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Research Paper

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ANTIMYCOBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS IN NIGER STATE, NIGERIA

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Abstract

Ten Nigerian medicinal plants *Abrus precatorius*, *Annona senegalensis*, *Anogeissus leiocarpus*, *Crateva adansonii, Detarium microcarpum, Faba spp, Neocarya macrophylla*, *Ocimum gratissimum*, *Securidaca longpenduculata* and *Terminalia avicennioides* used by traditional medicine practitioners for the management of infectious and chronic diseases such as tuberculosis and whooping cough were investigated for *in vitro* antimycobacterial activity against attenuated strains of *Mycobacterium bovis* (BCG). Hexane and methanol extracts of the plant materials were obtained by maceration. The antimycobacterial activity was determined by the broth microdilution method. The hexane extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* showed strong inhibitory activity at 312µg/ml. Eight of the ten plant extracts showed moderate inhibitory activity in either hexane or methanol extract at 1250µg/ml. While the hexane and methanol extracts of *Detarium microcarpum* and *Neocarya macrophylla* did not exhibit any significant activity. These observed activities could be associated with secondary metabolites in these plants. This study demonstrates the efficacy of Nigerian medicinal plants as potential agents in the management of the tuberculosis disease.

Key words: Antimycobacterial activity, Nigeria, Medicinal plants, Secondary metabolites, Tuberculosis

Introduction

Since the beginning of human civilization plants have been used to treat common infectious diseases. Some of these traditional medicines are still very much in use as part of the health care delivery in Nigeria. Although antimicrobial drugs have saved many lives and eased the suffering of millions, multi-resistant syndrome has tremendously limited the benefits of some of these drugs in controlling infectious diseases. Indigenous plants are reservoirs of various metabolites which provide a limitless source of important chemicals that have diverse biological properties. Tuberculosis (TB) persists as a leading global cause of death (WHO, 2005). The World Health Organization declared tuberculosis as an emergency, which requires global action especially searching for new remedies or agents to complement current ones. The emergence of multi-drugs resistant strains of *M. tuberculosis* as

well as its opportunistic infection with Human Immunodeficiency Virus, have significantly led to increased pressure on the current chemotherapy. This scenario calls for the continuous search for the discovery of new, effective, and affordable anti-tuberculosis agents to deal with the present situation.

Globally, plants are used as the main constituents in the traditionally treatment of tuberculosis and related diseases (Mann, 2007). Several Nigerian plant extracts have been known with microbial activities (Adeleye et al., 2008; Ibrahim et al., 2005; Malcolm and Sofowora, 1969; Mann et al., 2007; 2008a, b, c). Furthermore, the potential of higher plant extracts as sources of novel anti-TB leads (Mitscher and Baker, 1998) have also been known. Bacillus Calmette Guerin (BCG), attenuated strains of *Mycobacterium bovis* has been used as a surrogate test organism for screening for antitubercular activity (Mann et al., 2008c). This study is to evaluate some Nigerian medicinal plants for activities against a strain of M. *bovis* (BCG) with the aim of identifying extract with potential efficacy for TB chemotherapy development.

Materials and Methods

Plant material

Plants were selected based on the ethnobotanical uses to treat tuberculosis or related symptoms. The plant parts used were obtained as described by traditional medical practitioners from a forest near Emitete, Niger State, Nigeria. Voucher specimens were deposited in the Herbarium at the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria and National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria with the Herbarium numbers indicated. Approximately 1kg of fresh plant material of each species was collected and air-dried.

Extraction and fractionation

Two hundred grams (200g) of each dried plant material was powdered and extracted by maceration with 70% methanol for 72h at room temperature (3 x 250mL). All the crude extracts were filtered and evaporated *in vacuo* (35^0C). The crude methanol extract of each plant was then partitioned with *n*-hexane-MeOH (3 x 250mL, 1: 1) to give *n* -hexane and MeOH solubles. All the partitioned extracts for each plant concentrated and dried *in vacuo*. Stock solutions were prepared in dimethyl sulphoxide (DMSO) at a concentration of 20mg/mL and stored at -20^0C until use. The soluble fractions hexane and methanol of each plant species were tested for antimycobacterial activity using M. *bovis* (BCG).

Phytochemical analysis

The plant extracts were phytochemically screened to detect the presence of secondary metabolites such as carbohydrates, saponins, tannins, terpenoids, anthraquinones, steroids and alkaloids in accordance with standard methods (Brain and Turner 1975; Harborne, 1975; Trease and Evans, 1989).

