



Original Research Article

## Evaluation of some medicinal plants for anti-tuberculosis activity from Adamawa state, Nigeria

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### ABSTRACT

Tuberculosis kills approximately two million people annually. This study aimed at evaluating some selected medicinal parts that are used traditionally in Adamawa State, to treat tuberculosis and related symptoms against a microorganism that causes respiratory ailments. In this investigation, for new anti-tuberculosis lead molecules, ten medicinal plants species were selected and investigated for antimycobacterial activities following report of their therapeutic use in traditional medicine to treat infectious diseases such as tuberculosis. The stem-bark of the plants was extracted with methanol using soxhlet extractor. The crude extracts were screened for antimycobacterial activity against *Mycobacterium tuberculosis* using the broth microdilution method. The extract of *Anogeisus leiocarpus* showed strong activity at 0.312 mg/mL followed by *Boswellia dalzielii* with an activity of 0.625 mg/mL. Extracts of *Acacia tortilis*, *Bombax constantum*, *Ceiba pentandra* and *Fiscus platyphylla* showed activity at 1.25 mg/mL, 2.5 mg/mL, 2.5 mg/mL and 5.0 mg/mL, respectively. However, the extracts of *Echinaceae angustifolia*, *Fiscus trichopoda*, *Fiscus sycomorus* and *Isobertinia doka* did not exhibit any significant antimycobacterial activity. The results from this study indicated that these six plants are viable potential sources of products active against *Mycobacterium tuberculosis*. This report also demonstrated the efficacy of Nigerian medicinal plants as potential agents in treatment/management of tuberculosis and related symptoms. The results have also validated traditional knowledge from the local people regarding the use of these plants to treat tuberculosis. Therefore, the six plants have potential to be developed on new anti-tuberculosis drugs.

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### 1. Introduction

Tuberculosis is a common infectious disease caused by various strains of *mycobacteria*, usually *Mycobacterium tuberculosis*. Initially, tuberculosis was called *phthisis* or *phthisis pulmonalis* and in most cases the disease is lethal to the infected victims. Tuberculosis is usually abbreviated as TB which stands for *Tubercle bacillus*, the bacillus causing tuberculosis (Kumar et al., 2007; Abanda et al., 2017; Adams, 2017; Akanbi et al., 2017; Allinson et al., 2017).

Tuberculosis is the second most common cause of

death among the infectious diseases after HIV/AIDS (Snider et al., 1994). The *M. tuberculosis* usually attacks the lungs and can also attack other parts of the body like kidney, spine and brain. *M. tuberculosis* is spread from one person to another through air that contains droplets generated by cough, sneeze or spits of a person infected with the bacilli (Konstantinos, 2010).

Tuberculosis has been present in humans since prehistoric time (Rothschild et al., 2001). However, it was not certain whether tuberculosis originated in bovines and then it passed on to humans or whether it diverged from a common ancestor

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(Pearce-Duvel, 2006). However, a comparison of the genes of *Mycobacteria tuberculosis* complex (MTBC) in humans to MTBC in animals proves that humans did not acquire tuberculosis from animals during animal domestication as was previously believed. Other organisms that are responsible for *M. tuberculosis* complex are *M. bovis* and *M. africanum* (Ansha et al., 2008). TB can cause infections that mimic other diseases such as pneumonia, neoplasm or fungal infections (Hamza et al., 2006). An infected person may be symptomatic with signs of pulmonary and other organs involvement (Kirimuhuzya et al., 2009). Treatment of TB requires antimicrobial drugs that can kill the microscopic pathogens such as rifampicin and isoniazid.

