

Antimycobacterial activity of *Byrsonima crassa* Nied leaf extracts

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RESUMO: Atividade anti-micobacteriana de extratos de folhas de *Byrsonima crassa* Nied.

Neste estudo, avaliamos a atividade antimicobacteriana dos extratos cloroformico e metanólico de folhas de *Byrsonima crassa* Nied. A atividade antimicobacteriana foi determinada pela microtécnica denominada *Microplate Alamar Blue Assay* (MABA) e os princípios ativos promissores foram identificados por análise espectrofotométrica. O extrato cloroformico com Concentração de Inibitória Mínima (CIM) de 62,5 µg mL⁻¹, mostrou ser ativo contra o bacilo da tuberculose. O valor de CIM do extrato metanólico foi de 1000 µg mL⁻¹. Análise fotoquímica de extrato de cloroformico de *B. crassa* mostrou que esta atividade anti-micobacteriana pode estar relacionada com presença de triterpenos.

Palavras-chave: *Byrsonima crassa*, Malpighiaceae, atividade anti-micobacteriana, *Mycobacterium tuberculosis*, MABA

ABSTRACT: In this study, the antimycobacterial activity of chloroformic and methanolic extracts obtained from *Byrsonima crassa* leaves was evaluated. Antimycobacterial activity was assessed through the microtechnique named *Microplate Alamar Blue Assay* (MABA) and the promising active principles were identified by spectrophotometric analysis. The chloroformic extract presenting 62.5 µg mL⁻¹ minimum inhibitory concentration (MIC) showed to be active against tuberculosis bacillus. The MIC value of the methanolic extract was 1000 µg mL⁻¹. For the chloroformic one, phytochemical analysis indicated that antimycobacterial activity might be related to the presence of triterpenes.

Key words: *Byrsonima crassa*, Malpighiaceae, antimycobacterial activity, *Mycobacterium tuberculosis*, MABA

INTRODUCTION

Tuberculosis (TB) remains an important public health problem worldwide, accounting for 8 million new cases per year. Its infectious agent, *Mycobacterium tuberculosis*, kills approximately three million people every year in the world (WHO, 2007). Despite the improvements in chemotherapy, the epidemiology of TB is severely affected by the development of multi-drug resistance in *M. tuberculosis* strains.

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* activity of plant-derived terpenoids against *M. tuberculosis* (Cantrell et al., 2001). The literature also reports the antimycobacterial activity of many classes of natural products such as alkanes, phenolics, acetogenic quinones, flavonoids, and triterpenes (Copp, 2003).

The search for active compounds in plants of the huge Brazilian flora is the principal aim of our research. *Byrsonima* species (Malpighiaceae) are used in Brazilian folk medicine mainly for the treatment of gastric disorders, diarrhea and infections. Phytochemical investigations of *B. crassifolia*, *B. microphylla*, *B. verbascifolia*, and *B. crassa* have

revealed the presence of sulfonoglycolipids, steroids, triterpenes, aromatic esters, amino acids, proanthocyanidins, and flavonoids (Mendes et al., 1999; Gueiss et al., 1995; Sannomiya et al., 2004).

Despite the popular use of *B. crassa* as a medicinal plant, there are no published reports on the antimycobacterial activity of its leaf extracts. Thus, we evaluated the anti-TB activity and identified the compounds present in leaf extracts of this species.

MATERIAL AND METHOD

Plant material

B. crassa Niedenzu (IK) leaves were collected in Porto Nacional City, Tocantins State, Brazil, and identified by Dr. Eduardo Ribeiro dos Santos, from the Federal University of Tocantins. The voucher specimen (number 3377) was stored in the Herbarium of the Federal University of Tocantins.

Extraction and phytochemical analysis

The air-dried powdered leaves (2.0 kg) of *B. crassa* were completely and successively extracted with chloroform (CHCl_3) and methanol (MeOH) at room temperature (48h for each solvent). Solvents were evaporated at 60°C under reduced pressure, yielding CHCl_3 (53.8 g) and MeOH extracts (158.3 g). An aliquot of CHCl_3 extract (3.34 g) was applied to a silica gel column (32.0 x 3.0 cm i.d.), eluted with hexane followed by addition of EtOAc. Compounds were structurally identified through ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy. The NMR spectra in deuterated chloroform (CDCl_3) were obtained in a Varian INOVA 500 spectrometer, operating at 500MHz for ^1H and 150 MHz for ^{13}C . Chemical shifts were given in δ (ppm), and tetramethylsilane (TMS) was considered the internal standard. These data were compared with those reported in literature (Kundu & Mahato, 1994).

