Artigo

EVALUATION OF ANTI-*Mycobacterium tuberculosis* ACTIVITY OF *Campomanesia adamantium* (MYRTACEAE)

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The anti-*Mycobacterium tuberculosis* activity of *Campomanesia adamantium* fruits extracts were evaluated. Six compounds, identified as flavanones and chalcones were quantified by HPLC-DAD-UV. Promising antitubercular activity was observed with ethyl acetate extract (MIC 62.5 μg/mL) and their fractions (MIC values ranging from 39 to above 250 μg/mL). The better MIC result of 39 μg/mL was associated with two fractions that contain bigger amounts of 5,7-dihydroxy-6, 8-di-C-methylflavanone and 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone. These compounds exhibited MICs >250 and 62.5 μg/mL, respectively, while their mixtures showed values ranging from 62.5 to 7.8 μg/mL, demonstrating a synergism between them.

Keywords: *Campomanesia adamantium*; anti-*Mycobacterium tuberculosis*; flavonoids.

INTRODUCTION

Tuberculosis (TB) has reemerged as one of the leading causes of death in the world, reaching a million deaths annually.[1] The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* and co-infections with HIV has aggravated this serious situation and, since resistance decreases the effectiveness of most available antitubercular agents,[2] there is an urgent need to develop new drugs to help reduce the global burden of tuberculosis, as has been well documented.[3]

Natural products and/or their semi-synthetic derivatives can lead to novel antimycobacterial drugs and might have in the future important roles in the chemotherapy of tuberculosis. The first step to obtain these products is its extraction from the plants, following by the achievement of biological tests with the extracts and fractions.[4]

The *Campomanesia adamantium* (Myrtaceae) species is a small tree with edible fruit, commonly known as guavira or guabiroba.[5] The fruit is widely used to make liqueurs, juices and sweets. The infusion of the guavira leaves is used in popular medicine as depurative, anti-diarrhoeic, cleanser, anti-rheumatic and to decrease the blood cholesterol.[6]

The phytochemical studies about *Campomanesia* genus are recent. Chemical investigation of seeds of *C. lineatifolia* led to the isolation and identification of β-triketone type compounds, named champanones.[7] Studies of the leaves in this *Campomanesia* species identified quercetin, myricetin and rutin by HPLC.[8]

In this way, the present paper describes the anti-*M. tuberculosis* activity of extracts, fractions and pure substances from *Campomanesia adamantium* fruits and the identification and quantification of flavanones and chalcones in these fractions, as well as their action against *M. tuberculosis in vitro*.

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EXPERIMENTAL

Plant material

The fruits of the *C. adamantium* were colleted in Jardim city, Mato Grosso do Sul State, Brazil (latitude 21° 25' 02.0'' S and longitude 056° 13'' 77.0'' W) and identified by M. Sobral of Universidade Federal de Minas Gerais. A voucher specimen (nº 5195 - Jardim) was deposited in the Herbarium Mato Grosso do Sul - HMS, Campo Grande, MS, Brazil.

General procedures

Sephadex LH-20 (Pharmacia, 60 cm x 2.5 cm) was used for GPC. TLC analyses were carried out on silica gel 60G (Merck) (20 cm x 20 cm x 0.2 mm) plates eluted with toluene: ethyl acetate: acetic acid 8:2:0.5 v/v/v. The spots were visualized by spraying with NP/PEG reagent, or 10% H_2SO_4 followed by heating at 110 ºC for 5 min. Column chromatographies were performed on silica gel (Merck, 40-63 μm and 63-200 μm) and Lichroprep RP18 (Merck, 40-63 μm). NMR spectra were recorded on a Bruker DPX 300 spectrometer.