Tuberculosis is more common in developing countries. About 80% of the population in many Asian and African countries test positive in tuberculin tests. Xpert MTB/Rif has recently been recommended for diagnosis of TB (Lebina et al., 2016). Till date, only BCG vaccine is available which is ineffective against adult pulmonary TB, which is the most common form of disease. Various unique antibodies have been developed to overcome drug resistance, reduce the treatment regimen, and elevate the compliance to treatment. Therefore, we need an effective and robust system to subdue technological drawbacks and improve the effectiveness of therapeutic drugs which still remains a major challenge for pharmaceutical technology (Mohammad et al., 2017). Hopes of totally controlling the disease have been dramatically dampened because of a number of factors, including the difficulty of developing an effective vaccine, the expensive and time-consuming diagnostic process, longer period of treatment, the increase in HIV-associated tuberculosis, and the emergence of drug-resistant TB strains in the 1980s (Lawn and Zumla, 2011). The recurrent resurgence to natural products chemistry as a major contributor to drug discovery process lies in the screening of biodiversity for new chemicals entities with novel biological activities (Rai and Gupta, 2006). Natural products research remained a valid and successful approach to drug discovery, especially when supplemented with the wealth information available through ethnobotanical and ethnopharmacology knowledge of the plants. This invasive and burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in the health care (Koehn and Carter, 2005).

Medicinal plants are plants with medicinal activity based on some ethnobotanical information. These plants have been used in the treatment of many diseases (Akiniyi and Tella, 1991) and clinical success of drugs obtained from plants has rekindled interest in research into medicinal plants as potential sources of new drugs. In some countries like China, India and Vietnam the research on medicinal plants has been

fully developed, and plant-based remedies have been incorporated as alternative or complementary medicines to supplement the modern drugs (Ogundaini, 2005).

There were several plant extracts that demonstrated significant inhibitory activity against microscopic pathogens like bacteria, fungi and viruses (Kone et al., 2004; Addy, 2005; Kubmarawa et al., 2007; Tanaka et al., 2010; Mohammadhosseini et al., 2017; Mohammadhosseini, 2017a; Mohammadhosseini, 2017b). These pathogens were responsible for many deadly infectious diseases like leprosy, HIV and tuberculosis. Some medicinal plants were used in folkloric medicine to treat respiratory diseases including symptoms of tuberculosis such plants may contain bioactive agents that are responsible for the observed therapeutic potentials (Uba et al., 2003; Gupta et al., 2010; Mann et al., 2007; Knoll et al., 2017; Long et al., 2017; McCulloch and Lin, 2017; Simkins et al., 2017; Yeo et al., 2017).

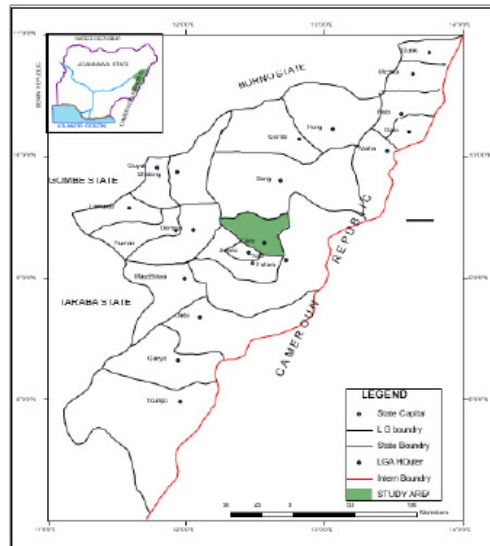
Tuberculosis is one of the oldest diseases in the world that remains a serious health concern globally. It attacks children and adults as well as privileged and less privileged persons. In fact, it cut across all segment of the population (Manchester, 1984). The treatment and management of tuberculosis with the already existing drugs has remained unsatisfactory due to multi-drug resistant of the microscopic pathogens which causes the disease. Therefore, research into medicinal plants for new and more potent drugs against the causative agent of tuberculosis has become inevitable.

The main aim of this research was to evaluate some medicinal plants for anti-tuberculosis activity with a view to identify the bioactive agents which can be used as drugs or drug leads in tuberculosis drug discovery.

