



## Antitubercular activity of the dried fruits of *Acacia nilotica* Linn Willd

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### Abstract

Ethnobotanical survey revealed that the dried fruits of *Acacia nilotica* Linn Willd of the family mimosaceae are used locally in various parts of Nigeria for the treatment of boil, cough and chest infections. This prompted the phytochemical and antitubercular screening of its fruit extracts. Preliminary Antitubercular activity of its aqueous methanolic extract was carried out by the use of BCG as test organism. It showed antitubercular activity with MIC of 1250 $\mu$ g/ml. Gradient column chromatographic separation of the whole extract yielded six fractions (FR1-FR6). These fractions were evaluated for antitubercular activities. Fractions FR2, FR3 & FR4 showed significant antitubercular activities with MIC of 78 $\mu$ g/ml.

Phytochemical spray with ferric chloride solution showed that the active fractions (FR2-FR4) are mainly polyphenolics (tannins). The antitubercular activity of this extract is being reported for the first time. Further studies to isolate and characterize the bioactive compounds are ongoing.

**Key words:** *Acacia nilotica*, Tuberculosis, mycobacterium tuberculosis, Bioguided column fractionation.

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### Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* with a mortality of about three million people per year. Current drugs for this disease are Isoniazid, Rifampicin, pyrazinamide and ethambutol. These drugs were developed over 50 years ago and the problem of resistance and low therapeutic efficacy are common with these medications (1). The long therapy involved during treatment with these drugs also promotes non-compliance among patients. Hence the need to develop other compounds that could become useful therapeutic agents in the treatment of tuberculosis.

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*Acacia nilotica* is a medium sized tree which belongs to the family mimosaceae (2). It grows widely in the northern part of Nigeria. Ethnobotanical survey revealed that when the fruit is boiled in water, it serves as a cough medicine and it's taken for chest complaints (3). These folkloric applications in cough and chest infections which are closely associated with tuberculosis (4, 5) prompted current investigations. We report in this study, the antitubercular activity of the aqueous methanolic extract of the fruit of *Acacia nilotica* Linn Willd.

### **Materials and methods**

The fruits of *Acacia nilotica* Linn Willd were purchased from Suleja market and identified by NIPRD taxonomist. A voucher specimen (NIPRD/H/6134) has been deposited at NIPRD herbarium for future reference.

#### *Preparation of extracts and fractions*

20g of the pulverized fruit was macerated in 200ml 70 % v/v aqueous methanol for 24 hours at room temperature. The extract was vacuum filtered using whatmann filter paper no.4. The extract was concentrated using rotary evaporator under vacuum at 450 C. Phytochemical screening was performed by standard procedures (6) on the extract to determine the various secondary metabolites in the extract. Activated silica gel (50gm) was packed by wet packing method into a column. The extract (2.6g) which was adsorbed on silica gel (60-120 mesh) and dried was loaded on the column. Gradient elution was performed with 100 ml of each mobile phase mixture in a series. The elution was performed under gravity. The mobile phase consists of hexane, ethyl acetate, methanol and water starting with 100 % hexane and 10 % increment in the next more polar component. The final elution was performed with 70 % methanol in water until the loaded sample was exhausted. The eluates were monitored by thin layer chromatography using normal phase precoated silica gel KC 5 plates. The mobile phase consists of a mixture of Ethyl acetate: Hexane (7:3). The eluates were combined based on the similarity of TLC fingerprint to give six fractions (FR1-FR6).

#### *Evaluation for antitubercular activity.*

Determination of antimycobacterium activity was carried out on BCG strain using the method described by NIAID/NIH Washington, USA which is summarized below (7,8).

100mg of the extract was dissolved in 1ml Dimethyl Sulphoxide. The solution was centrifuged for 20 minutes at 13,000 rpm. A 1: 10 dilution of the extract in 7H9 middle brook broth was made. 50µl of media fraction was introduced into wells 2-12, while 100 µl of the extract was delivered into well 1. 50 µl of the extract was

transferred from well 1 to 2, after thorough mixing, 50  $\mu$ l was transferred from well 2 to 3. The process was repeated through to well 11. 50  $\mu$ l of inoculum was added to all wells and incubated for 14 days. DMSO was used as control.

## Results

Phytochemical screening of the extract showed the presence of alkaloids, saponins, tannins and carbohydrate. The percentage yield of the cold maceration extractive process was 35.6 %, while the chromatographic column fractions yields are stated in Table 1. On spraying with Ferric chloride solution, fractions FR2-FR4 showed blue black colorations.

The preliminary antitubercular activities of the aqueous methanolic extract and its

**Table 1. MIC determination of aqueous methanolic extracts and column fractions of *Acacia nilotica* Linn Willd fruits.**

SAMPLE	YIELD	MIC ( $\mu$ g/ml.)
Whole extract	35.6 %	1250
FR.1	0.0235g	1468
FR.2	0.648g	78
FR.3	0.301g	78
FR.4	0.773g	78
FR.5	0.227g	156
FR.6	0.499g	312

chromatographic fractions are as shown in table 1.

## Discussion and conclusion.

The antimycobacterium assay showed that the whole extract of *Acacia nilotica* had an MIC of 125  $\mu$ g/ml while the most active fractions (FR2-FR4) are 16 times more active than the crude extract with MIC of 78 $\mu$ g/ml. The prominent blue colorations shown in fractions 2-4 on spraying indicate the presence of polyphenolics which is most likely related to the presence of tannins reported from the phytochemical screening of whole extract. This class of compound is possibly responsible for the antimycobacterium activity of *Acacia nilotica* seeds. Some tannins such as epigallocatechin galloyl esters have been shown to have antimolluscicidal activity (9), but have not been reported previously to be associated with antimycobacterium activity.

Previous workers have shown the effects of the extract of *Acacia nilotica* on other pathogenic organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus Subtilis* and *Candida albicans* (10). These microorganisms are

implicated in the opportunistic infections associated with HIV/AIDS. This previous observation coupled with our findings that it has significant activity against *Mycocardium tuberculosis* further justify the relevance of this plant in the treatment of cough, chest infections and other related infections. Further studies are ongoing in our laboratory to isolate and fully characterize the bioactive compounds in this plant.

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