

Bactericidal Effect of the Leaf Extract from *Musa* spp. (AAB Group, Silk Subgroup), cv. “Manzano” Against Multidrug-Resistant *Mycobacterium tuberculosis*

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ABSTRACT Air-dried leaves of a *Musa* spp. AAB, cv. “Manzano” plant, known as *Ja’as* in the Maya culture, were sequentially extracted with hexane, ethyl acetate, and methanol; the resulting extracts were investigated for their antimycobacterial activity against susceptible and drug-resistant strains of *Mycobacterium tuberculosis* (MTB) using the Microplate Alamar Blue Assay. Both the n-hexane extract (HE) and ethyl acetate extract (EE) showed potent activity against both strains of MTB, with the EE exhibiting the strongest activity and a Minimum Inhibitory Concentration of 12.5 and 6.25 µg/mL against susceptible and drug-resistant strains, respectively. Both extracts also demonstrated a mycobactericidal effect and a very good selectivity index when tested for cytotoxic activity on Vero monkey kidney cells, using the Sulforhodamine B assay. Our results demonstrate the efficiency and selectivity of *Musa* spp. AAB, cv. “Manzano” against MTB strains and support its traditional use as remedy against tuberculosis in Maya traditional medicine.

KEYWORDS: • antimycobacterial • banana • tuberculosis

INTRODUCTION

TUBERCULOSIS (TB) IS AN INFECTIOUS DISEASE caused mainly by *Mycobacterium tuberculosis* (MTB). TB is among the first 10 causes of death worldwide, and it is estimated that 1.7 billion persons have a latent TB infection. There are ~10 million new TB cases of TB worldwide and an estimated 1.6 million individuals died as a result of TB in 2017.¹ Inappropriate anti-TB treatments have led to the emergence of multidrug-resistant (MDR, resistant to at least first-line drugs isoniazid and rifampin), extensively drug-resistant (XDR, resistant to at least isoniazid, rifampin, one fluoroquinolone, and one of three injectable second-line drugs), and totally drug-resistant (TDR, resistant to all first- and second-line drugs) strains of MTB.^{1–3} Therefore, the

emergence of these strains urgently requires the development of novel, fast-acting, and highly effective anti-TB drugs.

Plants and plant products have long been used as treatment for disease conditions with symptoms of TB in different traditional medicines around the world. Medicinal plants have become subject to scientific investigations worldwide and their active components provide a potential alternative for the development of new drug candidates in the treatment of TB.^{4–6} Various components of the banana (genus *Musa*) plant have been used in traditional medicine for the treatment of health problems such as diarrhea, dysentery, intestinal lesions, diabetes, uremia, nephritis, gout, hypertension, cardiac disease, and TB.^{7,8}

We have recently reported that the leaf and rhizome extracts of *Musa* × *paradisica* var. Tabasco possesses activity against an MDR-MTB strain.⁹ On continuing with our search for anti-TB activity in banana, we wish to report the anti-TB activity of the leaf extracts of *Musa* spp. AAB, cv. “Manzano” when tested against susceptible and drug-resistant strains of MTB. The plant *Musa* spp. AAB, cv. “Manzano,” commonly known as *Ja’as* in the Maya culture, whose leaves are used to wrap “tamales” for cooking and serving, has also been documented for the use of its leaf sap¹⁰ and pseudostem sap^{11,12} for the treatment of TB. The remedy is prepared with the plant’s pseudostems and leaves, which are ground, squeezed, sweetened, and sneaked; its

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extract is refreshed overnight by the dew for human consumption on an empty stomach.¹³

The leaves of *Musa* spp. AAB, cv. “Manzano” were obtained in June 2017 from the Musaceae live collection maintained by “Centro de Investigación Científica de Yucatán” in the experimental fields of “Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias” in Uxmal, Yucatán, Mexico (20° 24' 27.72" Lat. N, and 89° 45' 06.66" Long. W, elevation 44.0 m above sea level).

The air-dried powdered leaves of *Musa* spp. ABB, cv. “Manzano” (15 g) were sequentially extracted with n-hexane, ethyl acetate, and methanol (3 × 150 mL), at room temperature by soaking the plant material for 24 h in an orbital shaker at 0.106 g. Each extract was filtered and evaporated the solvent *in vacuo*.

In vitro anti-TB activity was determined using the modified Microplate Alamar Blue Assay, carried out as previously described.¹⁴ Each of the extracts was dissolved with dimethyl sulfoxide (DMSO) and all the samples were tested using a concentration range of 200–1.56 µg/mL. The final concentration of DMSO in all assays was 2.5% or less, which is nontoxic for mycobacteria.¹⁴ The results are reported as the minimum inhibitory concentration (MIC). Rifampin or ofloxacin was included as positive controls. In addition, the uninoculated culture medium and DMSO were included as negative and solvent controls, respectively. All evaluations were carried out in three independent assays. Evaluation was performed utilizing two strains of MTB, that is, H37Rv ATCC 27294, susceptible (S-MTB) to all five (streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide) first-line anti-TB drugs, and the clinical isolate CIBIN 99 (MDR-MTB), known to be resistant to first-line drugs. Each of the previously mentioned strains was cultured in supplemented Middlebrook 7H9, and turbidity was adjusted to a McFarland No. 1 standard. This suspension was further diluted 1:50 with the same culture medium to obtain the test inoculum (~6 × 10⁶ colony-forming units/mL). In addition, the most active extracts (MIC of ≤25 µg/mL) were assayed for mycobactericidal activity using the method previously described in the literature.¹⁴

