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Anti-mycobacterial Activity of Extracts from Plants used in Mexico for the Treatment of Infectious Diseases

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ABSTRACT

Twenty seven extracts from eight medicinal plants used in Morelos, Mexico, for the treatment of several infectious diseases were screened for their *in vitro* anti-*Mycobacterium tuberculosis* (anti-MTB) activity on $H_{37}Rv$ strain of *Mycobacterium tuberculosis* (MTB), using the microplate Alamar assay test. Extracts (hexane, dichloromethane and methanol) from wild specimen of *Valeriana edulis* showed the most significant ($p<0.05$) activity against this strain (MICs values of 3.125, 25.0 and 50.0 µg/mL, respectively). On the other hand, hexanic and dichlorometanic extracts from *in vitro*-cultivated species of *V. edulis* also showed significantly activity against of *M. tuberculosis* (MICs values of 12.5 and 50.0 µg/mL, respectively). Most active extract (hexanic) obtained from *V. edulis* was also assayed against the drug-resistant strain (CIBIN 99), and the MIC value was 50 µg/mL. A qualitative TLC analysis of extracts from wild and *in vitro*-cultivated species of *V. edulis* showed that both content different chemical constituents. So, this last result explains the difference between anti-MTB activities of the extracts. Finally, dichloromethanic extracts from *Lepechinia caulescens* also showed a significantly activity against of *M. tuberculosis* (MIC 100 µg/mL). In conclusion, *V. edulis* and *L. caulescens* hexanic extracts are sources for the isolation of new or known compounds that could be used as leads for development of potential anti-MTB drugs.

KEYWORDS: Antimycobacterial activity, *Mycobacterium tuberculosis*, plant extracts, *Lepechinia caulescens*, *Valeriana edulis*.

INTRODUCTION

About one-third of the world's population infected and three million deaths per year are directly attributable to tuberculosis (TB). Currently, this health problem constitutes one of the primary causes of death or suffering cause in worldwide. Furthermore, multi-drug resistant strains of *M. tuberculosis* (MDR-MTB) as well as relationship between HIV/AIDS and TB have been main idea for the development of new anti-TB therapies [1]. According to World Health Organization reports, in 2003 there were 8.8 million new cases reported, killing 1.7 million of people worldwide. Based on a future trend, a total of 225 million new cases and 79 million deaths are expected from TB between 1998 and 2030 [2].

Although different types of anti-TB agents are available in world market for the treatment of this disease, there is a growing interest in herb remedies due unspecific side effects associated with synthetic therapeutics agents [3]. Because of natural products-derived scaffolds are therapeutic templates for the design of new therapeutic drugs using medicinal chemistry and computer-assisted design techniques, they have had a remarkable impact on the treatment of TB in comparison with classical FDA-approved drugs such as rifampin, kanamycin and cycloserine. Anti- *M. tuberculosis* (anti-MTB) compounds isolated from natural sources as plants, fungi and marine organisms have been found with different skeleton chemical forms and conformations [3].

In Mexico, a large number of plants have been used empirically in traditional medicine for the treatment of different diseases, including TB [4—10]. Furthermore, it exist a great background of research in natural products that has allow found new drugs with potential therapeutic uses. In this context, the main goal of current research was to evaluate anti-MTB effect of eight Mexican medicinal plants used for the treatment of different diseases (Table 1). Species selected for the study included *Valeriana edulis* (wild and *in vitro*-cultivated specimens), *Cochlospermum vitifolium, Tournefortia hartwegiana, Lepechinia caulescens, Laelia autumnalis, Cordia morelosana, Lepidium virginicum* and *Verbena carolina* [8]. The species were mainly selected for their ethnomedicinal uses in Mexican traditional medicine against parasitic infections. An *in vitro* assay was performed to proof anti-MTB activity using H37Rv strain, a drug-sensitive MTB strain, and drug-resistant strain (CIBIN 99)" by "An *in vitro* assay was performed to evaluate anti-MTB activity using a drug sensitive and drug resistant *M. tuberculosis* strains.

MATERIALS AND METHODS

Plant materials

Plant collection was carried out between July 2002 and October 2006 from their natural habitat in different regions of Morelos, México. Plants were collected and identified by Dr. P. Castillo-España and R. Ramírez and all voucher specimens were deposited at "Centro de Educación Ambiental e Investigación Sierra de Huautla" HUMO-Herbarium, Cuernavaca, Morelos, México. Different parts of collected plants were obtained and treated individually. An *in vitro*-generated specimen of *V. edulis* developed from a wild specimen was also assayed [11]. The details of medicinal plants features and their acquisition code number are listed in Table 1.

Preparation of extracts

Air-dried plant material (100 g from each species) were ground into powder and extracted exhaustively by maceration at room temperature with hexane, dichloromethane and methanol, respectively. Each solvent was replaced every 72 h during 3 times to allow a better extraction of metabolites. After a filtration process, the extracts were concentrated *in vacuo* at 40 °C, and the percentage yields determined.

Anti-Mycobacterium tuberculosis assay.

