYR), a treatment lasting up to 6–8 months. However, at present, many cases do not respond to the established therapies because of the presence and emergence of MDR strains, which are characterized by being resistant to RIF and INH (basic drugs for their treatment). MDR cases are treated with second-line drugs (Capreomycin, Kanamycin, Amikacin, Cyclocerin, Fluoroquinolones, Ciprofloxacin and Proteonamide, among others), but these agents have the disadvantage of not being specific, causing severe side effects, easily inducing resistance, poorly tolerated, expensive, entailing a prolonged treatment duration (up to 30 months) and scant treatment compliance [8,23-25].

One situation that greatly worsens the global panorama is XDR-TB, because there is no alternative for treating the disease and because these cases respond precariously to first- and second-line drugs [12,26,27]. XDR-TB is caused by *M. tuberculosis*, which is resistant to INH and RIF; it is also resistant to up to three injectable second-line drugs (capreomycin, kanamycin and amikacin), as well as to fluoroquinolones. These cases have been detected in numerous countries including Mexico, and they register a limited cure prognosis and high mortality rates [25,28,29]. Some factors that increase MDR and XDR cases worldwide comprise poor diagnosis and inadequate

second-line drug treatments, inadequate treatment adherence and a high incidence of HIV, rheumatoid arthritis and diabetes [20,29,30]. Approximately, 9% of all MDR cases is XDR and only 5% of XDR cases receives effective treatment; this fact clearly indicates that we face a serious health problem throughout the world. It was recently reported that cases of XDR-TB are on the rise. A total of 1,278 cases were studied and it was found that 7% was XDR-TB, while 43.7% turned out to be resistant to second-line drugs, 20% were resistant to injectable second-line drugs and 13% were resistant to fluoroquinolones [9,31-34].

The development of anti-TB drugs over the past 4 decades has been slack. At present, the only drugs for the treatment of TB approved by the U.S. Food and Drug Administration (FDA) are Rifabutin, Rifalazil and Rifapentine (all three RIF derivatives), drugs that are neither widely used nor widely distributed. About 10 new drugs are now under clinical investigation, among which we find the following: Moxifloxacin and Gatifloxacin (currently in phase II/III trial); DC-159a (preclinical phase); TMC207 (or R 207910 or compound J), a diarylquinoline in phase II trial), PA-824 and OPC-67683, a dihydroimidazoxazole derivative of Metronidazole that inhibits protein synthesis and the cell wall (both compounds now in a phase II trial). Linezolid (oxazolidinone class) and SQ109 (diaminade derivates of Ethambutol) are effective in treating cases of MDR- and XDR-TB (both drugs presently in phase II trials) [1,2,25,27,35]. The Bedaquiline fumarato (SirturoTM) was registered by FDA in December 2012 and has been recommended for adults with MDR pulmonary TB although at this time, the effectiveness and safety of the drug is unknown at this time. Linezolid, clofazimine, moxifloxacin, PA-824 and sutezolid are drugs not yet licensed for the treatment of MDR-TB nevertheless they have a promising future [4,7,8].

Mexican medicinal plants as a source for antimycobacterial compounds

Information concerning the antimycobacterial activity of Mexican plant extracts and secondary metabolites tested by Microdilution alamar blue assay (MABA) has been referred to in several bibliographic sources. In this manuscript we incorporated these extracts and pure compounds which showed a Minimal inhibitory concentration (MIC) of <50 μg/mL mainly against *Mycobacterium tuberculosis* H37Rv, a strain that is sensitive to all first-line drugs (INH, RIF, EMB, STR and PYR). The review also describes plant extracts and pure compounds that are active against NTM and MDR *M. tuberculosis* strains. It is relevant that recent information describes the antitubercular activity of some secondary metabolites evaluated an *in vivo* experimental model.

