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Inhibitory activities of selected South west Nigerian Medicinal Plants against *Mycobacterium tuberculosis*

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Summary

Introduction: Tuberculosis (TB), an infectious disease prevalent in the tropics especially in Africa and Asia is one of the highest causes of morbidity and mortality and a global concern. With increasing resistance of the pathogen, to existing anti-tuberculosis drugs and the synergy between TB infection and acquired immune deficiency syndrome (AIDS), the need for development of new drugs to cope with the infection is urgent.

Objective: Extracts from 16 plants identified and selected from the ethnomedicine of the Ijebus in Southwestern Nigeria as remedies for tuberculosis were evaluated for activity against *Mycobacterium tuberculosis, in vitro.*

Methodology: Plant extracts were screened against clinical isolate of *Mycobacterium tuberculosis* using agar plate method on Middlebrook 7H11 medium and observed for 12 weeks.

Results: The crude aqueous methanol extracts showed varying degrees of activity at concentrations of 0.025 – 100 mg/mL. *Ocimum grattisimum* (leaf) demonstrated the highest activity with minimum inhibitory concentration (MIC) of 0.025 mg/mL. Two standard anti-tuberculosis drugs; rifampicin and isoniazid, included in the assay had MIC values of 0.01 mg/mL and 0.0005 mg/mL, respectively.

Conclusion: The results of the study confirm the ethnopharmacological uses of some of the plants for TB indicating their potential as sources for the discovery of anti-tuberculosis drugs.

Keywords: *Mycobacterium tuberculosis, MTB clinical isolate, medicinal plants, SW Nigerian ethnomedicine*

Résumé

Introduction : La tuberculose (TB), une maladie infectieuse répandue dans les régions tropicales notamment en Afrique et en Asie, est l'une des plus grandes causes de morbidité et de mortalité de préoccupation mondiale. Avec une résistance accrue du pathogène aux médicaments antituberculeux existants, la synergie entre l'infection tuberculeuse et syndrome d'immunodéficience acquise (SIDA) démontraient la nécessité de développer de nouveaux médicaments pour faire face à l'infection est urgente.

Méthodologie : Des extraits de 16 plantes identifiées et sélectionnées à partir de l'ethnomédecine des Ijebus au sud-ouest du Nigeria comme remèdes contre la tuberculose ont été évalués pour leur activité *in vitro* contre les mycobactéries tuberculeuse. Les extraits de plantes ont été testés contre les isolats cliniques de Mycobactéries tuberculeuse en utilisant la méthode de la plaque de gélose sur milieu Middle brook 7H11 et observés pendant 12 semaines.

Résultats : Les extraits du méthanol aqueux brut ont montré des degrés d'activité à des concentrations variant de 0,025 à 100 mg / ml. Les feuille *d'Ocimum grattisimum* ont demontré la plus forte activité avec une concentration minimale inhibitrice (CMI) de 0,025 mg / ml, comparable au deux médicaments antituberculeux standard, la rifampicine et à l'isoniazide, inclus dans l'essai avec des valeurs de CMI de 0,01 mg / ml et 0,0005 mg / ml, respectivement.

En conclusion, les résultats de l'étude confirment les utilisations ethno-pharmacologiques de certaines plantes et indiquant leur potentiel source de découverte de médicaments antituberculeux.

Introduction

Tuberculosis (TB) an airborne, infectious disease caused by bacteria primarily affects the lungs and the infection remains one of the world's major causes of illness and death. Approximately one-third of the world's population, or two billion people, carry the TB bacteria, of which nine million become sick each year with "active" infection [1]. In 1993, the World Health Organization (WHO) declared TB a global health emergency [2]. Though TB is found globally, it is prevalent in resource-poor settings, particularlyin Asia and Africa. Over 90% of new TB cases are

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in Asia and Africa. Over 90% of new TB cases and deaths occur in developing countries, posing significant challenges to the livelihoods of individuals and developing economies because the infection affects persons in their productive years [3]. Drug-resistant TB, including multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) have become a major challenge to global TB control efforts [4]. In 2008, there were an estimated 440,000 cases of MDR-TB and as of March 2010, XDR-TB had been reported in 58 countries and territories [4]. The deadly synergy of TB with HIV/AIDS is threatening to destabilize gains recorded by the global TB control programme [3]. One of the key features of the United Nations Millennium Development Goals (MDGs) adopted by the United Nations (UN) was to halt and reverse TB incidence, prevalence, and deaths by 2015 [5]. This has generated an impetus for the urgent search for active molecules and/or structural prototypes for the effective management of TB from natural sources.