The activity of plant extracts against *M. tuberculosis* strains was tested using the microplate Alamar Blue assay (MABA) modified by Molina-Salinas and coworkers [6]. The strain of *M. tuberculosis* used for this assay were $H_{37}Rv$ (ATTC 27294), a strain sensitive at five first-line anti-TB drugs (streptomycin, isoniazid, rifampin, ethambutol and pyrazinamide) and drug-resistant strain (CIBIN 99), resistant to above anti-TB agents.

All organic extracts for anti-MTB bioassay were prepared at a concentration of 4 mg/mL in 100% of DMSO. The concentrations for plant extracts ranged from 100 to 0.195 µg/mL. Rifampin and Ofloxacin (Sigma-Aldrich Co., St. Louis, MO, USA) were used as positive controls with concentrations ranging from 2.000 to 0.062 µg of rifampin/mL and 16 to 0.50 µg of ofloxacin/mL. All assays were performed in duplicate.

Qualitative thin layer chromatography of the extracts from V. edulis

Qualitative TLC analysis of *V. edulis* extracts (*in vitro*-cultivated and wild specimens) was carried out on Silica gel 60 F_{254} (Merck®), using a dichloromethane:ethyl acetate (98:2) mixture as mobile phase. 50 µL of each extract (having a concentration of 1mg/mL) were added in the origin of the TLC plate.

RESULTS AND DISCUSSION

All plants investigated in this opportunity, except *V. edulis* (wild), were mainly selected by their anti-infection properties into Mexican folklore medicine (Table 1). The criterion to select screened plant species was their antimicrobial and anti-parasitic uses. This selection ensure to find potential natural sources for isolate new or known compounds that would be therapeutic agents in the treatment of this complex disease that, until this moment, remain killing people in worldwide.

Results of anti-MTB evaluations and minimal inhibitory concentration (MIC) values are shown in table 2. From the plant species evaluated, only *V. edulis* ssp. *procera* (wild and *in vitro*-cultivated specimens) and *L. caulescens* showed considerable anti-MTB activity. Hexanic extracts of *V. edulis* rhizomes from both wild and *in vitro*-cultivated specimens were the most potent samples showing MIC values of 3.125 and 12.5 µg/mL, respectively. In addition, dichloromethane extracts from both specimens showed MICs values of 25 and 50 µg/mL, respectively. Finally, methanolic extract of wild *V. edulis* showed a MIC value of 50 µg/mL. Thus, hexanic extract from *V. edulis* wild specimen was more active than *in vitro*-cultivated specimen for the evaluation. These results could be related with metabolic contents of each specimen since active compound might be overproduced in wild species more than *in vitro*-cultivated species. In order to test this hypothesis in a qualitatively manner, we did TLC analysis of extracts obtained from both types of *V. edulis* (Figure 1). This analysis showed that only

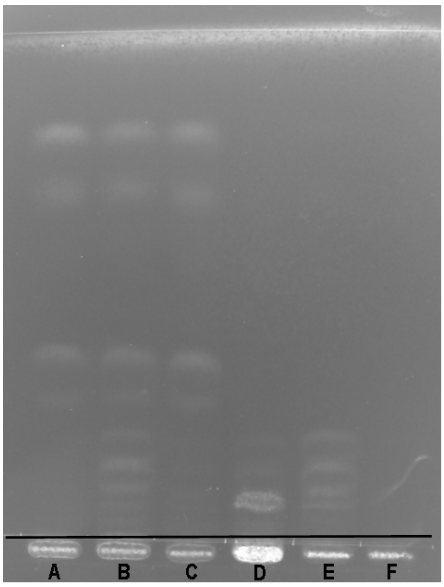

Figure 1. Thin Layer Chromatography of extracts from Valeriana edulis (Wild and Biotechnological species) carried out on Silica gel 60 F_{254} (Merck®), using a dichloromethane:ethyl acetate (98:2) mixture as mobile phas. Extracts from V. edulis (wild): A) Hexanic, C) dichloromethanic and E) methanolic. Extracts from V. edulis (biotechnologic): B) Hexanic, E) dichloromethanic and F) methanolic.

Table 1. Botanical and common names of plant species screened, their vouchers specimen numbers, parts extracted and traditional medicinal uses (Monroy-Ortiz and Castillo, 2007).

