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ANTIMYCOBACTERIAL COMPOUNDS FROM *NASTURTIUM OFFICINALE*

Rodrígo Quezada-Lázaro,[1] Eunice A. Fernández-Zuñiga,[1] Abraham García,[1] Elvira Garza-González,[2] Laura Alvarez,[3] María del Rayo Camacho-Corona[1*]

[1]Universidad Autónoma de Nuevo León, Facultad de Ciencias Químicas. Av. Universidad S/N, Ciudad Universitaria, C.P. 66451, San Nicolás de los Garza, Nuevo León, México.
[2]Universidad Autónoma de Nuevo León, Servicio de Gastroenterología y Departamento de Patología Clínica, Hospital Universitario Dr. José Eleuterio González. Madero y Aguirre Pequeño, Mitras Centro, C.P. 64460, Monterrey, Nuevo León, México.
[3]Universidad Autónoma del Estado de Morelos, Centro de Investigaciones Químicas, Avenida Universidad 1001, Col. Chamilpa, 62209, Cuernavaca, Morelos, México.
Corresponding author E-mail: maracamcor@hotmail.com

Abstract

Background: *Nasturtium officinale* is a plant used in Mexican traditional medicine to treat respiratory infections such as tuberculosis. In previous studies, it was found that the chloroform extract of the aerial parts of *N. officinale* showed good activity against one sensitive and four drug-resistant *Mycobacterium tuberculosis* H37Rv strains. Therefore, the present research was focused on the fractionation and identification of the antimycobacterial principles of this species.
Material and Methods: The chloroform extract was prepared and fractionated by column chromatography using silica gel and gradient of chloroform/methanol, yielding 14 fractions. Each fraction was analyzed by thin-layer chlromatography under UV light. The obtained fractions were further tested against *Mycobacterium tuberculosis* H37Rv strain using Alamar blue microassay.
Results: Of the 14 fractions assayed, only fractions 3 to 5 showed good inhibitory activity against *M. tuberculosis* H37Rv. The chemical composition of three fractions by GC-MS led to the identification of *E*-phytol as the most abundant and common component.
Conclusion: Antimycobacterial properties of the three active fractions were attributed to *E*-phytol and palmitic acid.

Key words: *Nasturtium officinale*, terpene, fatty acid, *Mycobacterium tuberculosis*, GC-MS

Introduction

According to the World Health Organization, of the estimated 9 million people who developed tuberculosis in 2013, 1.5 million died and 480 000 have developed multi-drug resistant tuberculosis (WHO 2014). The problem of multi-drug resistant strains of *Mycobacterium tuberculosis* together with an increased incidence of new tuberculosis cases, have drawn attention of researchers to working on the discovery and development of new anti-mycobacterial drugs. Over the last decade, our research group has directed effort to the quest for novel classes of anti-tubercular compounds from Mexican medicinal plants such as *Larrea tridentata* (Favela-Hernández et al., 2012), *Foeniculum vulgare* (Esquivel-Ferriño et al., 2012), *Citrus aurantifolia* (Sandoval-Montemayor et al., 2012) and *C. sinensis* (Esquivel-Ferriño et al., 2014). The screening of 36 extracts obtained from nine plants used for the treatment of tuberculosis led to the discovery of *Nasturtium officinale* with promising anti-mycobacterial properties. (Camacho-Corona et al., 2008),

N. officinale W.T. Aiton (Brassicaceae), commonly known as "berro", is naturally distributed in parts of Asia, Europe, and Africa. In Mexico, this species grows near rivers and wet regions. It is medicinally used to treat kidney, stomach, and liver diseases, diabetes, respiratory illnesses, and tuberculosis (Argueta-Villamar et al. 1994). It has been reported that the leaves of *N. officinale* contains vitamins, glucosinolates (Aires et al. 2013), terpenes (Amiri, 2012), phenolics and flavonoids (Martínez-Sánchez et al. 2008; Aires et al. 2013; Boligon et al., 2013). Biological evaluations revealed that it has antibacterial (Freitas et al. 2013; Iseri et al. 2014; Penecilla and Magno, 2011), antioxidant (Amiri, 2012; Martínez-Sánchez et al. 2008), antigenotoxic (Casanova et al., 2013), inhibitor of histamine release (Goda et al., 1999), anti-inflammatory (Sadeghi et al., 2014) and cardioprotective (Bahramikia and Yazdanparast, 2008) properties. Recently, our research group reported that the chloroform extract of the leaves of *N. officinale* exhibited activity against one sensitive and four mono-resistant *M. tuberculosis* strains with minimum inhibitory concentrations (MIC) in the range of 50 to 100 µg/ml (Camacho-Corona et al. 2008). Therefore, the aim of the present study was to fractionate the chloroform extract, evaluate the anti-mycobacterial activity of fractions, and analyze the active fractions by Gas Chromatography coupled with Mass Spectrometry (GC-MS) in order to identify the active compounds.