The fractions obtained from the ethyl acetate extract were analyzed by an Analytical HPLC (Varian 210) system, equipped with a diode array detector (DAD). Star WS software was used for chromatograms data treatment. The column was an RP18 (25 cm x 4.6 mm x 5 μm) reversed-phase type, with a small precolumn (2.5 cm x 3 mm) containing the same packing, used to protect the analytical column. Elution was carried out with a gradient solvent program of methanol/water/acetonitrile (40:50:10 in the beginning), taking 40 min to reach 80% methanol, 10% water and 10% acetonitrile, returning after that in 10 min to the initial conditions. The flow rate was 1.0 mL/min and the injected volume of the samples varied from 10 to

50 µL. All chromatographic analysis was performed at 22 ºC.

Spectroscopy-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore). The solvents employed in the other analyses were of analytical-grade.

Extraction and fractionation procedures

Fresh fruits material (852.0 g) was extracted at room temperature sequentially with ethyl acetate and methanol for 48 h with each solvent. Extracts were filtered and concentrated under vacuum, yielding the ethyl acetate (4.5 g) and methanol (11.0 g) extracts. A portion of the ethyl acetate extract (1.2 g) was fractionated by gel permeation column chromatography (GPC) (Sephadex LH-20) using ethyl acetate as solvent at 0.5 mL/min flow rate. Forty-five fractions of 7 mL were collected. The fractions were combined according to their behavior on thin layer chromatography (TLC) and resulted in 9 samples: F1 (48 mg), F2 (45 mg), F3 (97.4 mg), F4 (93 mg), F5 (156 mg), F6 (185.6 mg), F7 (195.0 mg), F8 (292.5 mg) and F9 (78.5 mg). These fractions were submitted to HPLC-DAD-UV analysis and tested against *M. tuberculosis*.

The compounds employed as standards were isolated from *C. adamantium* leaves (compounds 1 to 5) and *C. pubescens* fruits (compound 6), and used in the form of methanol solutions. The standards used for HPLC analysis were purified until purity suitable for this purpose. The ethanol extract of the leaves from *C. adamantium* was partitioned with water/hexane (1:1 v/v) and water/ethyl acetate (1:1 v/v), getting the hexane and ethyl acetate phases. The ethyl acetate phase was fractionated by GPC (Sephadex LH-20) by elution with methanol/ethyl acetate isocratic system. The substances **1-5** obtained were further purified by column chromatography on silica gel eluted with toluene/ethyl acetate/ethanol gradient system or on RP 18 column eluted with water/acetonitrile/methanol gradient system. Compound **6** was obtained from the hexanic extract of *C. pubescens* fruits. This extract was fractionated by column chromatography on silica gel, using hexane/ethyl acetate/ethanol as gradient solvent system. The fraction containing the substance **6** was purified by another column chromatography on silica gel (toluene/ethyl acetate/ethanol gradient system). The structures of the flavonoids **1-6** were unambiguously determined by means of spectroscopic methods (^{1}H, ^{13}C and 2D NMR experiments COSY, HSQC, HMBC and NOESY) and compared to those previously reported[9-14]. Compounds **5** and **6** were also tested against *M. tuberculosis.* The ^{1}H and ^{13}C NMR results and peak attributions are given below.

7-hydroxy-5-methoxy-6-C-methylflavanone (**1**):[9,10] NMR ^{1}H (300 MHz; C_3D_6O) δ_H (*J*=Hz; H): 5.49 (*dd*, *J*= 3.0, 12.0; H-2), 2.97 (*dd*, *J*= 12.0, 18.0; H-3aq), 2.69 (*dd*, 3.0, 18.0; H-3eq), 6.35 (*s*, H-8), 7.55 (*dd*, H-2/H-6), 7.40 (*m*, H-3'/H-5'/H-4'), 2.05 (*s*, CH_3), 3.77 (*s*, OCH_3). NMR ^{13}C (75 MHz; C_3D_6O) δ_c: 78.8 (C-2), 45.5 (C-3aq/C-3eq), 187.6 (C-4), 160.1 (C-5), 112.5 (C-6), 162.1 (C-7), 98.7 (C-8), 162.5 (C-9), 106.8 (C-10), 139.7 (C-1'), 126.3 (C-2'/C-6'), 128.7 (C-3'/C5'), 128.5 (C-4'), 7.2 (CH_3), 60.2 (OCH_3).

