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Original Research Article

Evaluation of the *In vitro* Antimycobacterial Activities of Some Nigerian Medicinal Plants

Abdulsalaam I. Alli[1]*, Stephen D. Oloninefa[2], Medinat O. Musa[3]

[1]*Department of Applied Biology, College of Science and Technology, Kaduna Polytechnic, Kaduna -Nigeria.*
[2]*Department of Biological Sciences, Faculty of Science and Computing, Kogi State University, Kabba-Nigeria.*
[3]*Microbiology Unit, Department of Applied Biology, College of Science and Technology, Kaduna Polytechnic, Kaduna -Nigeria.*

ABSTRACT

Various strategies which include the use of herbal medicines are be considered to reduce the burden and impact of tuberculosis and leprosy in Nigeria. Extracts obtained from four medicinal plants namely Rosary pea (*Abrus precatorius*), Hare's bean (*Desmodium ramosissimum*), Stonebreaker (*Phyllanthus amarus)* and Sweet-broom *(Scoparia dulcis)* were screened for their antimycobacterial activities against *Mycobacterium bovis* (BCG) strain using the broth micro dilution method. Phytochemical analysis of the extracts was evaluated using standard techniques. All the extracts were active with varying minimum inhibitory concentrations (MICs). The methanol extract of *Phyllanthus amarus* gave the most potent inhibitory effect with the same Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal Concentration of 62.5µg/ml and 62.5µg/ml respectively. Phytochemical screening of the extracts indicated the presence of alkaloids, saponins, tannins and terpenoids for all the extracts. The result of this investigation provides scientific support for the ethno-medicinal uses of some of these plants for the management of mycobacterial infections. The methanol extract of *P. amarus* extract merits further investigation in order to obtain pure compounds that can serve as lead in the development of antimycobacterial drugs.

Keywords: *Abrus precatorius, Desmodium ramosissimum, Phyllanthus amarus Scoparia dulcis,* Antimycobacterial activity, *Mycobacterium bovis* (*BCG*), Phytochemical analysis.

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Introduction

Mycobacterium is a genus in the domain bacteria which comprises of over 170 species and it is the only genus in the family Mycobacteriaceae of the order Actinomycetales in the class Actinomycetes.[1] The genus includes two important human pathogens, *Mycobacterium tuberculosis* and *Mycobacterium leprae* that cause tuberculosis (TB) and leprosy (Hansen's disease) respectively[2]. Tuberculosis and leprosy are two mycobacterial diseases that are still of major health challenge in Nigeria. Nigeria is reported to be the 6th among the 30 high tuberculosis burden countries in the world and 1st in Africa, accounting for 4% of the estimated cases globally.[3] Leprosy is still of significant public health concern in Nigeria, with over 3,500 new cases being diagnosed every year in the country and 25% of them cause irreversible deformities.[4] The treatment and break in transmission of these two diseases currently depends mainly on the use of chemotherapy.

*Corresponding author.
Email:ai.abdulsalaam@kadunapolytechnic.edu.ng
Tel: +2348023323435

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Despite the concerted efforts of the Nigerian National Tuberculosis and Leprosy Control Programme (NTBLCP) in collaboration with several other international agencies to reduce the burden and control of the transmission of tuberculosis and leprosy in the country, the strategies adopted are still hampered by several factors which include increasing poverty level and expensive antimycobacterial drugs, some of which are often not readily available to the poor rural populace.[5] The treatment of tuberculosis and leprosy also involves the use of a combination of antibiotics which may last up to at least six (6) months.[6] The prolonged use of the combination of these antibiotics, some of which are associated with adverse drug effects, is one of the reasons for treatment failure due to poor treatment compliance by patients in the rural areas.[7] It was reported that by the first quarter of 2022, the Nigeria government failed to meet her target of the World Health Organization road map of "End TB Strategy 2016-2035" due to lack of sustainability of the free-drug distribution. This was partly attributed to the COVID-19 pandemic that disrupted the supply and distribution of drugs globally.[8] The COVID-19 lock-down prompted an increase in the use of indigenous medicinal plants by the rural populace in Nigeria for the treatment of TB and leprosy due to the absence of sustained availability of effective therapeutic drugs.[9-11] Many researchers all over the world have reported on the antimycobacterial potentials of medicinal plants around their locality which they believe will offer great potential for controlling the scourge of TB and leprosy. A compendium of medicinal plants from Nigeria and other parts of Africa that can be used to control or cure tuberculosis was earlier prepared.[12] A comprehensive review of medicinal plants that can be used to cure leprosy was also reported.[13] Other reports of medicinal plants with antimycobacterial activities include those from Nigeria,[14] from South Africa[15] and from Uganda.[16,17] In addition, some of the researchers also reported that some of these medicinal plants could play significant roles in the discovery and

