

## Antitubercular activities of five Medicinal Plants against Two Strains of *Mycobacterium tuberculosis*

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### Summary

The antitubercular properties of aqueous methanol extracts of five plant were evaluated *in vitro* on Middlebrook 7H11 medium against two strains (clinical and H37Rv) of *Mycobacterium tuberculosis* simultaneously and observed for 12 weeks. Rifampicin and isoniazid were used as positive controls. Of the five plant extracts screened against *M. tuberculosis* H37Rv strain, *Hibiscus sabdariffa* (calyx) was the most active with MIC value of 3.125 mg/mL. The *M. tuberculosis* H37Rv strain was susceptible to rifampicin and isoniazid at MIC value of 0.04 mg/mL and  $5.0 \times 10^{-5}$  mg/mL, respectively. The aqueous methanol extract of *Hibiscus sabdariffa* (calyx), *Musa nana* (leaf) and *Psidium guajava* (stembark) had the highest inhibitory activity against *M. tuberculosis* clinical strain with an MIC of 0.025 mg/mL. An inhibition of growth by these plant extracts was observed in both *M. tuberculosis* clinical and H37Rv strains. This confirms their ethnomedicinal use of the plants for the management of tuberculosis and may be possible sources of discovery of anti-tuberculosis drugs.

Keywords: *Mycobacterium tuberculosis*; MTB H37Rv Strain; Medicinal plants; SW Nigerian ethnomedicine

## Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB) is a most common infectious disease and the fifth largest cause of mortality. Currently, there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* globally, including Nigeria.<sup>1</sup> One in 10 cases of TB infection has been reported to be resistant to treatment.<sup>2</sup> In addition, MDR-TB, resistant to rifampicin and isoniazid, now exceeds 0.5 million cases per year and in some areas accounts for up to 22% of TB cases<sup>3</sup>. Tuberculosis is the leading infectious cause of death among people with HIV/AIDS. It is estimated that TB kills someone approximately every 20 seconds — nearly 5,000 people every day<sup>4</sup>. This has generated an impetus for the urgent search for active molecules and/or structural prototypes for the effective management of TB from natural sources.

Plants have provided man's needs including shelter, clothing, food, flavours, fragrance and medicines<sup>5</sup>. There is still a heavy reliance of plants as sources of pharmaceutical drugs even in present day<sup>6</sup>. In Africa, plants are often exploited as remedies for management of diseases and infections including diarrhoea, dysentery, malaria, tuberculosis, bacterial and fungal infections and worm infestation in the various ethnomedicines<sup>7-12</sup>.

We have investigated Nigerian ethnomedicine for discovery of phytotherapeutic agents for the treatment of tropical infections and diseases<sup>9,13-15</sup>. As part of our continuing efforts, the present study reports the evaluation of antitubercular activities of Nigerian medicinal plants. This is in an effort to contribute to the plethora of natural compounds that will be investigated as possible leads for the discovery of compounds that could be used for treatment of tuberculosis.

## Materials and methods

### *Plant Collection and authentication*

Plant materials used for the study were collected

from Ibadan, Nigeria during the months of February and March. The plant parts were authenticated at the Forest Herbarium Ibadan (FHI), where voucher specimens were deposited.

### *Plant extraction*

Plant materials were air-dried under ambient conditions and ground into powdery forms with a hammer mill with a 5 KVA rotor. The powdered plant parts (200 – 300 g, respectively) were extracted by maceration in aqueous methanol (10:90) for 72 h at room temperature (28-32°C). Extracts were filtered and solvent removed in vacuum using a rotatory evaporator, thereafter crude extracts were stored in the refrigerator (4°C) until needed for analyses.

### *Mycobacteria*

*Mycobacterium tuberculosis* strains H37Rv and a clinical isolate were used in the present study. The MTB H37Rv strain is a drug sensitive reference strain. The clinical strain was isolated, identified and characterized in the TB Laboratory at Veterinary Public Health and Preventive Medicine Department, University of Ibadan, Nigeria, from a patient with advanced pulmonary tuberculosis. These organisms were preserved and maintained on Lowenstein-Jensen medium.