5,7-dihydroxy-6-C-methylflavanone (**2**):[11] NMR ^{1}H (300 MHz; C_3D_6O) δ_H (*J*=Hz; H): 5.53 (*dd*, *J*= 3.0, 12.0; H-2), 3.14 (*dd*, *J*= 12,0, 18,0; H-3aq), 2.79 (*dd*, *J*= 3.0, 18.0; H-3eq), 6.07 (*s*, H-8), 7.56 (*dd*, H-2/H-6), 7.41 (*m*, H-3'/H-5'/H-4'), 1.97 (*s*, CH_3). NMR ^{13}C (75 MHz; C_3D_6O) δ_c: 78.4 (C-2), 42.3 (C-3aq/C-3eq), 196.0 (C-4), 161.0 (C-5), 103.6 (C-6), 164.7 (C-7), 94.4 (C-8), 160.3 (C-9), 101.7 (C-10), 139.0 (C-1'), 126.6 (C-2'/C-6'), 128.6 (C-3'/C5'), 128.6 (C-4'), 7.0 (CH_3).

5,7-dihydroxy-8-C-methylflavanone (**3**):[12] NMR ^{1}H (300 MHz; C_3D_6O) δ_H (*J*=Hz; H): 5.53 (*dd*; *J*= 3.0, 12.0; H-2), 3.15 (*dd*, *J*= 12.0, 18.0; H-3aq), 2.80 (*dd*, *J*= 3.0, 18.0; H-3eq), 6.08 (*s*, H-6), 7.56 (*dd*, H-2/H-6), 7.43 (*m*, H-3'/H-5'/H-4'), 2.00 (*s*, CH_3). NMR ^{13}C (75 MHz; C_3D_6O) δ_c: 79.0 (C-2), 42.9 (C-3aq/C-3eq), 196.0 (C-4), 161.6 (C-5), 94.3 (C-6), 164.2 (C-7), 104.0 (C-8), 160.7 (C-9), 102.1 (C-10), 139.3 (C-1'), 126.4 (C-2'/C-6'), 128.5 (C-3'/C5'), 128.5 (C-4'), 6.2 (CH_3).

2´,4´-dihydroxy-6´-methoxychalcone (**4**):[9,10] NMR ^{1}H (300 MHz; C_3D_6O) δ_H (*J*=Hz; H): 7.74 (*d*, *J*= 15.5; α), 8.01 (*d*, *J*=15.5; β), 6.01 (*d*; *J*= 3.0; H-3'), 6.09 (*d*, *J*= 3.0; H-5'), 7.73 (*dd*, H-2/H-6), 7.44 (*m*, H-3/H-5/H-4), 3.98 (*s*, OCH_3). NMR ^{13}C (75 MHz; C_3D_6O) δ_c: 128.5 (C-α), 142.6 (C- β), 193.1 (C- β'), 106.0 (C-1'), 168.9 (C-2'), 97.0 (C-3'), 166.0 (C-4'), 92.3 (C-5'), 164.0 (C-6'), 136.3 (C-1), 129.2 (C-2/C-6), 129.8 (C-3/C-5), 130.9 (C-4), 56.4 (OCH_3).