## 2. Experimental

### 2.1. Collection and identification of the plants

Stem bark of the plants were collected from Girei Local Government area of Adamawa State, Nigeria (Fig. 1). The freshly gathered plants parts were conveyed in polyethene bags to the Modibbo Adama University of Technology, Yola and identified by a taxonomist who scientifically identified and classified the plants. Voucher specimens were prepared, labeled and deposited in the Herbarium of Botany Department, Modibbo Adama University of Technology, Yola namely: *Anogeisus leiocarpus* UHNP 2074, *Boswellia dalzielii* UHNP 2078, *Acacia tortilis* 2079, *Bombax constantum* UHNP 2138, *Celba pentandra* UHNP 2143, *Fiscus platyphylla* UHNP 3146, *Echinaceae angustifolia* UHNP 2149, *Fiscus trichopoda* UHNP 3147, *Fiscus sycomorus* UHNP 3148 and *Isoblerlinia doka* UHNP 2155, respectively. The photographs of the ten undertaken plants are shown in Fig. 2 and Fig. 3.



**Fig. 1.** Map of Adamawa state showing Girei local government.



Plant 1: *Acacia tortilla*



Plant 2: *Anogeissus leiocarpus*



Plant 3: *Bombax costatum*



Plant 4: *Boswellia dalzielii*



Plant 5: *Ceiba pentandra*



Plant 6: *Echinaceae angustifolia*

**Fig. 2.** The photographs of the studied plants (1-6).

Plate 7: *Ficus platyphylla*Plate 8: *Ficus sycomorus*Plate 9: *Ficus trichopoda*Plate 10: *Isoberlinia doka***Fig. 3.** The photographs of the studied plants (7-10).

## 2.2. Drying and pulverizing

The plants samples were dried under shade inside a room to avoid direct sunlight that could degrade some of the bioactive compounds in the plants parts. The parts were spread out and turn regularly to avoid fermenting and rotting. The well dried parts were then pulverised to a fine powder using mortar and pestle. The powders of each sample were weighed using analytical balance and kept at room temperature.

## 2.3. Extraction procedure

The weighed powdered of each samples were extracted using methanol in a Soxhlet extractor for 12 h. The extracts of each sample were filtered and concentrated at temperature of 40 °C and pressure of 55 mmHg using a rotary evaporator to obtain the crude extract.

## 2.4. Extract preparation

100-mg portion of each extract was dissolved in 1.0 mL of DMSO and centrifuged at  $13,000 \times g$  for 15 min. The centrifugate was further diluted 1:10 by dispensing 50  $\mu$ L of the DMSO concentrate into 450  $\mu$ L sterile 7H9/ADC broth to give a final concentration of 10 mg/

mL. Accurate weight of extracts was determined on a Mettler AB54 balance.

## 2.5. Antimycobacterial assay

The antituberculosis activity of the plant extracts was assessed using the broth microdilution method as described by [Oladosu et al. \(2013\)](#) to determine the minimum inhibitory concentration (MIC) for each extract.

## 2.6. Organism preparation

500  $\mu$ L of freshly-thawed stock of the test organism *Mycobacterium bovis* (BCG) was inoculated into 50 mL of sterile Middlebrook 7H9/ADC broth medium and incubated at 30 °C for 5-7 days. As determined using a UV spectrophotometer, the optical density (OD) of the resulting culture at 650 nm was approximately 0.2, equivalent to 109 CFU/mL.

## 2.7. Sample screening for antituberculosis (anti-Tb) activity

The microbroth dilution method was employed for the determination of anti-Tb activity of the extracts ([Oladosu et al., 2013](#)). Into each well of columns 2 to 12 of a 96-micro well plate, 50  $\mu$ L of sterile 7H9 broth

was transferred. To each of the well 1, 100  $\mu$ L of 10% DMSO was added. This step was followed by addition of 100  $\mu$ L of 25  $\mu$ g/mL solution of rifampicin and 100  $\mu$ L of each plant extract. It should be mentioned that rifampicin served as the positive control drug throughout the experiment. The solution was prepared by dissolving 250 mg of rifampicin powder in 10 mL DMSO and diluted 1: 1000 by dispensing 25  $\mu$ L of the stock rifampicin solution into 25 mL 7H9 Middlebrook broth.