### *Media preparation*

Middlebrook 7H11 agar (21 g, Difco Laboratories) was dissolved in 900 mL of distilled water containing glycerol. The solution was autoclaved at 121°C for 15 min and cooled to 55°C, the solution was then made up to 1000 mL with Middlebrook ADC enrichment fluid (Difco). The prepared samples of plant extracts in 7H11 medium were transferred into screw-capped glass bottles and solidified in slants at 32°C.

### Inocula preparation and Antitubercular studies

*Mycobacterium tuberculosis* clinical isolate and H37Rv strain were cultured and grown respectively on Lowenstein-Jensen medium, then sub-cultured in Middlebrook 7H9 broth supplemented with ADC enrichment fluid and incubated at 37°C for 2-3 weeks. A colony of *M. tuberculosis* was taken with a sterile inoculating loop and the isolate was transferred into a sterile screw capped tube in 20 mL of Middlebrook 7H9 broth. The tube was placed on a shaker for 5 mins and broth was added and adjusted to McFarland standard no.1. The cultures were then diluted to 1/1000 to reduce the bacteria load. The culture (80 µL) was inoculated into the 7H11 medium containing plant extracts under a bio-safety hood. They were then incubated for twelve weeks at 37°C. The same procedure was used for the standard drugs, rifampicin and isoniazid. Experiments were done in triplicates. The minimum inhibitory concentration (MIC) defined as the lowest extract concentration at which no mycobacterial growth was observed and estimated for all the extracts and drugs.

### Results

The plants, their plant families, common names and voucher specimen numbers are listed in Table 1. Percentage yields of the plant extracts in the range of 5.2-17.9 % are shown in Table 2. The minimum inhibitory concentrations of the five plant extracts and drug against Mtb clinical isolate and MTB H37Rv strain after 12 weeks of incubation at 37°C are also displayed in Table 2. Inhibition of MTB clinical isolate was observed for five medicinal plants with *Hibiscus sabdariffa* (calyx), *Musa nana* (Leaf) and *Psidium guajava* (stem bark) possessing the least MIC value (0.025 mg/mL), indicative of strong activity. Four plants inhibited the growth of MTB H37Rv strain among which *H. sabdariffa* (calyx) had the highest activity with an MIC value of 3.125 mg/mL. The mycobacteria were susceptible to anti-tuberculosis reference drugs; rifampicin and isoniazid (controls), with MIC values of 0.01 mg/mL and < 0.00005 mg/mL and 0.04 mg/mL and < 0.00005 mg/mL, for

Mtb clinical strain and H37Rv strain, respectively.

### Discussion

In the Nigerian ethnomedicine, plants are frequently used in the management of tuberculosis. A compilation of such plants, used commonly in the management of the illnesses by the Ijebus of southwest Nigeria, has been documented in a previous study<sup>11</sup>. The *M. tuberculosis* clinical and H37Rv strains used in this study were susceptible to extracts of *H. sabdariffa*, *M. nana*, *A. boonei* (leaves) and *P. guajava* (stem bark) at different concentrations.

*Hibiscus sabdariffa* had the highest inhibitory activities against both *M. tuberculosis* clinical isolate and H37Rv strain with MIC values of 0.025 and 3.125 mg/mL, respectively. The extract of *H. sabdariffa* calyces has been reported to exhibit antibacterial activities (MIC 0.30 –1.30 mg/mL) against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia marsecences*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Pseudomonas fluorescens*<sup>16</sup>. The high potency of this plant against these bacteria gives scientific basis for its use in folk medicine in the treatment of cough<sup>17</sup>. Sharaf and co-workers also showed that both the aqueous extract and the coloring matter of the calyces of *H. sabdariffa* are lethal to *M. tuberculosis*<sup>18</sup>. It is interesting to note that the antimycobacterial activity of *H. sabdariffa* extract against the clinical strain of *M. tuberculosis* was replicated with the *M. tuberculosis* H37Rv. Hatil has also reported folkloric use of *H. sabdariffa* in the treatment of cough and as mild laxative<sup>19</sup>.