Plant extracts for determination of antibacterial activities

Stock solutions of *B. crassa* MeOH and CHCl_3 extracts (10 mg mL^{-1}) were prepared in dimethyl sulfoxide (DMSO) and diluted to the final concentrations of 1000; 500; 250; 125; 62.5; 31.25; and 15.63 $\mu\text{g mL}^{-1}$.

Standard drug

Stock solution of isoniazid (10 mg mL^{-1}) was prepared in distilled water and diluted to the final concentrations of 1.0; 0.5; 0.25; 0.125; 0.06; 0.03; and 0.015 $\mu\text{g mL}^{-1}$.

Mycobacterial strains

M. tuberculosis H37Rv ATCC 27294, used in

the antimycobacterial activity analysis, was maintained on Lowenstein-Jensen medium.

Microplate Alamar Blue Assay (MABA)

Anti-TB activity of extracts diluted in DMSO was determined against *M. tuberculosis* H37Rv ATCC 27294, using the microplate alamar blue assay (MABA) recommended by Collins & Franzblau, 1997. Isoniazid was used as the reference drug. The minimum inhibitory concentration (MIC) of the extracts needed to inhibit 90% of the mycobacterial growth was measured in sterile 96-well microplates (Falcon 3072; Becton Dickinson, Lincoln Park, NJ). The visual MIC was defined as the lowest drug concentration that prevented the color change of Alamar Blue reagent (Accumed International, Westlake, Ohio) from blue to pink. Blue color in the well was interpreted as no mycobacterial growth and pink color was scored as growth. For crude extracts, a MIC value of < 125 $\mu\text{g mL}^{-1}$ was defined as promising activity against *M. tuberculosis*, according to Gu et al. (2004).

RESULT AND DISCUSSION

In the antimycobacterial activity assay, *B. crassa* CHCl_3 extract had a promising MIC value of 62.5 $\mu\text{g mL}^{-1}$ (Table 1). MeOH extract showed a MIC of 1000 $\mu\text{g mL}^{-1}$ (Table 1). MABA for *M. tuberculosis* were complete by the 8th day of incubation and the MIC obtained for isoniazid (0.03 $\mu\text{g mL}^{-1}$) was similar to that found by Franzblau et al. (1998).

TABLE 1. MIC values of isoniazid and plant extracts, determined through microplate alamar blue assay (MABA).

Control and tested extracts	MIC ($\mu\text{g mL}^{-1}$)
Isoniazid	0.03
Methanol extract	1000
Chloroform extract	62.5

MIC = Minimal Inhibitory Concentration for mycobacterial growth inhibition

As regards phytochemical analyses, silica gel column chromatography of *B. crassa* CHCl_3 extract and identification of fractions through ^{13}C NMR spectra yielded the triterpenes lupeol, α -amyryn, β -amyryn, their acetates, and α -amyrenone (Figure 1).

The MIC of lupeol isolated from *Chuquiraga ulcina* (Argentina) was 64 $\mu\text{g mL}^{-1}$ (Wachter et al., 1999). For Coop (2003), secondary metabolites of terpenoid origin are among the most promising classes of natural products with antimycobacterial activity. A literature review on plant products demonstrated that terpenoids have moderate to significant biological activity against *M. tuberculosis* (Coop & Pearce,