development of effective antimycobacterial agents.[18-20] Nigeria has a rich diversity of medicinal plants and vast majority of them have not been adequately investigated for their antimycobacterial potentials.[21] There is a continuous need to screen and validate the efficacy of such under reported medicinal plants in order to discover new treatments for mycobacterial infections. This investigation is designed to evaluate the antimycobacterial potentials of solvent extracts obtained from *Abrus precatorius, Desmodium ramosissimum, Phyllanthus amarus* and *Scoparia dulcis* which were earlier found to possess antibacterial activities.[22]

Materials and Methods

Collection, Identification and Authentication of the Plants
Ethnobotanical information on the test plants were obtained from traditional medical practitioners within Kogi State Central Senatorial District. The plants were collected from October to November, 2024 around Federal College of Education, Okene and the vicinity of Osara Dam in Adavi Local Government Area of Kogi State, Nigeria. The plants were identified at the Plant Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria where voucher specimens were also deposited.[22]

Extraction of Plant Materials
The leaves of *A. precatorius*, *D. ramosissimum* and *S. dulcis* were used while the entire aerial part of *P. amarus* was used. The plant materials were dried at ambient temperature under shade for 9 days. The dried plants were pulverized into fine powder using porcelain pestle and mortar. Methanol extracts of the powders were obtained by cold maceration of 100g of each plant powder with two successive 500 mL portions of 80% methanol in enclosed flasks for 48hr at room temperature with agitation twice a day.[23] The extracts were then filtered twice through Whatman No.1 filter paper. The filtrates obtained from each extraction of each plant powder were pooled together and concentrated to dryness using rotary evaporator (Rotary Evaporator RE-5A, Union Laboratories, England). The n-Hexane extracts were obtained by subjecting 100g of each plant powder to soxhlet extraction separately, followed by removal of the solvents under reduced pressure using a rotary evaporator (Rotary Evaporator RE-5A, Union Laboratories, England). Aqueous extracts of all the powders were obtained by decoction which involved mixing 100g of the powders with 1000 mL of distilled water and boiling for 20 min. Percentage yields were calculated. The extracts were subsequently transferred into clean screw-capped containers and stored in the fridge till when needed. [23]

Phytochemical Screening of the extracts
Screening for carbohydrates, tannins, alkaloids, saponins, flavonoids, steroids/terpenoids, cardiac glycosides and anthraquinone was carried out by standard methods as earlier described.[24]

Test Microorganism
Mycobacterium bovis (BCG strain) was sourced from the stock of the Dept. of Pharmaceutical Microbiology and Biotechnology, National Institute of Pharmaceutical Research and Development, Abuja. This strain was used as surrogate strain due to its low virulence which can be handled under BSL-2 containment facility.[25]

Preparation of Media/ Inoculum
All the media were prepared according to manufacturer's specifications. The *Mycobacterium bovis (BCG strain)* was tested for purity on 7H11 Middlebrook Agar and then grown in the 7H9 Middlebrook Broth at 37ºC for 7 days. The optical density of the resulting culture was 0.227 at 650nm.The culture was subsequently diluted appropriately (1:1000) to give approximately 10^5 CFU/ml.

