

***In vitro* Inhibition of *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* by Some Nigerian Medicinal Plants**

A. UBA^{1*}, K. IBRAHIM², E.B. AGBO¹ AND A.A. MAKINDE³

¹ *Biological Sciences Programme (Microbiology Unit), Abubakar Tafawa Balewa University, P. M. B. 0248, Bauchi, Nigeria.*

² *Department of Pharmaceutical Microbiology and Biotechnology, National Institute of Pharmaceutical Research and Development, P. M. B. 21, Garki, Abuja, Nigeria.*

³ *Dermatophilosis and Diagnostics Unit, National Veterinary Research Institute, Vom, P. M. B. 001, Jos, Plateau State, Nigeria.*

Some Nigerian plants used in traditional medicine to treat tuberculosis and/or some of its symptoms were screened for *in vitro* activity against *Mycobacterium smegmatis* and a clinical isolate of *Mycobacterium tuberculosis*. Only 3 of the 6 crude methanolic extracts of the 6 plant species exhibited inhibitory activities against *M. smegmatis*, while 5 inhibited the growth of *M. tuberculosis*. Three and four water extracts inhibited *M. smegmatis* and *M. tuberculosis*, respectively. Both methanol and water extracts of *Artemisia annua*, *Pterocarpus erinaceus* and *Piper guiniense* showed inhibitory activities against the two *Mycobacteria*. Methanol extracts of *Anogeissus leiocarpus* and *Piper guiniense* exhibited the highest activity against *M. tuberculosis* with a minimum inhibitory concentration of 0.1 mg/ml.

Keywords: Nigerian medicinal plants, *Mycobacterium* species, Inhibition

INTRODUCTION

Tuberculosis (TB) remains a serious public health problem in many regions of the world, especially the developing countries. Globally, approximately 8-10 million individuals develop clinical TB and about three million people die of the disease each year [1]. The annual incidence of the disease in the world is increasing [1,2]. Individuals infected with HIV are very susceptible to TB, and develop the disease before other manifestations become apparent. The HIV-tuberculosis co-infection has caused an impact on tuberculosis epidemiology all over the world in that the efficacies of the therapeutic schemes traditionally prescribed in the treatment of tuberculosis, such as isoniazid, rifampicin and pyrazinamide, have decreased due to the appearance of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains [3].

The control and prevention of the TB epidemic will depend largely on adequate treatment, and possibly on effective chemoprophylaxis [4]. Multi-drug resistant tubercle bacilli have emerged to various anti-TB drugs such as isoniazid, ethambutol, rifampicin and streptomycin [5,6] and, resistance to such drugs has been reported by the WHO to be on the

increase [1]. Drug resistance by *Mycobacterium tuberculosis* and other atypical mycobacteria pose serious problems to local and global TB control programmes. Resistant TB is very difficult to treat and requires more and different medications for a longer period of treatment and it is also more expensive and not as successful [2]. Therefore the search for novel antibacterial agents active against mycobacteria is made very urgent.

Plants, being a biologically and chemically diverse resource that constitutes an effective source of both traditional and modern medicine, may provide important leads to the development of antimicrobial agents active against MDR tubercle bacilli. Previous studies indicated that a number of plants contain substances that possess antimycobacterial activities [6-11]. A large proportion of the African population uses plant materials to treat TB and TB-like symptoms as a substitute for modern medicines. Despite the traditional use of plants in Africa to treat TB or its symptoms, little attention has been given to the search for their *in vitro* antimycobacterial activities. We therefore investigated the

* Author to whom correspondence may be addressed.

antimycobacterial activity of some Nigerian plants used in traditional medicine.

EXPERIMENTAL

Plant Materials

Six plant species were selected for the study on the basis of their use in traditional medicine to treat TB or TB-like symptoms such as cough, fever, chest pain or blood in the sputum. Information about the use of the plants was obtained from oral interview with traditional healers. All plants except *Artemisia annua* were collected from Bauchi, Northern Nigeria and were identified at the herbarium of the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where herbarium specimens were deposited. *Artemisia annua* was collected from Niger Republic and identified at the School of Biological Sciences, University of Wales Swansea, UK. Table 1 shows the plants under study and their traditional uses.