5,7-dihydroxy-6,8-di-C-methylflavanone (**5**):[13] NMR ^{1}H (300 MHz; C_3D_6O) δ_H (*J*=Hz; H): 5.39 (*dd*; *J*= 3.0, 12.0; H-2), 3.00 (*dd*, *J*= 12.0, 18.0; H-3aq), 2.77 (*dd*, *J*= 3.0, 18.0; H-3eq), 7.46 (*dd*, H-2/H-6), 7.35 (*m*, H-3'/H-5'/H-4'), 2.00 (*s*, CH_3-6), 2.01 (*s*, CH_3-8). NMR ^{13}C (75 MHz; C_3D_6O) δ_c: 79.6 (C-2), 44.0 (C-3aq/C-3eq), 197.5 (C-4), 163.9 (C-5), 104.8 (C-6), 158.7 (C-7), 104.1 (C-8), 159.9 (C-9), 103.1 (C-10), 140.2 (C-1'), 126.8 (C-2'/C-6'), 129.4 (C-3'/C5'), 129.2 (C-4'), 8.1 (CH_3-6), 7.4 (CH_3-8).

2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone (**6**):[14] NMR ^{1}H (300 MHz; C_3D_6O) δ_H (*J*=Hz; H): 7.98 (*d*, *J*=15.0, 7.0; α), 7.83 (*d*, *J*=15.0, 7.0; β), 7.64 (*dd*, H-2/H-6), 7.39 (*m*, H-3/H-5/H-4), 2.12 (*s*, CH_3-3'), 2.15 (*s*, CH_3-5'), 3.65 (*s*, OCH_3), (5.4 (*s*, OH-4'). NMR ^{13}C (75 MHz; C_3D_6O) δ_c: 126.7 (C-α), 142.9 (C- β), 193.4 (C- β'), 108.9 (C-1'), 162.0 (C-2'), 106.7 (C-3'), 159.4 (C-4'), 109.1 (C-5'), 158.8 (C-6'), 135.3 (C-1), 128.9 (C-2/C-6), 128.4 (C-3/C-5), 130.2 (C-4), 7.6 (CH_3-3'), 8.2 (CH_3-5'), 62.3 (OCH_3).

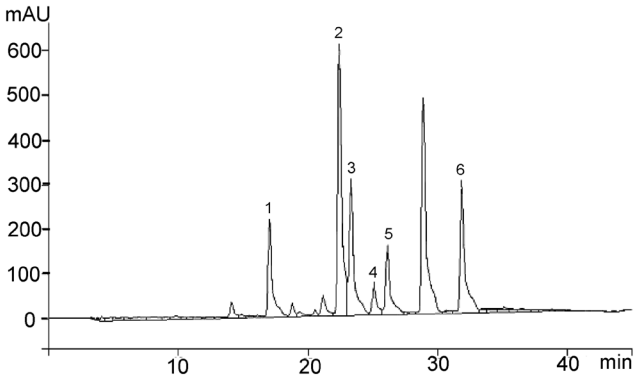

Figure 1. *Chromatography profile of ethyl acetate extract from the fruits of C. adamantium. Detection at 296 nm*

Quantification of flavanones and chalcones

The estimation of flavanones and chalcones content in the samples was performed by external calibration. The standards were dissolved separately in spectroscopy-grade methanol in order to obtain stock solutions, which were diluted into 5 concentrations for each one. Aliquots (50 µL) of all dilutions were analyzed by HPLC-DAD (n=3). For each standard, the mean areas under the chromatogram peaks, for the different concentrations, were plotted versus the concentration values. The equation parameters (slope and interception) of the linear least squares regression of the plots were used to obtain concentration values for the samples. The results are expressed as mg pure substance/g fraction (Table 1).

Stability study

The stability of the working standard solutions was tested at 22 ºC (working temperature) and –20 ºC (storage temperature). The stability

of relevant components in the samples was evaluated during all the storage steps (*i.e.* at room temperature and at –20 ºC). Good stability was defined as less than 2% loss of the initial drug concentration in the stated time.