Using a multichannel pipette, 50  $\mu$ L was carefully removed from well 1 to 2, with well 2 mixed thoroughly, and the process was continued up to well 11, from which 50  $\mu$ L was then withdrawn and discarded. The wells were inoculated with 50  $\mu$ L of diluted BCG culture and incubated at 30 °C for a period of seven days. After the incubation period the wells were stained with tetrazolium dye. The reduction of tetrazolium salt from a colorless or weakly-colored compound to a brightly-colored derivative in the wells is an indication of lack of activity by the tested sample, whereas activity is confirmed if the dye remains colorless.

The concentration of a test extract in the last well in which there has been no growth, out of all wells containing the extract (counting from the highest concentration used), is regarded as the MIC of the extract.

### 3. Results and Discussion

#### 3.1. Ethnobotanical uses of the studied plants

*Acacia tortillis*: The stem bark of the plant is traditionally used to treat cough. The stem bark is chewed for cough remedy (Kubmarawa et al., 2013).

*Anogeissus leiocarpus*: The plant is used for the treatment of diabetic ulcers, general body pain, blood clots, asthma, coughing and tuberculosis (Barku et al., 2013). It also possesses considerable anthelmintic properties (Waterman et al., 2010).

*Bombax costatum*: The roots of these plants are used to treat tuberculosis. Decoction of the aerial plants is taken regarding its anti-tuberculosis characteristics (Kubmarawa et al., 2013).

*Boswellia dalzielii*: This plant is a tree plant very popular among the locals as a potent source of ethnomedicine. The extract from its leaves is used for the treatment of diarrhea in poultry. The root decoction is used for wound healing (Etuk et al., 2006). The fresh bark is eaten to induce vomiting and relieve symptoms of giddiness and palpitations. The root decoction of the plant boiled along with *Hibiscus sabdariffa* is used for the treatment of syphilis. The fragrant gum resin from the plant is used locally for fumigation of clothes and houses and as a deodorant (Etuk et al., 2006).

*Ceiba pentandra*: The aerial part of *C. pentandra* has been traditionally prescribed to treat chest pain.

Decoction of the aerial plants is taken orally for chest pain. The combination of aerial parts of *C. pentandra*, *F. cycomorus* and *J. curcas* is taken for tuberculosis (Kubmarawa et al., 2013).

*Echinaceae angustifolia*: This plant is used traditionally to treat cough and malaria. Infusion of the corresponding stem bark is taken for cough and malaria (Kubmarawa et al., 2013).

*Ficus platyphylla*: The stem bark of *F. platyphylla* is used traditionally to treat malaria in different countries of African continent (Nadembega et al., 2011). They have also found effective in the treatment of tuberculosis (Kubmarawa et al., 2007). The extracts of *F. platyphylla* have also been reported to inhibit gastrointestinal motility (Amos et al., 2002).

*Ficus sycomorus*: The leaves are used in Hausa ethnomedicine of northern Nigeria to treat dysentery (Hutchinson and Dalziel, 1954; Ahmadu et al., 2007). The stem bark and leaves are also used in treating diarrhea, cough and chest conditions in South Africa (Anthony and Olubunmi, 2014).

*Ficus trichopoda*: The aerial parts are used to treat tuberculosis and pile. The ground aerial parts of *F. richopoda* are taken with water for tuberculosis. The infusion can also be taken for tuberculosis. The paste of the aerial part is applied on pile (Kubmarawa et al., 2013).

*Isobertinia doka*: The tree has been used by traditional medical practitioners for the treatment of diabetes, ulcer, wounds and cough (Abdulkadir et al., 2011).