Preliminary Antimycobacterial Susceptibility Test
Antimycobacterial susceptibility screening was carried out by the Microliter Broth Dilution Method using 96- well microtitre plates as described.[26] One hundred milligram (100 mg) of each extract was dissolved in 5ml of 10% dimethylsulphoxide (DMSO). The extract supernatants were sterilized using Corning sterile syringe filter (0.2µm pore size). Fifty microliter (50µL) of each extract supernatant was then added to 450 µL of 7H9 medium/Albumin Dextrose Complex (1:10 dilution) in separate tubes and centrifuged at1300rpm for 20 mins to remove precipitation as much as possible and to give a stock concentration of 1000 µg/ml each. In a 96- well microtitre plate, 50ml of Middlebrook 7H9 medium was added to wells 2-12 of each row except well one (1). In the first well of each row was added 100ml extracts. 50 ml of each extract was carefully transferred from well 1 to 2, mixed thoroughly by pipetting up and down four times and 50ml was then transferred to well 3. This was repeated through wells 4 to 12 of each row of the plate and the final 50 ml discarded from well 12. This two-fold serial dilution further diluted the extracts to give final testing concentrations of 1000, 500, 250,125, 62.5, 31.25, 15.6, 7.81, 3.9, 1.95, 0.98 and 0.49 µg/ml. To each well was added 50 µl of the standardized inoculum. The same procedure was repeated for the positive control using rifampicin with the initial concentration of 32 µg/ml which was diluted as described above for extracts to give final testing concentrations of 32, 16, 8,4,2 1, 0.5, 0.25, 0.125, 0.06, 0.03 and 0.015 µg/ml. Sterile medium without extract and DMSO served as negative controls. The plates were covered and placed in sealed plastic bags, the surface of the bags were decontaminated and placed in a 37^0C incubator for 7 days. Thereafter, each plate was visually read and the column number of the row at which no apparent growth was seen was recorded. The plates were again surface decontaminated and returned to the incubator and re-examined after 14 days.

Determination of Minimum Inhibitory Concentration (MIC)
A modification of the Microtitre Broth dilution with Methyl Thiazolyl Tetrazolium Chloride (M.T.T) as growth indicator was adopted to determine the minimum inhibitory concentrations as earlier described.[27] In this method, 96-well microplates were prepared with fresh Middlebrook 7H9 culture medium as previously described for the preliminary antimycobacterial susceptibility testing above. Two-fold serial dilutions of the extracts in the medium with inoculum were used to confirm the MIC values. The plates were covered and placed in sealed plastic bags, the surface of the bags were decontaminated and placed in a 37^0C incubator for 7 days. The same procedure was repeated for both the positive and negative controls. After the period of incubation, 25µL of freshly prepared Methyl Thiazolyl Tetrazolium chloride (2.5 mg/ml) was added to each well of the plate being used to determine the MIC values and reincubated at 37°C for 48hr for colour development. The result was read with microplate reader (Hinotek Microplate Reader, Model: AMR-100, China) as soon as a pink colour became visible in the untreated control wells. The MIC values were defined as the lowest extract concentrations that prevented the colour change of M.T.T reagent from blue to pink. The assay was repeated twice.

Determination of the minimum bactericidal concentration (MBC)
To determine the bactericidal activity of the extracts, two-fold dilution of the extracts were prepared with fresh supplemented Middlebrook7H9 broth culture medium as earlier described in two six-well rows of a microplate.[27] Fifty microliters (50 µL) of the standardized inoculum suspension was inoculated into each of the wells which correspond with the extract dilutions with no visible growth in the developed MIC microplates and incubated for 7 days. During this procedure, special care was taken to maintain the original relative position of each extract concentration in the new plates. The plates were developed with MTT reagent after the period of incubation as described above for MIC determination. The MBC corresponded to the minimum extract concentrations that did not cause change of colour of M.T.T reagent from blue to pink. The assay was also replicated twice. [27]