In vitro cytotoxic activity on the Vero cell line (ATCC CCL-8) was determined by the Sulforhodamine B method.¹⁵ Extracts were dissolved with DMSO and tested at a concentration range of 400–25 µg/mL. The concentration of DMSO (maximal 1%) did not affect the viability of the cells.¹⁶ The results were expressed as the concentration of extract that was lethal for 50% of the cells (median Cytotoxic Concentration [CC₅₀]). Docetaxel and the culture medium together with DMSO were utilized as positive and solvent controls, respectively. All evaluations were performed in three independent assays, and values were calculated using GraphPad Prism ver. 5 software.

The testing of all extracts for anti-TB activity demonstrated significant activity (MIC values of 6.25–50 µg/mL) (Table 1), in that crude extracts are considered to possess high potential when exhibiting MIC values below 100 µg/mL, moderate when values are 100 < MIC < 625 µg/mL, and low when the values are MIC > 625 µg/mL.¹⁷ Among the extracts,

TABLE 1. BIOLOGICAL ACTIVITIES OF LEAF EXTRACTS FROM *MUSA* SPP. AAB, CV. “MANZANO”

Leaf extracts	Anti-TB activity MIC µg/mL		Vero cells CC ₅₀ µg/mL
	S-MTB (SI)	MDR-MTB (SI)	
HE	25 (15.5)	12.5 (31.1)	388.50
EE	12.5 (7.2)	6.25 (14.4)	89.98
ME	50	25	>400
Rifampin	0.06	100	—
Ofloxacin	0.25	0.50	—
Docetaxel	—	—	1.68

Anti-TB: antituberculosis activity; CC₅₀, median cytotoxic concentration; EE, ethyl acetate extract; HE, n-hexane extract; MDR-MTB, *Mycobacterium tuberculosis* CIBIN 99 (resistant); ME, methanol extract; MIC, minimum inhibitory concentration; S-MTB, *M. tuberculosis* H37Rv (susceptible); SI, Selectivity Index defined as CC₅₀ Vero cells/MIC MTBs.

the strongest activity was shown by the ethyl acetate extract (EE), with MIC values of 12.5 and 6.25 µg/mL against the S-MTB and MDR-MTB strains, with the drug-resistant strain twice as susceptible to the EE than the susceptible strain. Testing of the most active extracts for mycobactericidal activity showed that the minimum bactericidal concentration values were identical to the MIC values. This is particularly relevant, because anti-TB agents with bactericidal activity are desirable to prevent relapse and reduce to the risk of developing resistance in MTB.¹⁸

Although plants used medicinally are widely assumed to be safe, many are potentially toxic. Toxicity studies help to ensure that the observed biological activity of the plant extract is not due to a general metabolic toxic effect. A good selectivity index (SI) is an indication of a large safety margin between the concentration of the extract that is able to kill the mycobacteria and the concentration that is toxic to mammalian Vero cells; in this case, a remedy or a candidate compound to be used medicinally must have an SI of ≥10.^{5,19} In this investigation, the two extracts with the strongest mycobactericidal activities revealed very good SI values (Table 1), even though the EE appeared to be more cytotoxic (CC₅₀ of 89.98 µg/mL) than the hexane extract (CC₅₀ of 388.50 µg/mL). It is noteworthy that the HE (n-hexane extract), with moderate mycobactericidal activity, exhibited the highest SI values in susceptible (SI = 15.54) and MDR (SI = 31.08) MTB strains.

The anti-TB activity of the HE (MIC = 12.5 µg/mL) and EE (MIC = 6.25 µg/mL) from leaves of *Musa* spp. AAB, cv “Manzano” is 50 and 100 times more potent than that recently reported for the hexane extract of the air-dried leaves of *Musa paradisiaca* var. Tabasco vs. the MDR-MTB strain.⁹ Even though leaf extracts from *M. paradisiaca*, *M. acuminata*, and *M. sapientum* have been reported to show antibacterial (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Citrobacter* spp., *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Enterococcus faecalis*, and *Staphylococcus aureus*), and leishmanicidal activities.^{20,21} To date, knowledge about the biological activity and phytochemical composition of *Musa* spp. AAB, cv. “Manzano” is limited or nonexistent.

With the emergence of drug-resistant strains of MTB, length of the therapy, the high levels of drug toxicity, and their various adverse effects, there is an urgent need to search for novel and more effective anti-TB agents. The use of medicinal plants, or natural plant products, for the treatment of TB represents an important option due to their use in the practice of traditional medicine and their large chemical diversity, respectively.²² Our results demonstrate the efficacy and selectivity of using the HE and EE of air-dried leaves of *Musa* spp. AAB, cv. “Manzano” against susceptible and drug-resistant strains of MTB.

In addition, our results support the traditional use of *Musa* spp. AAB, cv. “Manzano” to treat TB referred in Maya medicine.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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