Mycobacterium species

The extracts were screened for antimycobacterial activity against a clinical isolate of *Mycobacterium tuberculosis* and a strain of *Mycobacterium bovis* (BCG) using a broth microdilution method. The bacterial culture of *Mycobacterium tuberculosis* clinical isolates was obtained from the Diagnostic Laboratory of National Institute for Pharmaceutical Research and Development (NIPRD), Garki – Abuja, Nigeria and the activity evaluation against *M. tuberculosis* was done at the TB Research Section, Zankli Medical Laboratory, Garki-Abuja, Nigeria. BCG was obtained from the National Institute of Allergy and Infectious Diseases (NIAID), TB Research Section, NIH, Maryland, USA and cultured at the Department of Microbiology and Biotechnology, NIPRD, Abuja, Nigeria.

Inocula preparation

The inocula preparation of the clinical isolates of *Mycobacterium tuberculosis* and BCG were grown on Lowenstein Jensen medium (LJ) and subcultured in Middlebrook 7H9 broth supplemented with Albumin Dextrose Complex (ADC) at 37^oC for 7days to yield activity grown cells of *M. tuberculosis* and BCG. The activity grown *M. tuberculosis* and BCG cultures had its optical density adjusted to between 0.2 - 0.3 at a wavelength of 650 nm using

Beckman counter DU520 UV-Visible spectrophotometer. The cultures were diluted at 1/1000 by adding 25µL cell culture to 25 mL Middlebrook 7H9 broth

Determination of antimycobacterial activity

Antimycobacterial activity susceptibility testing was conducted using the broth microdilution method (BMM) in 96 well microtitre plates as earlier reported by Mann et al. (2008). The same procedure was repeated for the control, Rifampicin with an initial concentration of 32µg/mL and subsequent dilution to the final testing concentrations of 1, 0.5, 0.25, 0.125, 0.06, 0.03µg/mL. Appropriate solvent, growth and sterile controls were carried out with Rifampicin as positive control. All plates were incubated initially at 37^0C for 7 days. The plates were re-incubated at 37^0C for further 2 weeks. After this incubation, any well that turned cloudy was recorded as positive for growth. The minimum inhibitory concentration (MIC) was taken as the lowest extract concentration at which no mycobacterial growth was observed.

Results and Discussion

Selection of medicinal plants for the study

Plants were selected based on the performance index (*Ip*>0) of plants used to treat TB or related symptoms as reported in the survey by Mann et al. (2007). Performance index is an analytical tool for assessing the effectiveness of medicinal plants (Betti, 2004). The value of performance index obtained for a particular medicinal plant is indicative of its usefulness and the correctness of the information is often checked from the frequency of use by different healers across the region and also from the medicinal uses reported in literature.

Phytochemical analysis of the extracts

The results of the phytochemical analysis (Table 1) indicate the presence of saponins in all the plants extracts screened, while steroids, tannins, terpenes, and anthraquinones were observed in most of the plants tested.

Table 1: Phytochemical screening results of the crude methanolic extracts

Plant species	A	An	Cb	S	St	T	Tp
Abrus precatorius	+	+	+	+	+	+	+
Annona senegalensis	+	+	-	+	+	+	+
Anogeissus leiocarpus	-	+	+	+	-	+	+
Crateva adansonii	+	+	+	+	-	+	+
Detarium microcarpium	+	+	+	+	-	+	-
Faba spp	+	+	+	+	+	-	+
Neocarya macrophylla	+	-	+	+	+	+	-
Ocimum gratissimum	+	+	-	+	+	+	+
Securidaca longpenduculata	+	+	+	+	+	+	+
Terminalia avicennioides	-	+	-	+	-	+	+

Key: (+) present, (-) - absent, A-Alkaloids, An- Anthraquinone, Cb- Carbohydrate, S-Saponin,, St-Steroids, T-Tannin, Tp-Terpenoids

Extractive values and antimycobacterial activity of the extracts

The crude methanolic extracts from the ten plants were separately partitioned with *n*-hexane-methanol mixture to give the extractive values in Table 2. The extractive values of the ten selected plant species for the hexane soluble fractions ranged from (0.99-43.6%), while those of methanol soluble fractions ranged from (56.4- 99.01%). The details are shown in Table 2.