Plants still remain a major resource for the discovery and development of chemotherapeutic agents. The use of medicinal plants for the management of diseases dated back to the earliest times [6]. The presence of tropical biome with a large expanse of the equatorial forest in Africa, couple with a large percentage of endemic plants gives credence to exploitation of plants as remedies for management of infectious diseases in the continent [7-13].

As part of our continuing efforts and interest in the investigation of Nigerian ethnomedicine for discovery of agents for the treatment of tropical infectious diseases [9,12,14-15], we report herein the evaluation of selected medicinal plants in Southwest Nigeria for anti-TB properties. This is in an effort to contribute to the plethora of natural compounds that could be investigated as possible leads for the discovery of anti-tuberculosis agents.

Materials and methods

Ethical consideration

The study is an *in vitro* anti-mycobacterial evaluation of medicinal plants; it neither involved the use of human participants nor of laboratory animals. However, good laboratory practices were strictly adhered to during the investigation.

Plant material collection and authentication

The plant parts used were collected in the months of February and March from Ibadan, Nigeria. The plants were authenticated at the Forest Herbarium Ibadan (FHI), where voucher specimens were deposited.

Extraction of plant materials

Authenticated plant parts were air-dried under ambient conditions. Thereafter, plant materials were ground into powder with a mill. The powdered plant parts (200 – 300 g each) were extracted by maceration in aqueous methanol (10:90) for 72 h at room temperature (28-32°C). After removal of solvent with the rotatory evaporator, extracts were stored in the refrigerator (4°C) until needed for analyses.

Mycobacterium isolate

Mycobacterium tuberculosis clinical isolate was used in the study. The clinical strain was isolated, identified and characterized in the TB Laboratory at Veterinary Public Health and Preventive Medicine Department, University of Ibadan, Nigeria. The organism was preserved and maintained on Lowenstein-Jensen medium.

Media preparation

Middlebrook 7H11 agar (21 g, Difco Laboratories) was dissolved in 900 mL of distilled water containing glycerol. The solution was autoclaved at 121°C for 15 min and cooled to 55°C, the solution was then made up to 1000 mL with Middlebrook ADC enrichment fluid (Difco). The prepared samples of plant extracts in 7H11 medium were transferred into screw-capped glass bottles and solidified in slants at 32°C.

Inoculum preparation and antimycobacterial assay

The clinical isolate of *M. tuberculosis was* cultured and grown on Lowenstein-Jensen medium and then sub-cultured in Middlebrook 7H9 broth supplemented with ADC enrichment fluid and incubated at 37°C for 2-3 weeks. A colony of *M. tuberculosis* was taken with a sterile inoculating loop and transferred into a sterile screw capped tube which had 20 mL of Middlebrook 7H9 broth. The tube was placed on a shaker for 5 min and broth was added and adjusted to McFarland standard 1. The cultures were then diluted to 1/1000 to reduce the bacteria load. The culture (80 μL) was inoculated into the 7H11 medium containing plant extracts under a bio-safety hood. They were then incubated for 12 weeks at 37°C, a modification of the method by Lall and Meyer [16] was used. The same procedure was used for the standard drugs, rifampicin and isoniazid. Experiments were done in triplicates. The minimum inhibitory concentration (MIC, lowest extract concentration at which no mycobacterial growth was observed) was determined by serial dilution of the plant extracts and standard drugs.

Table 1: Sixteen study plants, their names, families and voucher specimen numbers

Plant names	Family	Common name	Parts	Voucher number
Acanthus montanus (Nees) T. Anders	Acanthaceae	False thistle	Leaf	FHI 107833
Aframomum melegueta K. Schum	Zingiberaceae	Guinea grains	Seed	FHI 107831
Alstonia boonei De Wild	Apocynaceae	Alstonia	Stem bark	FHI 107826
Baphia nitida Lodd.	Papilionaceae	Cam wood	Stem bark	FHI 107827
Cocos nucifera Linn.	Palmae	Coconut	Husk	FHI 107825
Cola acuminata (P.Beauv.) Schott & Endl.	Sterculiaceae	Cola nut	Seed	FHI 107835
Costus afer Ker-Gawl.	Zingiberaceae	Ginger Lily	Stem	FHI 107833
Cymbopogon citratus (Nees) Stapf	Gramineae	Lemon grass	Leaf	FHI 107376
Garcinia kola Heckel	Guttiferae	Bitter kola	Leaf	FHI 107834
Glyphaea brevis (Spreng.) Monachino	Tiliaceae		Leaf	FHI 107829
Lonchocarpus cyanescens (Shumach & Thonn) Benth	Papilionaceae	Indigo vine	Leaf	FHI 107377
Ocimum grattisimum L.	Labiatae	Fever plant	Leaf	FHI 108378
Pycnanthus angolensis (Welw) Warb	Myristicaceae	African nutmeg	Stem bark	FHI 107832
Spondias mombin L.	Anarcadiaceae	Hog plum	Leaf	FHI 107764
Vitellaria paradoxa Gaertn F.	Sapotaceae	Shea Butter	Leaf	FHI 107828
Ficus exasperata	Moraceae	Sand paper tree	Leaf	FHI 108379

