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Priliminary anti-tuberculosis screening of *Acacia nilotica* L.: A Nitrogen fixing tree

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ABSTRACT

Tuberculosis is considered a re-emerging disease and one of the most important health problems worldwide. Present investigation was the first attempt and the main objective of this research was to test the effect of *Acacia nilotica* L. leaf and stem bark extracts against *M. tuberculosis* H_{37}Rv strain. Nitrate Reductase Assay (NRA) method was used to determine the MIC range of plant extracts against the mycobacterial strain. Both bark and leaves methanolic and ethanolic extracts of *Acacia nilotica* L. was observed to possess anti-tuberculosis activity. However, out of them leaves methanolic extract possessed highest antimycobacterial activity as determined by their respective MIC range (0.980 – 3.92 mg/ml).

Introduction

Tuberculosis (TB) is a contagious infectious disease mainly caused by Mycobacterium tuberculosis. It is an aerobic pathogenic bacterium that usually establishes its infection in the lungs (Ducati et al., 2006). TB is the leading cause of death worldwide from a single pathogen,claiming more adult lives than diseases AIDS, malaria, diarrhea, leprosy and all other tropical diseases combined (Zumla et al., 1998). 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). Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties (Heinrich et al., 2001). One of them, Acacia nilotica (L.) wild ex Del, a nitrogen fixing tree, belongs to the family Leguminosae commonly known as Babool or Kikar. It is a multipurpose legume tree, found in India, Australia and Africa.

The stem bark and leaves of Acacia nilotica L.(8 years old) were collected in April

2011 from Haatoj, Jaipur district, Rajasthan (India) .The specimen was authenticated by the department of Botany, University of Rajasthan and the voucher specimen (Voucher No. RUBL 20432) was deposited for future reference in the Botany Department Herbarium.

For the extraction plant samples were dried at room temperature and powdered via.mortar and pestle. Further, the plant samples were successively extracted with 80% ethanol (100 ml/ gm dry weight) on a water bath for 24 hrs (Subramanian & Nagarajan, 1969) ethanol and methanol using soxhlet apparatus. The solvents were evaporated using a rotary vacuum- evaporator at 50ºC. The extracts used for the detection of anti-TB activity.

The test mycobacteria strain was Mycobacteria tuberculosis (H37 RV) strain with (ATCC 27294), SMS Medical College, Jaipur, Rajasthan, India. Mycobacteria strains were rejuvenated on Lowenstein Jensen (LJ) slants for 14 days at 370C using standard procedures.

NRA tests are rapid and inexpensive and could be good alternatives than the conventional Proportion method (PM), resazurin microplate assay (REMA) in low-resources countries. Therefore for the detection of anti-TB activity of plant samples, NRA was performed as described by Angeby in 2002 with the slight modifications.The M.tuberculosis H37Rv strain (ATCC 27294) was cultured at 37°C in Lowestein-Jensenn medium until log phase growth; then a cell suspension was prepared at a concentration of about 2x106 UFC /ml and further diluted 1:20 in Middlebrook 7H9 (Becton Dickinson and Co., Sparks MD, USA) medium. The later was supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase). 100 µl of sterile medium was introduced in to well 2-11 row B-G of a sterile 96 well microtiter plate to each well. 100 µl of plant extract was added into 5th well of each row. Serial two fold dilution of plant extracts were prepared directly into micro titre plate.100 µl of inoculum was added into each media containing well. 200 µl of sterile water was added to all outer periphery wells to avoid evaporation during incubation. The plate was covered with its lid, replaced in the original plastic bag and incubated at 37° C under a normal atmosphere. After 7 days of incubation, 0.5 ml freshly prepared reagent mixture (one part 50% conc. HCl, 2 parts 0.2% sulphanliamide and 2 parts 0.1%N -1 naphthylethylenediamine di hydrochloride) as added in to control. The result was classified as negative, no color change or positive and depending on the color change, from 1+ (pink) to 4+ (Deep red to violet).

If the result in the control (drug free well) was at least 2+ positive, the drug containing well would developed color, otherwise the whole plate was reincubated and the procedure was repeated at day 10 , day 14 and finally at day 21. A strain

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was consider -ed resistant if the extract containing well produce a change in colour that was similar to or more intense then that in the control. A strain was considered susceptible, if there was no change in the extract containing well was less than that in control well. The NRA was easier to implement since it needs no additional material for laboratories.

Earlier only research reported on the antimycobacterial activity of methanolic stem bark extract of Acacia nilotica L. (Marrita et al., 2011), but they only shows the bacterial Numerical growth units (GUs) 19613 on the conc.1 mg/ml. They have not done experiment on low conc. But we, for the first time, show that a more refined anti-TB activity on low conc. 0.5 mg/ml (Table 1), however our article also reported foremost on the both leaves methanolic and ethanolic extracts. The data suggest that methanol and ethanol extracts from Acacia nilotica L. was observed to possess antimycobacterial activity; however, ethanolic extract possessed higher antimicrobial activity then methanolic in case of stem bark and it shows maximum in methanolic leaves extract than ethanolic leaves extract, as determined by their respective MIC range.

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Table 1: The MIC range values of ethanolic and methanolic extracts of *Acacia nilotica* L. against strain of *mycobacterium tuberculosis*

Alcoholic / Explants	MIC range (mg/ml) Ethanolic extract	MIC range (mg/ml) Methanolic extract
Bark	0.50 – 1.00	0.078 – 0.325
Leaves	0.23 – 0.85	0.980 – 3.92