Published in final edited form as:
J Ethnopharmacol. 2014 August 8; 155(1): 524–532. doi:10.1016/j.jep.2014.05.059.

Some Nigerian Anti-Tuberculosis Ethnomedicines: A Preliminary Efficacy Assessment

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Abstract

Ethnopharmacological significance—Nigerian herbalists possess indigenous ethnomedicinal recipes for the management of tuberculosis and related ailments.

Aim of the study—To carry out a collaborative preliminary modern scientific evaluation of the efficacy of some Nigerian ethnomedicines used by traditional medicine practitioners (TMPs) in the management of tuberculosis and related ailments

Materials and methods—Ethnomedicinal recipes (ETMs) were collected from TMPs from locations in various ecological zones of Nigeria under a collaborative understanding. The aqueous methanolic extracts of the ETMs were screened against *Mycobacterium bovis*, BCG and *Mycobacterium tuberculosis (M. tb.)* strain H_{37}Rv using the broth microdilution method.

Results—Extracts of ETMs screened against BCG showed 69% activity against the organism. The activities varied from weak, ≤ 2500μg /mL to highly active, 33μg /mL 64% of the extracts were active against *M. tb.* The activities of the extracts against *M.tb.* varied from weak, ≤ *2500*μg /mL to highly active, 128μg/mL. There was 77% agreement in results obtained using BCG or *M. tb.* as test organisms

Conclusion—The results show clear evidence for the efficacy of the majority of indigenous Nigerian herbal recipes in the ethnomedicinal management of tuberculosis and related ailments. BCG may be effectively used, to a great extent, as the organism for screening for potential anti-*M. tb. agents.* A set of prioritization criteria for the selection of plants for initial further studies for the purpose of antituberculsis drug discovery research is proposed.

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Keywords

African ethnomedicines; cough; anti-*Mycobacterium* activity; *M.tb.*; *M.bovis* (BCG)

1.0 Introduction

1.1 Tuberculosis as a global health problem

Tuberculosis (TB) is a chronic bacterial infection caused by the bacillus, *Mycobacterium tuberculosis* and easily transmitted from person to person through the air by droplet nuclei (Moulding, 1988). Tuberculosis remains a leading cause of death in the world from a single infectious agent. It is estimated that one-third of the world's population is infected with the tubercule bacillus and about 80% of individuals diagnosed with the disease every year live in the 22 most populous countries (Dye *et al.*, 1999; Dye, 2006). Effective treatment of TB has been hampered by the emergence of drug resistant strains of *M. tuberculosis*. Particularly, ominous is the emergence of multi-drug resistant TB (MDR-TB) and extensively-drug resistant TB (XDR-TB), which has been accelerated by the rise of Human Immune Virus/Acquire Immune Deficiency Syndrome, HIV/AIDS (CDC, 2006; Smith and Moss, 1994). Despite the introduction of Directly-Observed Therapy Short course (DOTS) by WHO in 1995 (The Economist, 1995), a control strategy to detect and cure TB, millions of TB patients continue to perish (Whalen, 2006).

1.2 Antituberculosis drug discovery efforts and the need for more vigorous drug discovery efforts

Though there are many efforts being made to discover new drugs to treat TB, these efforts are not a major focus of many pharmaceutical companies. There are some notable successes from pharmaceutical companies as exemplified by the recently FDA-approved Bedaquiline The reasons for the lack of more vigorous investments by the industry are mainly economic as the countries most in need of new anti-TB drugs are primarily developing countries whose populations are not able to buy expensive drugs that would arise from the costs of investing huge sums to develop. Current antituberculosis chemotherapy demands the taking of up to four drugs simultaneously over a period of six months which leads to poor adherence by patients and demands close supervision of patients to mitigate the development of drug-resistance. MDR-TB and XDR-TB, which require therapy for up to two full years with multiple poorly-active second-line drugs, have compounded the problem of achieving success and have a high percentage of treatment failure (Gandhi *et al.*, 2010; Mitnick *et al.*, 2003). Many of the drugs used in the treatment of MDR-TB and XDR-TB also have serious toxic effects (Carroll *et al.*, 2012). New drug scaffolds and drugs need to be found and developed which will reduce the current long duration of therapy, reduce the pill burden, successfully treat MDR-TB and XDR-TB, be co-administrable with anti-HIV and anti-diabetes drugs and exhibit less toxic side effects (Barry, 2003).