Table 2: Percentage yield and minimum inhibitory concentrations against ***Mycobacterium tuberculosis*** of the plant extracts

Plants/Drugs	Parts	Percentage yield of plant extracts (%)	MIC (mg/mL)
Acanthus montanus (Nees) T. Anders	Leaf	17.88	3.120
Aframomum melegueta K. Schum	Seed	0.75	0.050
Alstonia boonei De Wild	Stem bark	11.90	0.100
Baphia nitida Lodd.	Stem bark	8.14	1.560
Cocos nucifera Linn.	Husk	11.82	> 100**
Cola acuminata (P.Beauv.) Schott & Endl.	Seed	13.47	> 100**
Costus afer Ker-Gawl.	Stem	7.19	6.250
Cymbopogon citratus (Nees) Stapf	Leaf	5.32	> 100**
Garcinia kola Heckel	Leaf	7.20	> 100**
Glyphaea brevis (Spreng.) Monachino	Leaf	12.13	> 100**
Lonchocarpus cyanescens (Shumach & Thonn) Benth	Leaf	10.10	> 100**
Ocimum grattisimum L.	Leaf	12.33	0.025
Pycnanthus angolensis (Welw) Warb	Stem bark	6.18	> 100**
Spondias mombin L.	Leaf	13.22	0.200
Vitellaria paradoxa Gaertn F.	Leaf	17.73	> 100**
Ficus exasperata	Leaf	12.90	> 100**
*Rifampicin			0.010
*Isoniazid			< 0.00005

*= *Drug*
** = *not active at the highest concentration (100 mg/mL) tested*

Results

The 16 plants investigated, their plant families, common names and identification (FHI) numbers were presented in Table 1. The percentage yield of the extracts and MIC of plant extracts and drug against MTB clinical isolate after 12 weeks of incubation was shown in Table 2. Yields of the plant extracts were in the range of 5.2-17.9 %. *Ocimum*

grattisimum (leaf) had the highest activity with an MIC of 0.025 mg/mL. The anti-TB reference drugs; rifampicin and isoniazid, have MIC of 0.01 mg/mL and < 0.00005 mg/mL, respectively.

Discussion

In Nigerian ethnomedicines plants are frequently used in the prophylactic and chemotherapeutic management of TB, other infections and diseases especially those endemic to the region. In the assay, *O. grattisimum* (leaf) was the most active (MIC 0.025 mg/mL) against *M. tuberculosis* clinical isolate. *Aframomum melegueta* (seed), *Spondias mombin* (leaf), *Acanthus montanus* (leaf), *Alstonia boonei* (stem bark), *Costus afer* (stem) and *Baphia nitida* (stem bark) also had inhibitory activities against the organism. *Cola acuminata* extracts have been reported to be devoid of activities against *M. tuberculosis* [17]; this is in agreement with our finding that it also showed no activity at the highest concentration (100 mg/mL) tested as displayed in Table 2. In addition, *A. melegueta* seed extract was active against MTB with an MIC value of 0.05 mg/mL as shown in Table 2. In another report, *A. melegueta* inhibited the growth of *M. chelonei, M. intracellulare, M. smegmatis* and *M. xenopi* with an MIC of 10-15 μg/mL [18]. However, the study reported by Adeleye and co-workers [19], indicated that *A. melegueta*, and *G. kola* were ineffective against *M. tuberculosis. Acanthus monatus* had MIC of 3.12 mg/mL in the present study, previous assessment of the antimicrobial properties of *A. montanus* showed that the aqueous root extract had moderate antimicrobial activity against clinical isolates of *P. aeruginosa* and *S. aureus* [20].

In conclusion, seven of the 16 plants demonstrated anti-TB properties against the clinical isolate of *Mycobacterium tuberculosis* used. This has confirmed the ethnomedical uses of some of the plants in the traditional management of TB in Southwest Nigeria. Bioassay-guided fractionation and isolation of active compounds is on-going to enable identification of compounds responsible for the anti-tuberculosis property in the active plant extracts.

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