Table 2: The plant extracts and their corresponding percentage yields and antimycobacterial activity against BCG

Plants Species /Herbarium number[a]	Extract	Amount (g)	% Recovery	MIC (μg/ml)
Abrus precatorius 932	Hex	3.2	21.33	1250
	MeOH	11.8	18.67	NA
Annona senegalensis (5737)	Hex	6.3	28.3	NA
	MeOH	15.9	71.7	1250
Anogeissus leiocarpus 167	Hex	0.40	3.8	312
	MeOH	10.00	96.2	1250
Crateva adansonii 6967	Hex	4.6	31.0	NA
	MeOH	10.21	69.0	1250
Detarium microcarpium 900676	Hex	0.5	3.4	NA
	MeOH	14.2	96.6	NA
Faba spp *	Hex	8.2	43.6	1250
	MeOH	10.6	56.4	NA
Neocarya macrophylla (5740)	Hex	0.24	1.6	NA
	MeOH	14.6	98.4	NA
Ocimum gratissimum 1285	Hex	7.1	24.1	1250
	MeOH	22.3	75.9	NA
Securidaca longpenduculata (5742)	Hex	4.4	25.9	NA
	MeOH	12.6	74.1	1250
Terminalia avicennioides (5735)	Hex	0.20	0.99	312
	MeOH	20.00	99.01	1250
Rifampicin				0.03

Key: Hex-Hexane, MeOH-Methanol, NA-Not Active; [a]Herbarium numbers of ABU are Unbracketed, while those of NIPRD are bracketed, *Number not assigned

Eight plants of ten exhibited some degree of antimycobacterial activity. *Detarium microcarpium* and *Neocarya macrophylla* did not exhibit any significant activity for both hexane and methanol extracts. Six plants' extracts showed moderate inhibitory activity either in hexane or methanol fractions. Only the hexane fraction of extracts of *A. leiocarpus* and *T. avicennioides* showed activity at 312 μg/mL (Table 2) against BCG. The present results are in conformity with those of Mann et al. (2008c) where the *n*-hexane extracts of *A. leiocarpus* and *T. avicennioides* showed the most significant inhibitory activity at 312.5 and 200 μg/mL respectively against BCG. This study also shows the antimycobacterial potential of these two plants in comparison to the other medicinal plants of Niger state, Nigeria. This fraction deserves special attention in the exploration of chemotypes with anti-TB activity. It is pertinent to observe that hexane fractions of some plants contain essential oils which are biocidal against a broad range of organisms such as bacteria, fungi, protozoa, insects, plants, and viruses (Rosa et al., 2003). In particular it possesses the greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal, and biliary systems, as well as on the skin. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant, and anticarcinogenic properties (Lopes et al., 2000). It is generally recognized that the antimicrobial action of essential oils depends on their hydrophilic or lipophilic character. Terpenoids may serve as an example of lipid-soluble agents that affect the activities of membrane-catalyzed enzymes, for example their action on respiratory pathways. The mode of action of antimicrobial agents depends on the type of microorganism under consideration and is mainly related to their cell wall structure and the outer membrane arrangement. It has been proved that the effectiveness of the antibacterial agent generally increases with its lipophilic properties as a result of the action on cytomembranes. The hexane fractions of the two plants having the most active fractions contain terpenoids and saponins, which may be responsible for the associated activity (Table 2). Among the plants found to possess inhibitory activity, a few were also reported to be active (Uba et al., 2003). The review of antimycobacterial natural products showed that metabolites such as terpenes, monoterpeniods, diterpenes, sesquiterpenes and triterpenes, steroids and alkaloids were found to possess antimycobacterial activity (Copp, 2003). These metabolites were found to be abundant in the extracts of these plants studied. Therefore, they were reported to possess potential structural skeletons that could provide useful scaffolds / templates for the development of new antimycobacterial drugs. In *Abrus precatorius*, the active compounds associated with these activities have been isolated from the aerial parts of this plant as abruquinone B and abruquinone G, particularly abruquinone B exhibited antitubercular, antiplasmodial and cytotoxic activities (Limmatvapirat et al., 2004). Katerere and co-workers isolated

imberbic acid with potent activity against *Mycobacterium fortuitum* and *S. aureus* from the stem bark of *Terminalia spp.* (Katerere et al., 2003). This observation makes these plants with good activity interesting for further investigation.

Conclusion

This study illustrates that the hexane extracts of Nigerian medicinal plants could be good sources of metabolites with antimycobacterial activities worthy of further investigation.

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