In the scientific literature, several papers describe the extracts and the pure compounds obtained from numerous worldwide medicinal plants that exhibit significant *in vitro* activity against *M. tuberculosis* H37Rv (MIC ≤5 μg/mL). These compounds have been isolated and characterized by using several methodologies, such as bio-guided assays and have been evaluated by the BACTEC 460 radiorespirometric method or by MABA assay [36-38]. Some examples of these secondary metabolites are 1′-acetoxychavicol acetate with MIC=1μg/mL (isolated from *Alpinia galanga*), ergosterol-5,8-endoperoxide (isolated from *Ajuga remota*), the (E)-phytol (obtained from *Leucas volkensii*) and micromolide (isolated from *Micromelum hirsutum*), with MIC values of 1.0, 2.0, and 1.0μg/mL, respectively [39-44].

Within this context, several groups in Mexico are also contributing to the knowledge of the potential that medicinal plants represent in the search for new anti-TB drugs, and the present review attempts to illustrate the progress that has been made in the evaluation of about 100 species of the Mexican medicinal flora.

The hexanic extract of *Flourensia cernua* (*hoja sen or hojasé*), a medicinal plant from Northeastern Mexico showed an MIC=50 μg/mL against *M. tuberculosis* H37Rv and an MIC=25 μg/mL against the MDR clinical isolate of *M. tuberculosis* CIBIN/UMF15:99 [42]. The non-polar fraction of the decoction showed an MIC=6.25 μg/mL against the same MDR strain [43].

From *Piper sanctum*, commonly known as *hoja santa or acuyo*, the following compounds were isolated: 2-oxo-14-(3′,4′-methylenedioxyphenyl)tetradecane (1) and 2-oxo-16-(3′,4′-methylenedioxyphenyl)hexadecane (2), with an MIC=6.25 μg/mL; 5,6-dehydro-7,8-dihydromethysticin (3) with an MIC=4 μg/mL; cepharanone B (4) with an MIC=12 μg/mL; cepharadione B with an MIC=32 μg/mL and piperolactam A (5) with an MIC=8 μg/mL) [44].

Other papers that explore potential antimycobacterial activity of medicinal plants from South and Central Mexico and that are used to treat TB and illnesses related to the disease describe the presence of compounds with significant antimycobacterial activity *in vitro*. About 60 medicinal species have been tested by MABA assay; the hexanic extracts of some of these, such as *Lantana hispida*, *Chamaedora tepejilote*, *Thymus vulgaris*, *Artemisia ludoviciana*, *Juniperus communis*, *Aristolochia taliscana* and *Aristolochia elegans* inhibited the growth of *M. tuberculosis* H37Rv with MIC of <100 μg/mL against *M. tuberculosis* H37Rv [45-52].

Oleanolic acid (6) isolated from *L. hispida*, showed an MIC=25 μg/mL against *M. tuberculosis* H37RV. Ursolic acid (7), farnesol (8) and squalene (9) were isolated from the hexanic extract of *C. tepejilote*, inhibited the growth of *M. tuberculosis* and showed an MIC=50 μg/mL. On the other hand, the mixture of the ursolic and oleanolic acids [6,7] isolated from the hexanic extracts of *A. ludoviciana* and *T. vulgaris* exhibited an MIC=12.5 μg/mL against *M. tuberculosis* H37Rv. It is noteworthy that the antimycobacterial activity of ursolic and oleanolic acids isolated from several plants, such as *Aspidosperma quebracho-blanco*, *Junellia tridens*, and *Valeriana laxiflora*, among other species, has been described, showing an MIC=15 μg/mL or 32 μM for ursolic acid and an MIC=30 μg/mL or 64 μM for oleanolic acid when evaluated by the BATEC 460 radiorespirometric system, with MIC values of 41.9 and 28.7 μg/mL, respectively, when was evaluated by MABA assay [36,53-55].

Another species that inhibited the growth of *M. tuberculosis* H37Rv was *A. taliscana rizhome* (hexanic extract), which showed an MIC=50 μg/mL. By bioassay-guided fractionation, three neolignans were isolated and identified as licarin B, eupomatenoid-7 and (-)-licarin A (10–12), with important antimycobacterial activity against *M. tuberculosis* H37Rv (MIC <25 μg/mL). (-)-Licarin A (12) was the most active compound, displaying activity against four monoresistant variants of *M. tuberculosis* H37Rv and 12 MDR *M. tuberculosis* clinical isolates with a MIC ranging from 3.12–25 μg/mL; this compound also inhibited the growth of the following four NTM strains: *M. fortuitum*, *M. smegmatis*, *M. chelonae* and *M. avium* (MIC <6.25 μg/mL). Compounds 10 and 12 showed a median Lethal dose (LD50) of >1,706 mg/kg in male BALB/c mice [50,56,57].