Determination of anti-*M. tuberculosis* activity

The anti-*M. tuberculosis* activity of extracts and fractions was determined using the Microplate Alamar Blue Assay (MABA) as analytical method.[15] Stock solutions of the tested compounds were prepared in dimethyl sulfoxide and diluted in broth medium Middlebrook 7H9 (Difco), supplemented with oleic acid, albumin, dextrose, and catalase (OADC enrichment - BBL/Becton-Dikinson, Sparks, MD, USA), to obtain final drugs concentration ranges of 0.15 to 1600 µg/mL. The isoniazid were solubilized with distilled water according to the manufacturers' recommendations (Difco laboratories, Detroit, MI, USA) and used as standard drug. Suspensions of *M. tuberculosis* $H_{37}Rv$ ATCC 27294 were prepared so that their turbidities matched with standard scale McFarland no. 1. They were further diluted 1:25 in broth medium Middlebrook 7H9 (Difco) supplemented with OADC and added to each well of microtiter plate, together with the essayed compounds. Minimal inhibitory concentration (MIC) of these compounds necessary to inhibit 90% of growth of *M. tuberculosis* $H_{37}Rv$ ATCC 27294 were determined in triplicate in sterile 96-well plates (Falcon 3072; Becton Dickinson, Lincoln Park, NJ), by reading the fluorescence with excitation at 530 nm and emission at 590 nm in a SPECTRAfluor Plus (Tecan) microfluorimeter.[16] For standard test, the MIC value of isoniazid was determined each time.[16] The acceptable MIC of isoniazid ranges from 0.015 to 0.05 µg/mL.[16]

RESULTS AND DISCUSSION

The ethyl acetate and methanolic extracts from *C. adamantium* fruits and 9 fractions (F1-F9) of ethyl acetate extract were prepared as described in the Experimental, and analyzed by HPLC (Figure 1). Table 1 shows the identified compounds in the fractions of the ethyl acetate extract of *C. adamantium* fruits.

Identification and quantification of compounds were performed by NMR spectroscopy and HPLC analysis, respectively, resulting in: 7-hydroxy-5-methoxy-6-C-methylflavanone (**1**), 5,7-dihydroxy-6-C-methylflavanone (**2**), 5,7-dihydroxy-8-C-methylflavanone (**3**), 2´,4´-dihydroxy-6´-methoxychalcone (**4**), 5,7-dihydroxy-6, 8-di-C-methylflavanone (**5**), 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone (**6**), with purities of 93 to 97% (Figure 2 and Table 1).

HPLC analysis showed baseline separation for the compounds of interest. The identification of **1**-**6** was made by comparison with retention times of standards and by adding standards to the samples. A diode array detector was employed in the determination of compounds and in the evaluation of interference in all peaks of the chromatograms. Average standard errors for the peak areas of replicate injections (n=3) were smaller than 2%, showing good repeatability of the calibration curves. The peak areas were determined at different wavelengths, according to the compound: 284 nm for the species **1**, 296 nm for **2** and **3**, 350 nm for **4**, 299 nm for **5**, and 340 nm for **6**.

The anti-*M. tuberculosis* $H_{37}Rv$ activity was evaluated (Table 1) for extracts, fractions and compounds **5** and **6**. Tosun *et al*.[17] considered inactive the plant extracts that could not prevent growth of *M. tuberculosis* up to concentration of 200 µg/mL, and according to Gu *et al*.[18] a sample with MIC value ≤128 µg/mL is defined as active. In this sense, we considered a promising result the MIC of 62.5 µg/mL found in ethyl acetate crude extract. Within the 9 fractions from ethyl