1.3 TB in Nigeria and the need to investigate the efficacy of ethnomedicines and medicinal plants in Nigeria for the purpose of discovering new TB drugs

Tuberculosis was declared a national emergency in Nigeria in June 2006. The country was ranked one of the most highly TB burdened countries in the world with an estimated incidence of all forms of TB at 311 per 100,000 population (WHO, 2008).

In Nigeria, a large percentage of the population, particularly in the rural areas, depend on traditional medicines for their primary health care. Traditional medicine is a broad term used to describe non-western medicine. Ethnomedicine is a form of traditional medicine that includes the use of plants for healing by humans (Iwu, 2002). Ethnomedicine is a preferred choice for many people as it is readily available and more affordable. Plants have contributed significantly as starting points for the development of modern drugs (Khazir *et al.*, 2013Newman *et al.* 2005, Newman and Cragg, 2007) as evidenced by taxol in cancer and artemisinin in malaria. This may be attributed to their chemical diversity, biochemical specificity, possession of a greater number of chiral centres than in synthetic or combinatorial libraries, and evolutionary pressures to create biologically active compounds by interactions with different proteins and biological targets (Queiroz *et al.*, 2009; Wolfender, 2009). Plants therefore, represent potential sources of new drugs acting through novel mechanisms in the search for new and more potent and safe antituberculosis agents. There are a number of natural plant metabolites that have been reported to have inhibitory or bactericidal activities *in vitro* against *Mycobacterium tuberculosis* at micromolar concentrations (Copp, 2003; Copp and Pearce, 2007; Okunade *et al.*, 2004). Such reports carry hope of success in fully planned isolation and synthetic strategies to discover new antituberculosis drugs in plants. It is estimated that there are about 250,000 – 500,000 plant species and only about 10 per cent of these has been phytochemically investigated for the purpose of determining biological activity of their components (Hostettmann *et al.*, 1996). A very high percentage of these unstudied plants are endemic to Africa and Asia. Nigeria's bio-resource is massive and diverse and is divided into various climatic zones that include marine mangrove, rainforest, Sudan savannah, derived savannah and the Mediterranean. Nigeria possesses over 5000 plant species and also has a culture and history that is very rich in ethnomedicine.

1.4 The aim of the study

The aim of the work reported here was to initiate a collaborative and preliminary sample study of Nigerian ethnomedicines used by the traditional medicine practitioners (TMPs), living across various ecological zones in the Country, for the management of coughs including bloody cough (tuberculosis), and to evaluate the scientific basis for the use of these traditional remedies.

2.0 Materials and methods

2.1 Study sites

Eight states of the Federation located in various climatic zones (Fig. 1) were visited between August 2005 and February 2006 for the purpose of interviewing individual traditional medicine practitioners (TMPs) about their experience of treating TB and collection of herbal

anti-TB recipes and medicines. Ethnobotanical studies were carried out in four geographical regions of Nigeria comprising the South West, South South, South East and North Central. This survey included Lagos, Ogun, Oyo, Edo, Enugu, Niger, Plateau, Kaduna states and the Federal Capital territory, Abuja.

2.1.1 Interviews—The TMPs were interviewed using copies of the same questionnaire for all the TMPs. The questionnaire was titled "The effectiveness of Nigerian Traditional Medicines for the Treatment of Tuberculosis" and included in it were the following sections: (a) Personal details of the healer, (b) Questions about the healer and his/her practice, (c) Questions about tuberculosis and (d) Herbal remedy. Information was also collected on (i) the nomenclature; botanical, common and native names of the plants used, (ii) part of the plant used (stem, leaves or roots), (iii) special time of collection, (iv) the habitat and mode of growth of plant (wild, cultivated), and (v) mode of collection and drying of plant. The questionnaire requested the TMPs, Table 1 to provide their bio-data, knowledge about TB, their anti-TB recipes, dosage and duration of treatment, plant collection guidelines and procedure for preparing the medicines. In most of the interviews, the TMPs could only communicate in their local language, and a person was at hand to translate and complete the questionnaire in English.

(c) Many of the TMPs could not be reached for recollection and collection of their respective ETM formulation plant details. Nevertheless, such ETMs have been included and the names and location of the TMPs concerned are in Table 1 to guide further investigation.