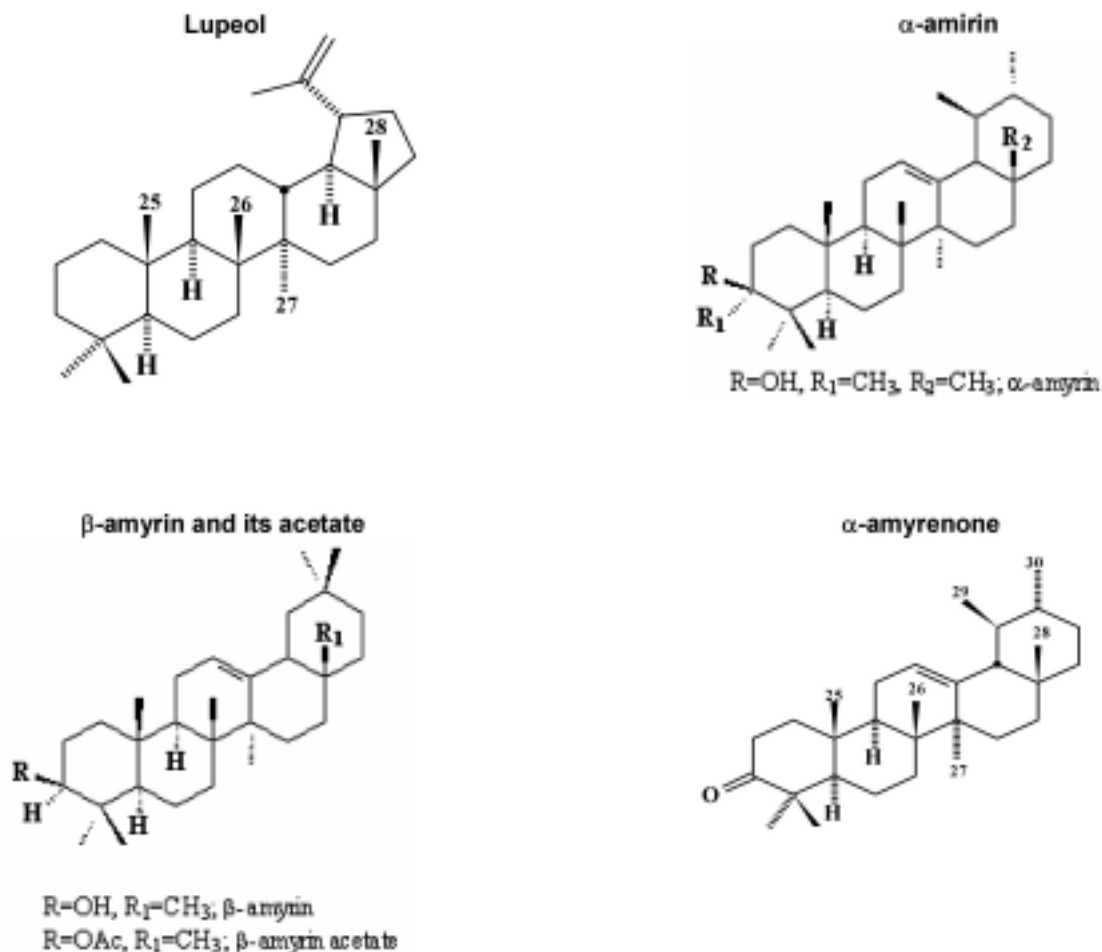


FIGURE 1. Lupeol, α-amyrin, β-amyrin, their acetates, and α-amyrone.

2007). That review covered active compounds in five major terpenoid groups: monoterpenes, sesquiterpenes, diterpenes, triterpenes, and phytol, its derivatives and structural analogs. According to Cantrell et al. (2001), isolated compounds that have MIC of 64 $\mu\text{g mL}^{-1}$ or lower are considered promising. For crude extracts, MIC should be equal to or lower than 125 $\mu\text{g mL}^{-1}$ (Gu et al., 2004). Thus, the MIC value of 62.5 $\mu\text{g mL}^{-1}$ determined here for *B. crassa* chloroform extract is a promising isolated compound.

In a previous study, Cardoso et al. (2006) reported that methanol leaf extract of *B. crassa* had mutagenic activity in *Salmonella typhimurium* TA 98; however, no mutagenicity was observed in its chloroform extract.

Our results suggest that the triterpenes in *B. crassa* leaves may be the compounds responsible for the observed antimycobacterial activities. The high lipophilicity of terpenes is probably one of the factors that allow them to penetrate the mycobacterial cell wall. In addition, the considerable activity of the chloroform extract against *M. tuberculosis* is probably due to the synergic action of the mixture of these identified terpenes.

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