Results and Discussion

Test Plants
The selected test plants, their local names (Ebira), voucher specimen numbers and the current ethnomedicinal uses in Ebiraland are listed in Table 1.[22]

Table 1: Ethnobotanical Information of The Test Plants

Test plant	Local name (Ebira)	Voucher No	Part used	Ethno-medicinal uses
Abrus precatorius	Ohinohine-orupa	932	Leaves	Tuberculosis, Cough, Sore throat, Aphrodisiac and Diabetes
Desmodium ramosissimum	Ema (oweyi)	879	Leaves	Tuberculosis, Diarrhea, Dysentery, Fever, Pulmonary troubles, Cough, Venereal, diseases and Jaundice.
Phyllanthus amarus	Avi–ogogirema	3073	Aerial	Tuberculosis, Diarrhea, Venereal infection and Poison antidote
Scoparia dulcis	Ohinohine-sesere	555	Leaves	Tuberculosis, Cough, Sore throat, Gonorrhea and Diabetes

Phytochemical Screening of Test Plants Extracts

A total of twelve (12) extracts from the four (4) test plants were screened. Phytochemical screening of the selected test plants' extracts detect the presence of phytochemicals such as alkaloids, flavonoids, tannins, cardiac glycosides, terpenoids and steroids as shown in Table 2. These phytocompounds have earlier been reported to possess antibacterial actions against Mycobacterium species, by inhibiting the bacteria's growth and replication, and sometimes, causing cell death.[28]

Determination of Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal Concentration (MBC) of the crude extracts against Mycobacterium bovis (BCG)

The result of the determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of the crude extracts against *Mycobacterium bovis* (BCG) is presented in Table 3. All the extracts except both the aqueous and methanol extracts of *Desmodium ramosissimum* displayed antimycobacterial activity against the test organism at the highest concentration of 1000 µg/mL employed in this study (Table 3). The methanol extract of *Abrus precatorius* was more active (M.I.C. of 62.5 µg/mL) compared to both its aqueous and hexane extracts (250 and 125 µg/mL respectively) as shown in Table 3. This extract is very reach in alkaloids (Table 2) and may be responsible for the observed antimycobacterial activity as earlier reported for the methanol extracts of *Psoralea carylifolia* and *Sanguinaria canadensis*.[28] The observed antimycobacterial activity may also be due to the presence of other bioactive constituents, such as flavonoids, saponins and tannins which was earlier reported to be responsible for the antimycobacterial activity of both the aqueous and methanol extracts of *Alpinia galanga* and *Oldenlandia umbellate*.[29]

Table 2: Phytochemical screening of the various test plant extracts

Test plant	**Extracting solvent**	**Extract yield**	**Phytochemicals**								
			Alkaloid	**Anthra-quinone**	**Cardiac glycoside**	**CHO**	**Flavanoids**	**Phenol**	**Saponin**	**Steroid/ Terpenoid**	**Tannin**
Abrus precatorius	Aqueous	**2.74**	-	+	+	+	+	-	+	-	+
,,	Hexane	**0.85**	-	-	-	-	-	-	-	+	-
,,	Methanol	**4.3**	+	+	+	+	+	-	+	-	+
Desmodium ramosissimum	Aqueous	**0.80**	-	-	-	-	+	+	-	-	+
,,	Hexane	**1.86**	-	+	-	-	-	+	-	+	-
,,	Methanol	**6.58**	-	-	-	+	+	+	+		+
Phyllanthus amarus	Aqueous	**3.10**	+	-	-	+	+	+	+	-	+
"	Hexane	**1.82**	+	-	-	-	-	-	-	+	-
"	Methanol	**3.64**	+	-	+	+	+	+	+	+	+