Materials and Methods

General Experimental Procedures

Silica gel (70-230 mesh, Merck®) was used for column chromatography (CC). Fractions were monitored using pre-coated silica gel Aluminum foils (Fluka) and UV light at 254 and 364 nm. Analysis of active fractions were performed on a HP Agilent Technologies 6890 gas chromatograph equipped with a MSD 5973 quadrupole mass detector (HP Agilent) in electron impact mode at 70 eV. On the other hand, the anti-tubercular assay was performed using Middlebrook 7H9 broth base (BBL™, MGIT™, Becton Dickinson) in 96-well microtitre plates sealed in a plastic bag. Institutional ethics committee approved this study.

Plant Material, Extraction, Fractionation and GC-MS Analysis

N. officinale was purchased from the Central de Abastos of Monterrey, Nuevo León, Mexico (November 21, 2011). A voucher sample (024774) was deposited at the Herbarium UNL at Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, México. The leaves were removed and dried at room temperature. The dried material was powdered (525 g) and macerated with hexane (3 L) for 24 h and then with chloroform (3 L) for 72 h. The hexane extract was discarded from the present study because it exhibited no biological activity against *M. tuberculosis*. On the other hand, solvent was removed from the chloroform extract on a rotary evaporator under reduced pressure obtaining a greenish oily residue (22 g, 4.19%). The organic extract was subjected to a silica gel (440 g) CC eluted with chloroform and a stepwise gradient of chloroform/methanol as mobile phase (F1-F3 $CHCl_3$ 100%; F4-5 $CHCl_3$/MeOH 99:1; F6 98:2; F7 97:3; F8-9 95:5; F10 93:7; F11 90:10; F12 80:20; F13 60:40; F14 MeOH 100%). Fractions (100 ml each) were monitored by thin-layer chromatography and combined in 14 fractions according to their spot pattern similarities. The spots were viewed under UV light and the plates were sprayed with ceric sulphate. Chemical composition of active fractions was performed on a GC-MS.

Volatile compounds were separated on a HP Agilent Technologies 6890 gas chromatograph equipped with a mass spectrometer (MSD 5973) using a dimethylpolysiloxane HP-1 fused silica capillary column (25m x 0.2 mm i.d.; film thickness, 0.3 µm). The carrier gas was helium with a flow rate of 1 ml/min, the injector and detector temperatures were 250°C; injection in split mode (5:1). The oven temperature was held at 60 ºC for 1 minute, and then programmed from 60 to 255 ºC at 5°C/min and finally held at 255ºC for 45 minutes. The mass spectra were recorded on a Hewlett-Packard selective quadrupolar type mass spectrometer model 5973; ionization was obtained by electronic impact under a potential of 70 eV. Identification of volatiles was performed comparing their mass spectra with those of the National Institute of Standards and Technology NIST 1.7 library. In addition, standard solutions of C7-C40 alkanes were used to obtain the retention indices of compounds and were compared with literature data (Adams, 2009). Semi-quantitative data were calculated from the GC peak areas without using correction factors and were expressed as relative percentage (peak area %) of the total volatile constituents identified.

Microplate Alamar Blue Assay for anti-Mycobacterial Activity

The activity of fractions against *M. tuberculosis* H37Rv was tested using the Microplate Alamar Blue Assay (MABA) as previously described (Camacho-Corona et al., 2008). Ethambutol was used as positive control.