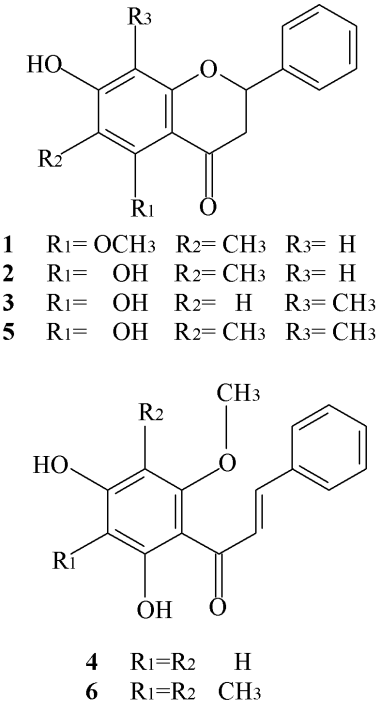

Figure 2. *Structures of the compounds identified in fractions of ethyl acetate extract from C. adamantium. Compounds: 7-hydroxy-5-methoxy-6-C-methylflavanone (***1***), 5,7-dihydroxy-6-C-methylflavanone (***2***), 5,7-dihydroxy-8-C-methylflavanone (***3***), 2´, 4´-dihydroxy-6´-methoxychalcone (***4***), 5,7-dihydroxy-6, 8-di-C-methylflavanone (***5***), 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone (***6***)*

acetate extract, F6 and F7 presented good inhibition activity on the growth of *M. tuberculosis*, with MIC of 39.1 µg/mL (Table 1).

The fractions F1 to F3, with MICs >250 and equals to 125 and 125 µg/mL, respectively, did not show any of the **1** to **6** quantified compounds. The fraction F4 showed 34.7 mg/g of the compound **6** (2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone) and a MIC of 125 µg/mL. The fractions F5, F6 and F7 showed a mixture of **6** and **5** (5,7-dihydroxy-6, 8-di-C-methylflavanone). The MIC of 62.5 µg/mL was verified for F5 and of 39.1 µg/mL for F6 and F7. The greater MIC value for F5 may be explained by the presence of compound **5** in lower concentration for F5 than for F6 and F7. The fractions F6 and F7 presented the best results against *M. tuberculosis* $H_{37}Rv$ ATCC 27294. Both contain the greatest quantities of compounds **5** and **6**, respectively 5,7-dihydroxy-6, 8-di-C-methylflavanone (**5**) and 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone (**6**), considered ensemble. The chalcone seems to have the greater activity, because F8, with high content of flavanone and less chalcone, has a MIC greater than F6 and F7.

Compounds **5** and **6** were tested separately and exhibited MIC >250 and equal to 62.5 µg/mL, respectively, while their mixtures, in several ratios, showed different MICs according to the ratio. A MIC of 62.5 µg/mL was verified for the mixture (5+6) in ratio 8:2, a MIC of 15.6 µg/mL for the ratios 3:7 and 1:1, a value of 31.25 µg/mL for the ratio 7:3 and the better activity, namely 7.8 µg/mL, for the ratio 2:8. It is clear the occurrence of a synergism between compounds **5** and **6** when mixed, especially in certain ratios.

The F9 showed a MIC of 62.5 µg/mL and this fraction contain a complex mixture of compounds from **1** to **4**, identified as: 7-hydroxy-5-methoxy-6-C-methylflavanone (**1**), 5,7-dihydroxy-6-C-methylflavanone (**2**), 5,7-dihydroxy-8-C-methylflavanone (**3**) and

Table 1. MIC values and identified compounds of bulk extracts and fractions of *C. adamantium* fruits. F1 to F9: fractions of ethyl acetate extract

Samples	MICs (μg/mL)	Compounds (mg/g of fraction)
methanol extract	1000	
ethyl acetate extract	62.5	
F1	> 250	ND
F2	125	ND
F3	125	ND
F4	125	**6** (34.7)
F5	62.5	**5** and **6** (43.7 and 247.3)
F6	39.1	**5** and **6** (123.7 and 330.0)
F7	39.1	**5** and **6** (147.9 and 290.3)
F8	125	**5** and **6** (349.0 and 147.6)
F9	62.5	**1**,**2**,**3** and **4** (53.7, 175.0, 60.4 and 12.3)
Compound **5**	>250	
Compound **6**	62.5	
Mixture **5** + **6** (2:8)	7.8	
Mixture **5** + **6** (7:3)	31.25	
Mixture **5** + **6** (1:1)	15.6	
Mixture **5** + **6** (3:7)	15.6	
Mixture **5** + **6** (8:2)	62.5	

ND= Not detected. Compounds: 7-hydroxy-5-methoxy-6-C-methylflavanone (**1**), 5,7-dihydroxy-6-C-methylflavanone (**2**), 5,7-dihydroxy-8-C-methylflavanone (**3**), 2´, 4´-dihydroxy-6´-methoxychalcone (**4**), 5,7-dihydroxy-6, 8-di-C-methylflavanone (**5**), 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone (**6**).

