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Artigo

In vitro anti-*Mycobacterium tuberculosis* activity of some Brazilian "Cerrado" plants

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RESUMO: "Atividade anti-*Mycobacterium tuberculosis in vitro* de algumas plantas do "Cerrado" Brasileiro". O objetivo deste trabalho foi realizar uma seleção de algumas plantas de uma determinada região Brasileira com atividade contra *Mycobacterium tuberculosis*. Extratos clorofórmicos e metanólicos de 37 espécies de plantas distribuídas em 17 famílias do "Cerrado" Brasileiro foram avaliadas contra *M. tuberculosis* $H_{37}Rv$ e a Concentração Inibitória Mínima (CIM) foi determinada pelo uso do Microplate Alamar Blue Assay (MABA). Extratos brutos de dezesseis plantas apresentaram valor de CIM ≤ 125 µg/mL e três de 31,2 µg/mL. Estes resultados sugerem que o "Cerrado" Brasileiro deve possuir um recurso de plantas com constituintes ativos anti-*M. tuberculosis* que podem ser extraídos por solventes polares e apolares.

Unitermos: Plantas do Cerrado Brasileiro, *Mycobacterium tuberculosis*, atividade anti-tuberculose, MABA.

ABSTRACT: The aim of this work was to carry out a screening of some plants of this Brazilian region with activity against *Mycobacterium tuberculosis*. Chloroform and methanol extracts of 37 plant species distributed among 17 families from Brazilian "Cerrado" which were tested against *M. tuberculosis* $H_{37}Rv$ and the Minimum Inhibitory Concentration (MIC) was determined by the use of Microplate Alamar Blue Assay (MABA). Crude extracts from sixteen plants showed MIC value of ≤ 125 µg/mL and three 31.2 µg/mL. These results suggest that the Brazilian "Cerrado" may be a source of plants that have activity anti-*M. tuberculosis* constituents that can be extracted by polars and apolars solvents.

Keywords: Brazilian "Cerrado" plants, *Mycobacterium tuberculosis*, antitubercular activity, MABA.

INTRODUCTION

Tuberculosis (TB) continues to be the leading cause of worldwide death due to an infectious agent. The rapid spread of multidrug resistant TB (MDRTB) strains around the world have showed the urgent need for the development of new TB drugs to shortening the duration of the treatment and the fight against MDRTB strains (Tripathi et al., 2005).

Natural products and/or their semi-synthetic derivatives can lead to novel antimycobacterial drugs and may have important roles in the chemotherapy of tuberculosis. Some recent reports have demonstrated the *in vitro* bioassay activity of plant-derived terpenoids against *M. tuberculosis* (Higuchi et al., 2008; Cantrell et al., 2001). The literature also reports the antimycobacterial activity of many classes of natural products: such as alkanes, phenolics, acetogenic quinines, flavonoids and triterpenes (Copp, 2003).

Having savannah as vegetation, Brazilian Central Cerrado, is one of the major biogeographical regions of the world with more than 7,000 native species of vascular plants (Mendonça et al., 1998). Many of these plants are commonly used as natural drugs by the people who live in the Cerrado area to treat several illnesses (Almeida et al., 1998), including some verbal relates for TB treatment. The aim of this work was to carry out a screening of some plants of this Brazilian region with activity against *Mycobacterium tuberculosis.*

MATERIAL AND METHODS

Plant materials

The fresh leaf, bark, fruits, chapter and scapes

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Table 1. Plants from Brazilian "Cerrado" and screening of MIC values of their chloroform and methanol extracts against *Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27264.

Plants	Plant Part	$CHCl_3$ MIC (µg/mL)	MeOH MIC (µg/mL)
Anacardiaceae			
Anacardium humile	Leaf	2000	500
Mangifera indica	Leaf	4000	2000
Apocynaceae			
Harcornia speciosa	Leaf	2000	4000
Bromeliaceae			
Ananas ananassoides	Leaf	2000	2000
Bromélia balansal	Fruits	2000	2000
Compositae			
Articun lappa	Leaf	NR	4000
Cucurbitaceae			
Wilbrandia ebracteata	Leaf	NR	2000
Cyperaceae			
Caesalpinia ferrea	Leaf	NR	4000
Dilleneaceae			
Curatella americana	Bark	62.5	500
Davilla elliptica	Leaf	62.5	4000
Davilla nitida	Leaf	125	2000
Eriocaulaceae			
Eriocaulon ligulatum	Scapes	1000	4000
Leiothrix flavescens	Scapes	125	2000
Syngonanthus artrothichus	Chapter	4000	NR
Syngonanthus artrothichus	Scapes	1000	NR
Syngonanthus macrolepsis	Scapes	1000	4000
Euphorbiaceae			
Alchornea glandulosa	Leaf	4000	4000
Alchornea triplinervia	Leaf	4000	4000
Leguminosae			
Indigofera suffruticosa	Leaf	1000	125
Indigofera truxilensis	Leaf	NR	500
Loganiaceae			
Strychnos pseudoquina	Leaf	125	4000
Malpighiaceae			
Byrsonima basiloba	Leaf	125	250
Byrsonima coccolobifolia	Leaf	NR	1000
Byrsonima crassa	Leaf	125	1000
Byrsonima crassa	Bark	2000	1000
Byrsonima fagifolia	Leaf	62.5	500
Byrsonima intermedia	Leaf	250	2000
Melastomataceae			
Miconia cabuku	Leaf	250	31.2
Miconia rubiginosa	Leaf	250	31.2
Mouriri pusa	Leaf	4000	2000
Nyctaginaceae			
Guapira noxia	Leaf	> 250	31.2
Neea theifera	Leaf	62.5	250
Simaroubaceae			
Quassia amara	Bark	250	NR
Vitaceae			
Cissus suscicaulis	Leaf	62.5	NR
Vochysiaceae			
Qualea grandiflora	Bark	62.5	1000
Qualea multiflora	Bark	125	500