On the other hand, from the hexanic extract of *A. elegans*, *fargesin* (13) and (8R,8′R,9R)-cubebin (14) were isolated; these compounds

showed an MIC=50 µg/mL against *M. tuberculosis* H37Rv. Fargesin also inhibited the growth of three monoresistant variants of *M. tuberculosis* H37Rv (RIF-R, STR-R and EMB-R) and demonstrated an MIC of <25 µg/mL and the MDR *M. tuberculosis* clinical isolate (SIN4) exhibited an MIC=50 µg/mL. (8R,8´R,9R)-Cubebin was active only against two MDR *M. tuberculosis* clinical isolates (SIN4 and CIBIN/UMF15:99); MIC values were 50 µg/mL [49].

Continuing with the medicinal flora of Central and Southern Mexico, from the active extract of *A. brevipes* rhizome (MIC=12.5 g/mL) 9-methoxytariacuripyrone (15), 7,9-dimethoxytariacupyrone (16) and aristolactam I (17) were isolated; these compounds inhibited the growth of *M. tuberculosis* H37Rv with an MIC values of <50 µg/mL. Moreover, these compounds were active against four monoresistant variants of *M. tuberculosis* and against the following three MDR *M. tuberculosis* clinical isolates: MMDO, MTY147 and SIN452 [58]. The biotransformation of 9-methoxytariacuripyrone (16) with Saccharomyces cerevisiae led to the formation of 5-amino-9-methoxy-3,4-dihydro-2H-benzo-[h]chromen-2-one (18); this compound was most active against *M. tuberculosis* H37Rv with MIC=3.125 µg/mL [59].

Some compounds (19-29) with MIC values <32 µg/mL that were isolated from *Rumex hymenosepalus, Cosmos pringlei, Iostephane heterophylla* and *Ipomoea tricilor* are described in Table 1 [60]. Tyrianthinic acids I and II (30,31), as well as tyrianthins 8 and 9 (32,33) isolated from the methanolic extract of *I. tyrianthina* roots, showed an MIC= 25 µg/mL against *M. tuberculosis* H37Rv [61].

From the active CH_2Cl_2:MeOH (1:1) extract, obtained from *Buddleja cordata* bark (MIC=50 µg/mL), 2[4´-hydroxy-phenyl]-ethyllignocerate (34) was isolated; this compound showed an MIC=64 µg/mL [62]. From the CH2Cl2:MeOH (1:1) extract of *Arracacia tolucensis* (aerial parts), osthol (35) and suberosin (36) were obtained with MIC values of 32 and 16 µg/mL, respectively against *M. tuberculosis* H37Rv [63].

The hexanic extracts of *Juglans mollis* and *Carya illinoinensis* collected in Northeastern Mexico were active against *M. tuberculosis* H37Rv with an MIC=50 and 31 µg/mL, respectively [64]. The hexanic extracts from *Citrus aurantifolia*, *C. sinensis* and *Olea europea* were active only against mono-resistant variants of H37Rv (INH-R) with MIC=25 µg/mL and the chloroformic extract from *Nasturtium officinale* was active against RIF-R, INH-R and EMB-R of H37Rv (MIC=50 µg/mL) [65]. Escobarine A and B (37,38), obtained from the *Calliandria californica* (root, collected in Baja California Sur), showed an MIC=25 and 50 µg/mL against *M. tuberculosis* H37Rv and an MIC=12.5 and 100 µg/mL against MDR (CIBIN/UMF15:99) clinical isolates, respectively [66]. The hexanic, CH2Cl2 and MeOH extracts from *Diospyros anisandra* bark (native plant from the Yucatan Peninsula) showed an MIC=50, 12.5 and 25 µg/mL against *M. tuberculosis* H37Rv and MIC=25, 6.25 and 12.5 µg/mL against MDR *M. tuberculosis* clinical isolates CIBIN/UMF 15:99, respectively [67]. By bioguided fractionation from the active extract plumbagin (39), maritinone (40) and 3,3´-biplumbagin (41) were isolated; these compounds showed an MIC<3.13 µg/mL against *M. tuberculosis* H37Rv and the MDR *M. tuberculosis* clinical isolate CIBIN 99. In addition, zeylanone (42) was obtained, with an MIC of 25 and 12.5 µg/mL against *M. tuberculosis* H37Rv and the MDR *M. tuberculosis* clinical isolate CIBIN 99, respectively [68].