2´,4´-dihydroxy-6´-methoxychalcone (**4**).

In respect of the identified compounds, chalcones have been reported to possess many therapeutic properties, including anti-inflammatory, antimicrobial, antifungal, antioxidant, cytotoxic, antitumor and anticancer activities.[19] *M. tuberculosis*, *M. avium* and *M. bovis* are strongly inhibited by licochalcone obtained from *Glycyrrhiza inflata* (Fabaceae), with MICs in the range of 5-20 mg/L. A number of *Glycyrrhiza* species are used as throat demulcents.[20]

The literature reports that 2´,4´-dihydroxy-3´-5´-dimethy-6´-methoxylchalcone, isolated from *Dalea versicolor*, exhibit, alone and in synergy with known antibiotics (berberine, erythromycin and tetracycline), activity towards the human pathogen *Staphylococcus aureus* and the opportunistic *Bacillus cereus.*[20]

Salem and Werbovetz described leishmanicidal and trypanocidal activities of this same chalcone isolated from *Psorothamnus polydenius.*[14]

Lin *et al*.[21] synthesized and evaluated a series of flavonols, flavanones and chalcones for inhibitory activity against *M. tuberculosis* $H_{37}Rv$. The authors also relate that the presence of a halogen substituent on ring A of 2´-hydroxychalcone led to an increase in anti-tubercular activity. Compounds with a halogen at the 3-position demonstrate stronger anti-*M. tuberculosis* activity than those with a halogen substituent at the 2- or 4-position. Chalcones with a 2´-hydroxyl group in ring B and a 3-chloro- or 3-iodo-group in ring A have the highest activity, inhibiting 90 to 92% growth of *M. tuberculosis* $H_{37}Rv$ at a drug concentration of 12.5 mg/mL. The activity of 2'-hydroxychalcone (61% inhibition) was enhanced by adding a chlorine atom (89%) or a methoxy group (78%) to 4'-position of ring B. Introduction of an additional substituent, such as methoxy, amino, bromo or carboxyl groups, on ring B led to a dramatic decrease or a complete loss of activity.

According Cantrell *et al*.,[22] compounds that exhibit a MIC of 64 μg/mL or lower are considered promising. In this sense, MIC of 39.1 μg/mL for F6 and F7 and 62.5 μg/mL for F5 and F9 are very interesting, because these samples are enriched fractions, not isolated compounds. On the other hand, the mixture of pure compounds 5+6 in a ratio of 2:8 showed a very promising antitubercular activity with MIC of 7.8 μg/mL.

These MIC values are very larger than the MIC of 0.03 μg/mL of the reference drug Isoniazid, but are comparable or better than the MIC of pyrazinamide (20-100 μg/mL),[23] another first-line anti-tuberculosis drug.

In conclusion, the bioassay-directed fractionation of the ethyl acetate extract from *C. adamantium* fruits yielded fractions which promising anti-TB activity. The compounds identified within these fractions, namely, 7-hydroxy-5-methoxy-6-C-methylflavanone (**1**), 5,7-dihydroxy-6-C-methylflavanone (**2**), 5,7-dihydroxy-8-C-methylflavanone (**3**), 2´,4´-dihydroxy-6´-methoxychalcone (**4**), 5,7-dihydroxy-6, 8-di-C-methylflavanone (**5**), 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone (**6**), probably contribute for this activity against *M. tuberculosis*. The synergism between the compounds **5** and **6** depends on their concentration ratio.

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