Plant name (Family)	Common name	Part used	Voucher number	Traditional uses
Cochlospermum vitifolium Willd. Ex Sprengel (Bixaceae)	Pánicua	Bark, stem bark	14628	Diabetes, hepatitis and related diseases, hypertension, antiseptic
Cordia morelosana Standley (Boraginaceae)	Anacahuite, palo prieto	Flowers, bark	in process	Respiratory diseases, cough, diarrhea, kidney inflammation, fever
Laelia autumnalis (Lex.) Lindley (Orchidaceae)	Flor de San Diego	Aerial parts	22025	To avoid abortion, antiseptic
Lepechinia caulescens (Ortega) Epling (Lamiaceae)	Betónica, Brenilla, Bretónica	Aerial Parts, Seeds, Leaves	20386	Kidney diseases, diarrhea, vomit
Lepidium virginicum L. (Brassicaceae)	Chilacaquilitl, lentejilla	Whole plant	25673	Diarrhea, stomachache, vomit, to expulse the intestinal parasites, cough, headache
Tournefortia hartwegiana Steudel (Boraginaceae)	Tlachichinole, hierba rasposa	Leaves, aerial parts	20382	Diabetes, diarrhea and kidney pain
Valeriana edulis ssp. procera *(Kunth) Meyer (Valerianaceae)*	Raíz de gato, valeriana mexicana	Rhizomes, roots	22446	Insomnious, headache, stress, heart diseases, hypertension, rheumatism, muscular pain
Verbena carolina L. (Verbenaceae)	Verbena, axixipatli	Leaves, aerial parts	25674	Dysentery, diarrhea, stomachache

Table 2. In vitro inhibition of M. tuberculosis of organic extracts obtained from several medicinal plants used in Mexican folk medicine for the treatment of several diseases

Plant	Part used	Extract	[a]MIC (µg/mL) *M. tuberculosis* $H_{37}Rv$
C. vitifolium	Bark	Hexanic	N. A.
		Dichloromethanic	N. A.
		methanolic	N. A.
T. hartwegiana	Aerial parts	Methanolic	N. A.
L. autumnalis	Aerial parts	Methanolic	N. A.
C. morelosana	Flowers	Methanolic	N. A.
L. virginicum	Aerial parts	Hexanic	N. A.
		Dichloromethanic	N. A.
		Methanolic	N. A.
V. carolina	Aerial parts	Hexanic	N. A.
		Dichloromethanic	N. A.
		Methanolic	N. A.
V. edulis subsp. Procera	Rhizome	Hexanic	3.125
(wild species)		Dichloromethanic	25.0
		Methanolic	50.0
V. edulis subsp. Procera	Rhizome	Hexanic	12.5
(biotechnological species)		Dichloromethanic	50.0
		Methanolic	N. A.
L. caulescens	Leaves	Hexanic	N. A.
		Dichloromethanic	N. A.
		Methanolic	N. A.
	Stem bark	Hexanic	N. A.
		Dichloromethanic	N. A.
		Methanolic	N. A.
	Roots	Hexanic	100
		Dichloromethanic	N. A.
		Methanolic	N. A.
	Flowers	Hexanic	100
		Dichloromethanic	N. A.
		Methanolic	N. A.
Rifampin			0.062

[a] N.A., not active.

hexanic extracts have similar phytochemical profile, while dichloromethanic and methanolic extracts of both specimens have different types or less quantity of active compounds.

On the other hand, we decided to evaluate the more active extract (hexanic) obtained from *V. edulis* against the drug-resistant strain (MDR) CIBIN 99, a clinical isolate of *Mycobacterium tuberculosis* resistant to all five first-line antituberculosis drugs. The MIC value determined was 50 µg/mL. Our results support the potential for identifying new compounds effective against MDR strains because we identified a plant species that has acceptable antituberculosis activity against both the sensitive and the resistant strains. In addition, there are some reports about isolation of sesquiterpenoid, monoterpenoid and iridoid scaffolds from genus *Valeriana* that could be involved in biological effect for this evaluation [12].

Previously studies about some species of Valeriana have demonstrated the useful in traditional medicine as sedative agents such as *V. officinalis*, *V. wallichii*, *V. fauriei* and *V. angustifolia*. This biological activity is related to the presence of valepotriates and other terpenoids derivates [12]. However, Gu and coworkers [13] reported that above-ground biomass and hexanic and dichloromethane extracts from *V. laxiflora* roots possess further anti-MTB activity besides having sedative effect. Bio-guided fractionation of these extracts led the isolation of active compounds as the new iridolactone [(4*R*,5*R*,7*S*,8*S*,9*S*)-7-hydroxy-8-hydroxymethyl-4-methylperhydrocyclopenta[c]pyran-1-one] and the new lignan [(+)-1-hydroxy-2,6-bis-epi-pinoresinol] as well as betulinic acid, betulin, ursolic acid, oleanolic acid and other triterpenoid derivates, with MIC values that ranged among 15.5 and 127 µg/mL [13].

Despite these results, hexanic extracts of both wild and *in vitro*-cultivated *V. edulis* showed better inhibitory effect for MTB being more potent than *V. laxiflora*.

Finally, hexanic extracts of *L. caulescens* flowers and roots also showed a relevant anti-MTB activity with MIC 100 µg/mL. In addition, it's important to mention that Ávila-Acevedo and colleagues [14] demonstrated that essential oil constituted with borneol, camphor and trans-caryophyllene possessed a significantly anti-*Vibrio cholerae* activity with MIC 4 µg/mL.

To best of our knowledge, no previous research has been developed about anti-tuberculosis properties of all plant species assayed here. Bio-guided fractionations of active extracts are in progress in order to isolate and characterize the lead compounds.

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