#### 3.2. Evaluation of antimycobacterial activity

The result of the antimycobacterial activity of the ten plants are shown in Table 1. The results concerning the antimycobacterial activities of the crude extracts of the ten plants revealed that only six plants, namely *Anogeissus leiocarpus*, *Boswellia dalzielii*, *Acacia tortilis*, *Bombax constantum*, *Ceiba pentandra* and *Fiscus platyphylla* inhibit the growth of the bacterium with minimum inhibitory concentration (MIC) within the range of 312 mg/mL to 5.0 mg/mL. Extract of *Anogeissus leiocarpus* showed strong activity at 0.312 mg/mL followed by *Boswellia dalzielii* with activity of 0.625 mg/mL. Extracts of *Acacia tortilis*, *Bombax constantum*, *Ceiba pentandra* and *Fiscus platyphylla* showed activity at 1.25 mg/mL, 2.5 mg/mL, 2.5 mg/mL and 5.0 mg/mL, respectively. On the other hand, extracts of *Echinaceae angustifolia*, *Fiscus trichopoda*, *Fiscus sycomorus* and *Isobertinia doka* did not exhibit any significant antimycobacterial activity. The result of the antimycobacterial activity of *Anogeissus leiocarpus* agreed with that of Mann et al. (2009) who reported that the MIC of *Anogeissus leiocarpus* being 0.312  $\mu$ g/mL. In addition, the result of the antimycobacterial activity of *Boswellia dalzielii* agreed with that of Nvau et al. (2011) who reported

**Table 1**

Antimycobacterial activity of the plant extracts concentrations (mg/mL).

Plant species	1 (5)	2 (2.5)	3 (1.25)	4 (0.625)	5 (0.312)	6 (0.156)	7 (0.07)	8 (0.03)	9 (0.015)	10 (0.007)	11 (0.0035)	12 (OVC) <sup>a</sup>
<i>A. tortilis</i>	- <sup>b</sup>	-	-	+ <sup>c</sup>	+	+	+	+	+	+	+	+
<i>A. leocarpus</i>	-	-	-	-	-	+	+	+	+	+	+	+
<i>B. costatum</i>	-	-	+	+	+	+	+	+	+	+	+	+
<i>B. dalzielii</i>	-	-	-	-	+	+	+	+	+	+	+	+
<i>C. pentandra</i>	-	-	+	+	+	+	+	+	+	+	+	+
<i>E. angustifolia</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>F. platyphylla</i>	-	+	+	+	+	+	+	+	+	+	+	+
<i>F. sycomorus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>F. trichopoda</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>I. doka</i>	+	+	+	+	+	+	+	+	+	+	+	+
Rifampicin (25 µg/mL)	-	-	-	-	-	-	-	-	-	+	+	+

<sup>a</sup>Well 12 served as Organism viability control (OVC)<sup>b</sup>- = Active, inhibition of organism, (Rifampicin mic = 0.04-0.09 µg/mL)<sup>c</sup>+ = No active (Rifampicin mic = 0.04-0.09 µg/mL)

that the MIC of *Boswellia dalzielii* was 5000 µg/mL. The results have validated traditional knowledge from the local people regarding the use of these plants to treat tuberculosis and its related symptoms. The present study demonstrates that Nigerian plants could be good sources of compounds with antimycobacterial activities worthy to be investigated. Furthermore, the column chromatographic investigation of the most active extract is currently being carried out to isolate and characterize the most active compound(s).

#### 4. Concluding remarks

The results of this study have shown that there is a potential to develop compounds against *Mycobacterial tuberculosis* from *Anogeisus leiocarpus*, *Boswellia dalzielii*, *Acacia tortilis*, *Bombax constantum*, *Celba pentandra* and *Ficus platyphylla*. The findings of this work validate the potential use of the studied plants in the treatment of tuberculosis by traditional medicine practitioners and further emphasizes that there is a strong positive correlation between the anti-mycobacterial activity results and traditional knowledge on plants used for Tuberculosis and related diseases in ethnomedicine. This study further develops the pharmaceutical prospects of these plants.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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