Some compounds (13 alkaloids, 9 flavonoids, 2 quinones, 9 triterpenes and 2 diterpenes), obtained from several plant species were tested against *M. tuberculosis* H37Rv, against mono-resistant variants of *M. tuberculosis* and against MDR of *M. tuberculosis*. The most active compounds (with an MIC of <25 µg/mL) were the following: dihydrochelerythrine (43), isolated from *Bocconia arborea*; 6-methoxy dihydro chelerythrine (44), isolated from *B. arborea*; 6-methoxy dihydro chelirubine (45), isolated from *B. arborea*; liriodenin (46), isolated from *Stephania dinklagei*; peracetylstrictosidine lactam (47), obtained from *Cephaelis dichroa*; phaeanthine (48), isolated from *Triclisia patens*; 5,7-dihydroxy-6-methyl-8-prenylflavanone (49), isolated from *Eysenhardtia platycarpa*; pinostrobin (50), isolated from *Teloxys graveolens*; 1-hydroxy-benzoisochromanquinone (51), isolated from *Psychotria camponutans*; aloe-emodin (52), obtained from *Stephania dinklagei*; epi-oleanolic acid (53), isolated from *Celaenodendron mexicanum* and 5α-lanosta-7,9(11)24-triene-3µ,24-diol (54), isolated from *Guarea rhopalocarpa*. 6-Methoxydihydrochelirubine (45) inhibited only the growth of MDR clinical isolates *M. tuberculosis* M-20, M12 and 345, showing an MIC=12.5 µg/mL; 6-methoxydihydrocheleritrine (44) was active against MDR clinical isolates *M. tuberculosis* M20 (MIC=25 µg/mL) and 6-methoxydihydro sanguinarine (55), obtained from *B. arborea*, inhibited the growth of MDR clinical isolates from *M. tuberculosis* M-20 and -345 with MIC=12.5 and 25 µg/mL, respectively [69].

A furanolignan, identified as 2´,5´´-dimethoxysesamin (56), isolated from *Leucophyllum frutescens*, showed an MIC=63 µg/mL against *M. tuberculosis* H37Rv. Furthermore in this species, leubethanol (57), a serrulatane-type diterpene was isolated; this compound also inhibited the growth of *M. tuberculosis* H37Rv (MIC=12.5 µg/mL) and clinical isolates of *M. tuberculosis* CIBIN/UMF15:99 (MIC= 6.3 µg/mL) [70,71]. The following compounds were isolated from *Citrus aurantifolia*: 5-geranoxypsoralen (58), 5,8-dimethoxypsoralen (59), 4-hexen-3-one (60), citral (61), palmitic acid (62) and linoleic acid (63); these compounds showed an MIC <50 µg/mL against the reference strain (*M. tuberculosis* H37Rv) [72].

Different compounds were isolated from the hexanic extract of *Foeniculum vulgare*; the most active compounds was 2,4-undecadienal (64) showing MIC values of <50 µg/mL against *M. tuberculosis* H37Rv and three MDR clinical isolates of *M. tuberculosis* (M-10, M-15 and M-26) [73]. On the other hand, dihydroguaiaretic acid (65) and 4-epi-larreatricin (66), isolated from *Larrea tridentata*, showed an MIC of <50 µg/mL against *M. tuberculosis* H37Rv and against three MDR clinical isolates of *M. tuberculosis* (MIC of <50 µg/mL). 3´-Dimethoxy-6-O-demethylisoguaiacin (67), 5,4´-dihydroxy-3,7-8,3´-tetramethoxyflavone (68) and 5,4´-dihydroxy-3,7,8-trimethoxyflavone (69) were obtained from the same species; these compounds were active only against MDR clinical isolates of *M. tuberculosis* with MIC values of <50 µg/mL [74].