Preparation of Plant Extracts

Plant materials were collected fresh, dried under shade at room temperature and then ground into fine powder with the help of pestle and mortar. The powdered plant materials were taken to the Natural Products Research Laboratory, School of Biological Sciences, University of Wales Swansea, UK, where the methanol and water extracts of each plant were prepared. One kilogram of powdered plant material was extracted with 2 separate volumes of 2.5 L of methanol. Each volume was allowed to stand for 72 h. The extract was filtered through Whatman No.1 filter paper (England). The two extracts were combined and concentrated to dryness using a rotary evaporator (Janke & Kunkee,

Labortechnik). Water extracts were prepared by soaking 100 g of material in 500 ml of distilled water for 72 h. The extract was filtered through Whatman No. 1 filter paper and the filtrate was then concentrated to dryness *in vacuo* at room temperature.

Test Organisms

The antimycobacterial activity of the extracts was determined against *Mycobacterium smegmatis* ATCC 607 obtained from NIPRD Abuja, Nigeria and *M. tuberculosis* isolated from sputum samples obtained from patients attending TB and Leprosy Hospital Bayara in Bauchi, Nigeria. Isolation was done according to standard procedures on Lowenstein-Jensen (L-J) medium [12].

Determination of Antimycobacterial Activity

Each plant extract was screened for antimycobacterial activity by the minimum inhibitory concentration (MIC) method described by Canetti *et al.* [13] and Vareldzis *et al.* [14]. An inoculum weighing 2 mg (wet weight) from a solid growth was taken from an L-J culture slope within 2 wks of the culture initially becoming positive for growth. The inoculum was added to 0.4 ml of sterile distilled water in a 7 ml screw capped bottle. A loopful of the suspension was spread on the surface of each L-J slope containing the plant extract. Slopes were incubated at 37 °C and a preliminary reading made at 2 wks. A final reading was made at 4 wks. The methanol and water extracts were separately incorporated into 250 ml batches of L-J medium before into slants to obtain final concentrations of 0.1, 0.2, 1.0, 2.0, 5.0 and 10.0 mg/ml. The minimum inhibitory concentration (MIC) of each plant

Table 1: Plants species, traditional uses and herbarium specimen numbers

Plant species (Family)	Traditional uses	HSN
<i>Anogeissus leiocarpus</i> (D.C.) Guill & Perr. (Combretaceae)	Cough, fevers, leprosy	4617
<i>Artemisia annua</i> L. (Compositae)	Malaria, fevers, cough, tuberculosis	AG011
<i>Ficus carpensis</i> L. (Moraceae)	Wounds, cough	5111
<i>Khaya senegalensis</i> (Desr.) A. Juss. (Meliaceae)	Stomachache, fevers	5385
<i>Pterocarpus erinaceus</i> Poir. (Papilionoideae)	Tuberculosis, cough, fevers	5131
<i>Piper guiniense</i> Linn. (Piperaceae)	Cough, tuberculosis, colds, sore throat	5386

HSN: Herbarium Specimen Number

extract was taken as the lowest concentration that showed inhibitory activity. Control slants (medium without plant extracts) were also inoculated. The strain was considered resistant if there were twenty or more colonies on slants with plant extract and it was considered sensitive if there were less than 20 colonies.

RESULTS

Prolific growth of *M. smegmatis* and *M. tuberculosis* was evident on slants containing no plant extracts (control slants) within 4 wks. The numbers of colonies in the control series were 51 and 54 on slants 1 and 2 for *M. smegmatis* ATCC 607 and 43 and 41 on slants 1 and 2 for *M. tuberculosis*. The results of the screening of the plant extracts are presented in table 2.