*Psidium guajava* (stem-bark) had inhibitory activity against the clinical isolate and H37Rv strain of *M. tuberculosis* with MIC of 0.025 mg/mL and 25 mg/mL, respectively. This data is in agreement with other studies which demonstrated inhibition of *Mycobacterium phlei*, *S. aureus*, *B. subtilis*, *Sarcina lutea* and *Aeromonas hydrophila* of the water, alcohol and chloroform extracts of *P. guajava* leaves, roots and stem bark<sup>20-21</sup>. Paranee reported a

lowering of the frequency of cough induced by capsaicin aerosol in rats and guinea pigs by 35 and 54% ( $P < 0.01$ ), respectively, when treated with water extract of *P. guajava* leaves at doses of 2 and 5 g/kg, p.o. within 10 min as compared to the control<sup>22</sup>. In addition, two compounds (morin-3-O- $\alpha$ -L-lyxopyranoside and morin-3-O- $\alpha$ -L-arabopyranoside) with antimicrobial activity were isolated from *P. guajava* leaves.<sup>23</sup>

*Allium cepa* had an MIC value of 6.25 mg/mL against Mtb clinical isolate. This is agreement with the findings of Adeleye who reported that the ethanol and water extracts of *Allium ascalonicum*, *A. cepa* at 50 mg/mL inhibited the growth of *M. tuberculosis*<sup>24</sup>. The aqueous extracts of *A. cepa* had anti-tuberculosis activity against two multi-drug resistant *M. tuberculosis* isolates and *M. tuberculosis* H37Rv strain and no activity against the rapid grower *M. fortuitum*<sup>25</sup>. The antimycobacterial agent in *A. sativum* has been identified as allicin<sup>26</sup>. The results of the antifungal activity of aqueous extracts prepared from *A. cepa* and *A. sativum* evaluated against some *Candida sp.* indicated that onions and garlic might be promising in treatment of fungal-associated diseases<sup>27</sup>.

Conclusively, the five plants have shown antitubercular properties using two strains of MTB and justify the traditional use of these plants in folk medicine against TB in the South west Nigeria. We are continuing with the identification of the active compounds responsible for the observed activities in the plant extracts in a bid to contribute to agents that could be used as agents for development of anti-tuberculosis drugs and the results will be presented in another communication.

### Acknowledgement

The study received financial support from the University of Ibadan Senate Research Grant SRG/COM/2006/1C.

see Table 1.

see Table 2.

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Botanical name	Family	Common name	Part	Voucher number
<i>Allium cepa</i> L.	Liliaceae	Onion	Bulb	FHI 107763
<i>Alstonia boonei</i> De Wild	Apocynaceae	Alstonia	Leaf	FHI 107826
<i>Hibiscus sabdariffa</i> L.	Malvaceae	Red sorrel	Calyx	FHI 107263
<i>Musa nana</i> L.	Musaceae	Dwarf banana	Leaf	FHI 108262
<i>Psidium guajava</i> L.	Myrtaceae	Guava	Stem bark	FHI 107824

Table 1: Percentage yields and MIC values of plant crude extracts using two strains of *Mycobacterium tuberculosis*

Botanical name	Part	Percentage yield of plant extracts (%)	MIC (mg/mL)	
			<i>M. tuberculosis</i> H37Rv	<i>M. tuberculosis</i> clinical isolate
<i>Allium cepa</i> L.	Bulb	13.47	>100	6.25
<i>Alstonia boonei</i> De Wild	Leaf	6.30	100	1.56
<i>Hibiscus sabdariffa</i> L.	Calyx	7.20	3.125	0.025
<i>Musa nana</i> L.	Leaf	5.16	50	0.025
<i>Psidium guajava</i> L.	Stem bark	11.82	25	0.025
*Rifampicin			0.04	0.01
*Isoniazid			< 0.00005	< 0.00005

Table 2: Percentage yields and MIC values of plant crude extracts using two strains of *Mycobacterium tuberculosis*. \*= Drug