In vivo antitubercular activity of isolated compounds

Although in the literature natural compounds with significant antimycobacterial activity (MIC≤5 µg/mL, *in vitro* assay) are reported, it is notably that public and private research sectors in search of anti-TB drugs are focusing mainly on the evaluation of synthetic drugs. The antitubercular effect of the above mentioned natural compounds has not yet been confirmed in *in vivo* assays, probably because of the solubility of the natural product, the lack of pharmacokinetics, pharmacodynamics and toxicological studies, the inavailability of a sufficient amount of the pure compound and the high cost of carrying out the *in vivo* test, among others.

Recently, the antitubercular activity of (-)-licarin A (12) was reported. This compound is a neolignan that was isolated from the hexanic extract of the A. taliscana rhizome. It's *in vivo* activity was determined by using the experimental mouse model of progressive pulmonary TB [50,75,76]. TB was induced with *M. tuberculosis* H37Rv and clinical isolated MDR *M. tuberculosis* (CIBIN:UMF15:99, resistant to RIF, INH, EMB, STR and PYR). The neolignan showed a significant reduction of the pulmonary bacterial burden in mice infected with both mycobacterium strains; this effect was observed predominantly during the first month of treatment. On the other hand, the percentage of pneumonia was significantly reduced in mice treated with the compound; this effect was more significant in animals infected with *M. tuberculosis* H37Rv during the first and second months of treatment. In mice infected with the clinical isolate MDR *M. tuberculosis* (CIBIN 15:99) and treated with (-)-licarin A (12), the percentage of pneumonia was lower in the first and second months of treatment, 22.28% vs. 43.56% and 29.15% vs. 71.97%, respectively [50,77]. The subacute toxicity of the compound tested at 21 days in healthy Balb/C male mice showed that subcutaneous administration at 5 mg/kg does not induce alterations of basic biochemical and hematological parameters, neither of the weight and the behavior of the treated animals. The histological analysis of liver, kidney, spleen, lung, brain, heart and muscle of treated with (-)-licarin A (12) did not show abnormalities [50,77].

Following the same methodological procedure, the other compounds which were subjected to *in vivo* tests were the triterpenes mixture of ursolic and oleanolic acids (6,7); these compounds were isolated from the medicinal species *C. tepejilote* and *L. hispida* [43,45]. Both compounds in TB induced with H37Rv or MDR bacilli (CIBIN 15:99) reduced bacterial load and pneumonia. Remarkably, the animals treated with these triterpenoids showed an increased expression of interferon gamma and the tumor necrosis factor alpha in their lungs. Although these triterpenoids showed poor antimycobacterial activity, the immune-stimulatory effect enables the control of experimental pulmonary TB [78,79].

The *in vivo* activity of these natural compounds is patented and currently there are ongoing studies to determine their interactions with antitubercular drugs in clinical use.

Conclusion

Because of the worldwide health problem that TB represents, it is urgent to find new drugs that allow better control of the outbreak and arrest the emergence of patients with MDR and XDR TB, cases which have arisen alarmingly.

Geographical position and topography places Mexico in the fourth place in global biodiversity with approximately 25,000 plant species. In addition, there is also a great ethnobotanical tradition that has led to the calculation that approximately 15% of Mexican plants is used to cure various diseases; a number of these species has been screened in the search for the antimycobacterial potential of these plants. Progress in the knowledge of their properties has been achieved when antitubercular activities of some of the isolated pure (-)-licarin A, ursolic and oleanolic acids were tested. This has led to the patenting and future projections of clinical evaluation in humans.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

AJ-A, M M-F and SR-T planned coordinated, analyzed data and drafted the manuscript. G G-R designed the chemical structure. All authors have read and approved the final manuscript.

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