Methanol extracts of 3 of the 6 plants inhibited *M. smegmatis* and 5 inhibited *M. tuberculosis*, while water extracts of three of the plants inhibited *M. smegmatis* and 4 inhibited *M. tuberculosis*. Methanol extracts of three of the plant species, *A. annua*, *P. erinaceus* and *P. guiniense* inhibited the growth of both *M. smegmatis* and *M. tuberculosis*. Methanol extracts of *A. leiocarpus* and *P. guiniense* inhibited *M. tuberculosis* with minimum inhibitory concentrations of 0.1 mg/ml.

Ficus carpensis did not show inhibitory activity against any of the two test organisms. Only the methanol extract of *Khaya senegalensis* exhibited inhibitory activity against *M. tuberculosis* with an MIC of 5.0 mg/ml.

DISCUSSION

The plants screened in this study are used in traditional medicine in Nigeria to treat TB and/or chest related diseases. *A. annua* had been reported as a remedy for various fevers [15] and for TB treatment traditionally [16]. This study has shown that *A. annua* possesses *in vitro* antimycobacterial activity. Fagbohun reported the use of *Piper* species in Nigeria for the herbal management of tuberculosis [17]. In this study, *P. guiniense* was demonstrated to have *in vitro* activity against the two test organisms. The results of this screening further justifies the choice of these plants for their traditional use to treat TB or its symptoms and also reports their potential as sources of important leads for the development of active anti-TB agents.

The minimum inhibitory concentrations of the plant extracts screened ranged between 0.1 mg/ml and 5.0 mg/ml. Methanol and water extracts of *A. leiocarpus* and *P. erinaceus* and methanol extract of *P. guiniense* exhibited the lowest MICs (0.1 – 0.2 mg/ml). The MICs were in conformity with previous studies of antimycobacterial activities of plants extracts by Lall and Meyer [18] who reported an MIC range of 0.1 mg/ml and 1.0 mg/ml, and Newton *et al.* [11] with most MICs of 0.5 mg/ml. Some of the MICs obtained in this screening test especially those of *A. annua*, *P. guiniense* and *K. senegalensis* were higher than MICs those previously reported. This deviation could be explained because of the fact that individual plants within a species may vary according to a

Table 2: Antimycobacterial activity of the plant extracts

Plant species	Part tested	MIC of plant extracts (mg/ml)			
		<i>Mycobacterium smegmatis</i>		<i>Mycobacterium tuberculosis</i>	
		M	W	M	W
<i>Anogeissus leiocarpus</i>	SB	na	na	0.1	0.2
<i>Artemisia annua</i>	W	2.0	5.0	5.0	5.0
<i>Ficus carpensis</i>	SB	na	na	na	na
<i>Khaya senegalensis</i>	SB	na	na	5.0	na
<i>Pterocarpus erinaceus</i>	SB	0.2	2.0	1.0	1.0
<i>Piper guiniense</i>	SD	2.0	5.0	0.1	5.0

Na: not active at the highest concentration (10.0 mg/ml) tested; SB: Stem bark; W: Whole plant; SD: Seeds; M: methanol extract; W: Water extract.

number of factors including where the plant was grown, climate (temperature and rainfall), soil type, season in which the plant is collected, length of day and storage conditions [19]. These may collectively have pronounced effects on the nature, quality and quantity of secondary metabolites within the plant and, might therefore explain the variation in the MICs.

Both the methanolic and aqueous extracts of *F. carpensis* did not show inhibitory activity against the test organisms in this screening even at a concentration of 10.0 mg/ml. Although these plants were used in the treatment of TB and related diseases according to oral interview with traditional healers, it is possible that these plants are effective against colds, coughs or chest pain caused by agents other than *Mycobacterium* species. It is also important to highlight that some of the plants may have stimulant or modulatory effects on the immune system [18]. Furthermore, *in vitro* activity may not necessarily be the same with *in vivo* activity. Some potentially useful compounds may therefore be missed, as most of the screening tests do not mimic the *in vivo* environment [6]. All these call for further investigation into the plants in the area of immune modulators and to establish animal models.

CONCLUSION

The result of this study has demonstrated that African medicinal plants possess antimycobacterial activities worthy of investigation. Further investigations are now being conducted to purify and isolate the active component(s). Further research is also required to investigate the *in vivo* activities of the plants against *Mycobacterium* species.

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