Results

The chloroform extract obtained from the leaves of *N. officinale* was fractionated into 14 fractions, which were evaluated against *M. tuberculosis* H37Rv. According to the results, fractions 3, 4 and 5 exhibited mycobacterial growth inhibition with MIC values of 200, 100 and 100 µg/ml, respectively, whereas the other fractions did not show anti-mycobacterial activity (MIC > 200 µg/ml). Ethambutol showed a MIC value of 2µg/ml.

Considering that fractions 3-5 were the only active fractions against *M tuberculosis* H37Rv, a chemical analysis of these fractions by GC-MS (Table 1) was conducted. The chemical composition of fractions 3, 4 and 5 revealed that they are mainly constituted by terpenes (phytols) and cholestanes (cholesterol, sitosterol, etc.). The terpenes named *E*-phytol, *Z*-phytol, and isophytol are the common components of these three fractions, *E*-phytol being the most abundant compound. Additionally, fraction 5 is composed of fatty acids such as palmitic and stearic acids.

Table 1: Chemical composition of fractions 3 to 5 obtained from the chloroform extract of N. **officinale**

Fraction number and compound names	RT[a]	%[b]	RI[c]
Fraction 3			
E-Phytol	32.46	26.93	2101
Unknown	33.08	4.80	2135
Isophytol	33.54	7.47	2160
Z-Phytol	39.157	1.33	2495
2,4-bis(1-methyl-1-phenylethyl)-Phenol	47.46	6.13	2977
Cholesterol	64.457	2.93	3449
Campesterol	70.434	7.46	3446
γ-Sitosterol	77.215	34.66	3457
(3β,24*Z*)-Stigmasta-5,24(28)-dien-3-ol	78.21	1.63	3477
Fraction 4			
E-Phytol	32.45	58.0	2101
Unknown	33.09	10.20	2136
Isophytol	33.54	15.32	2160
Z-Phytol	39.15	2.98	2494
Fraction 5			
E-Phytol	32.44	10.0	2136
Unknown	33.09	1.66	2160
Isophytol	33.53	2.50	2272
Methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate	35.591	7.5	2308
Hexadecanoic acid (palmitic acid)	36.10	16.25	2560
(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester	40.356	50.83	2266
Octadecanoic acid (Stearic acid)	40.672	4.17	
			2270

[a] RT Retention time (min). [b] % Relative abundance from the peak area integration. [c] RI Retention index calculated for each compound.

Discussion

Previous studies on chemical composition of essential oil of *N. officinale* leaves reported as the major volatile constituents: myristicin, α-terpinolene, and limonene (Amiri 2012). Other study reported 2-phenylethyl isothiocyanate, pulegone, heptyl isothiocyanate and 4-phenyl isothiocyanate as the major components of essential oil of leaves (Afsharypour and Ma´soumeh, 2008). Chemical composition of essential oil of leaves is different from the volatile constituents of active fractions of chloroformic extract of *N. officinale* obtained from leaves.

It is interesting to note that the active fractions (3 to 5) are composed of mainly non-polar compounds, which presumably diffuse easily across the mycobacterial membrane because the mycobacterial wall is highly lipophilic. In our previous pharmacological studies, palmitic acid was reported to exhibit growth inhibition of *M. tuberculosis* H37Rv with MIC value of 50 μg/ml (Sandoval-Montemayor et al. 2012). Other research groups have reported that *E*-phytol is a potent antimycobacterial compound having a MIC value of 12.5 μg/ml (Chen et al. 2010). Considering these findings, the antimycobacterial properties of fractions 3 to 5 could be attributed to the presence of this terpene and the fatty acid. The antimicrobial activity of fatty acids could be reinforced by previous reports where certain fatty acids inhibit cellular growth of *M. tuberculosis* (Koch et al. 2010; Skalicka et al. 2010).

Conclusion

We concluded that anti-mycobacterial properties of *N. officinale* could be attributed in part to the presence of *E*-phytol (MIC = 12.5 μg/ml) and palmitic acid (MIC = 50 μg/ml). It is important to mention that it is possible that other compounds not identified in active fractions could contribute with the anti-mycobacterial activity of this plant.

Conflicts of interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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