(NR) - Not Realized
($CHCl_3$) - Chloroform Extracts.
(MeOH) - Methanol Extracts.

of plants were collected at Road of the Brejinho of Nazaré, State of Tocantins, Brazil and the species was identified by Dr. Eduardo Ribeiro dos Santos of Tocantins University. All voucher specimens were deposited at the Herbarium of the Universidade do Tocantins in Tocantins, Brazil.

Preparation of extracts and fractions

The air-dried and powdered leaves were exhaustively extracted with chloroform and methanol, successively at room temperature (48 h for each solvent). Solvents were evaporated at temperature of 60 ºC under reduced pressure to yield the chloroform extract and methanol extract.

Antitubercular activity assay

The antitubercular activity of crude extracts was determined using the MABA (Collins & Franzblau, 1997) as the analytical method. Stock solutions of the tested compounds were prepared in dimethyl sulfoxide

(Collins & Franzblau, 1997) and were diluted in Middlebrook 7H9 (Difco) broth supplemented with oleic acid, albumin, dextrose, and catalase (OADC enrichment - BBL/Becton-Dikinson, Sparks, MD, USA) to obtain final sample concentrations ranges of 0.15 to 1600 µg/mL. Isoniazid was solubilized with distilled water according to the manufacturers' recommendations (Difco laboratories, Detroit, MI, USA) and used as a positive control drug. *M. tuberculosis* $H_{37}Rv$ ATCC 27294 was grown for 7 to 10 days in Middlebrook 7H9 supplemented with OADC added of 0.05% Tween 80 to avoid clumps. Suspensions were prepared and their turbidities matched to a McFarland no. 1 (turbidity standard). After further dilution of 1:25 in Middlebrook 7H9 supplemented with OADC, the inoculum was added to each well of the 96 well microtiter plate (Falcon 3072; Becton Dickinson, Lincoln Park, NJ) together with the compounds. Samples were set up in triplicate. Cultures were in incubated for 7 days at 37 °C, and after additioned Alamar Blue for the reading. The minimum inhibitory concentration (MIC) was defined as the lowest concentration resulting in 90% inhibition of growth of *M. tuberculosis* (Collins & Franzblau, 1997) measuring the fluorescence (excitation/emission of 530/590 nm filters respectively) in a SPECTRAfluor Plus (Tecan) (Franzblau et al., 1998). For standard test, the MIC value of isoniazid was determined each time. The acceptable MIC of Isoniazid ranged from 0.015 to 0.05 µg/mL (Collins & Franzblau, 1997).

RESULTS AND DISCUSSION

The MIC values of 37 plant extracts against *M. tuberculosis* are shown in Table 1.

Tosun (2004) considered inactive the plant extracts that could not prevent growth of *M.tuberculosis* up to concentration of 200 µg/mL and according to Gu (2004) the MIC value of ≤128 µg/mL is defined as active against *M. tuberculosis*. From analyzed extracts, 12 chloroform extracts were considered promising and the MIC was ranged within 62.5 to 125 µg/mL. In these extracts, probably, the chloroform enabled the extraction of the apolar compounds, their lipophilic character allowed to penetrate the mycobacterial cell wall, determining the antimycobacterial activity. The terpenes are the apolar compounds frequently found within Cerrado plants and that anti-tuberculosis activity was extensively described (Copp, 2003; Aguiar et al., 2005). However, it was also verified prominent anti-*M. tuberculosis* activity in 4 methanol extracts with MIC value ranging from 31.2 to 125 µg/mL. The phenolic polar compounds and alkaloids with antimycobacterial activity are frequently isolated from Cerrado plants that also can pass through the cell wall of the bacteria (Aguiar et al., 2005).

These results suggest that the Brazilian "Cerrado" may be a source of plants that have active anti-*M. tuberculosis* constituents that can be extracted by polar and apolar solvents.

Further studies on isolation of bioactive fractions present in the active extracts of these plants will permit the establishment of a correlation between structure and antitubercular activity.

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