Scoparia dulcis	Aqueous	**2.28**	+	+	-	+	+	-	-	-	+
"	Hexane	**1.2**	-	-	-	-	-	+	-	+	-
"	Methanol	**5.67**	+	+	+	+	+	+	+	+	+

Key: CHO ⇒ Carbohydrate
+ ⇒ Present - ⇒ Absent

Table 3: Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal Concentration of the crude extracts against *Mycobacterium bovis* (BCG)

Test plant	Extracting solvent	M I.C. (µg/ml)	M.B.C. (µg/ml)
Abrus precatorius	Aqueous	250	1000
,,	Hexane	125	500
,,	Methanol	62.5	250
Desmodium ramosissimum	Aqueous	>1000	>1000
,,	Hexane	62.5	250
,,	Methanol	>1000	>1000
Phyllanthus amarus	Aqueous	125	250
"	Hexane	250	500
"	Methanol	62.5	62.5
Scoparia dulcis	Aqueous	125	500
"	Hexane	62.5	250
"	Methanol	125	500
Rifampicin (Control)		0.25	0.5

The n-Hexane extract of *D. ramossismum* was the only extract from this plant that demonstrated strong antimycobacterial activity against the test strain. The aqueous and methanol extracts of this plant did not inhibit the growth of the test strain at the highest concentration of 1000 µg/mL employed in this study (Table 3). The strong antimycobacterial activity of this n-Hexane extract may be due to the presence of appreciable quantities of steroids/terpenoids in the extract (Table 2) as previously observed for the n-Hexane fraction of *Costus speciosus*, *Cymbopogon citratus* and *Tabernaemontana coronaria*, [30] and for the n-Hexane extract of *Morinda citrifolia*.[31]

Methanol extract of *Phyllanthus amarus* exhibited higher antimycobacterial activity (MIC value of 62.5µg/mL) against the test strain compared to both its aqueous and n-Hexane extracts (125 and 250 µg/mL respectively) as shown in Table 3. The Methanol extract of *P. amarus* is particularly very rich in polyphenols as earlier reported.[32]The extract also contained other major classes of phytochemicals (Table 2). These phytochemicals could be acting in concert to inhibit the test organism. This assertion is supported by the report that plants effective against *M. tuberculosis* are rich sources of alkaloids, flavonoids, glycosides, diterpenoid, triterpenes, lipids, phenolic compounds, tannins, sterols etc.[33]

The methanol extract of *Scoparia dulcis* with MIC value of 62.5 µg/mL was the most active against the test organism compared to both its aqueous and n-Hexane extracts with MIC values of 125 and 250 µg/mL respectively. This extract contained appreciable quantities of steroids and terpenoids which may be responsible for the observed antimycobacterial activity as earlier reported for *P. carylifolia and S. canadensis*.[32] In addition the extract was the only extract that contains all the classes of phytochemical compounds tested for, therefore, the phytocompounds could be acting in synergy to effect the observed inhibition of the test strain as earlier reported.[34]

Conclusion

From the result of this study, it can be concluded that all the test plants' leave extracts demonstrated varied levels of antimycobacterial activities against the test mycobacterium strain. The most potent antimycobacterial activity in terms of the MIC and MBC value (62.5µg/mL each respectively) was exhibited by the methanol extract of *P. amarus* indicating that the extract may be bactericidal at this concentration. The MBC values of all the other extracts were considerably higher than their corresponding M.I.C. values indicating that they may be merely bacteriostatic. None of the extracts compared favourably in terms of antimycobacterial activity with Rifampicin (MIC and MBC of 0.25 and 0.5µg/mL respectively) that served as positive control. However, it should be noted that these are crude extracts that needs further purification. The results of this study provide scientific support for the ethnomedicinal uses of these plants for managing tuberculosis and leprosy. Further studies are advocated to isolate and characterize the bioactive principles responsible for the observed antimycobacterial activities of *P. amarus* in particular. The effort may provide lead compounds for the development of new and effective drugs for the treatment of mycobacterial infections.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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