2.2 Herbal recipes (ETMs) and material collection

Herbal recipes were fine or coarse powders or liquids. They were collected from the traditional healers as used by the indigenous people to treat TB symptoms such as fever, cough, and blood in the cough sputum etc. Eighty-six preparations were obtained based solely on the recommendations of the traditional healers and no attempt to determine the composition was made at the initial stage unless they volunteered to provide this. At this stage a token sum was paid for each recipe and a one page agreement of collaboration to participate in the research to help in the investigation of a global health problem was signed by the herbalist and a senior member of the research team. The agreement included a clause to the effect that publications arising from work on the unique recipe will include the herbalist's name. Traditional medicine practitioners (TMPs) whose recipes were found active *in vitro* against BCG/*M.tb.* were re-visited for the purpose of recollection of their recipes, the composition of the recipes and the provision for taxonomical identification purposes of plant samples used in the preparation of their recipes. The TMPs whose recipes exhibited activities were given letters giving indications about the results of screening the extracts of their recipes against *M. tb.* together with a slightly higher token sum for services. Fifty-six plant specimens were collected by the individual TMP for each ETM during the time of the team's visit. The plant samples were identified by the taxonomists at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja where voucher samples were deposited.

2.3 Preparation of Extracts

For recipes that were powders 10 g of raw material was extracted by soaking in 70 % aqueous methanol (100 ml), shaking occasionally for 24 hours. 70% aqueous methanol was used as the solvent of extraction, from experience (Mann *et al.*, 2008), in attempt to extract the whole range of compound polarities in the recipe samples. The extracts were then filtered and concentrated under reduced pressure using rotary evaporator to remove the methanol and some water. The concentrated extract was then lyophilized. For the aqueous recipes, 50 mL of each of the liquid was shaken vigorously for homogeneity and then freeze-dried. The resulting dry powder was transferred to a bottle, weighed and stored.

2.4 Determination of Minimum Inhibitory Concentration (MIC)

The broth microdilution method (Duckworth et al., 2012), which in a survey (Franzblau et al., 2012), was found to be the mostly used protocol in the field, was employed for the preliminary screening. All extracts were screened against both *M. tb.* strain H_{37}Rv and *Mycobacterium bovis* BCG after cells were grown to an optical density of 0.2-0.3 at 650 nm in 7H9/ADC/Tween consisting of Middlebrook 7H9 broth supplemented with 0.5% bovine serum albumin fraction V, 0.08% NaCl, 0.2% glucose, 0.2% glycerol and 0.05% Tween 80. The lyophilized plant extract was dissolved in 4 mL DMSO and made up with 7H9/ADC/Tween to a final DMSO concentration of 4%. Each plant extract-medium was vortexed and 100 μL of it dispensed into the empty first column (first row) of a 96-well microtitre plate while each of all the other wells (two to twelve of the column) contained 50 μL of 7H9/ADC/Tween. Two-fold dilutions were performed by sequential transfer of 50 μL from the first column through to column 11 leaving column 12 for the drug-free control. The mycobacterial culture was diluted 1:1000 in 7H9/ADC/Tween, and 50 μL added to all wells giving a final volume of 100 μL. in each well. Isoniazid (INH) was used as positive control. The microtitre plate was incubated for 7-10 days at 37°C, after which the growth or inhibition of growth was read by direct recording of visual growth. All of the MIC determinations and extractions were done in duplicate with the BCG screening at NIPRD, Abuja, Nigeria while the *M. tb.,* H_{37}Rv strain, screening was carried out at TRS, LCID, NIAID/NIH Bethesda, MD USA. The MIC was reported as the highest concentration of extract resulting in complete inhibition of visual growth.

3.0 Results and Discussion

3.1 Ethnobotanical demography and information

Most of the healers interviewed were men with ages ranging from 30 and above. Most of them were also from a family of traditional healers and had been practicing for most of their lives. They also had mostly learnt the practice from an elderly family member confirming the well-known fact that the healing art in most cultures survives through family inheritance and training. Most of the traditional healers also indicated that symptoms such as fever, cough and blood in the sputum together were indications for the treatment of tuberculosis. A number of the recipes contained multiple plant components and decoction was the method used by most of the practitioners in the preparation of these recipes. Most of the plants were obtained from the wild. Some healers recommended special seasons or times for collection of the plant e.g. the dry season or during the day, because they believed that the plants were

more potent at the time chosen for collection. The majority of the healers stored the plant parts in aerated sacks. Though the TMPs are not aware of the molecular composition of their herbs, they have learnt over the years that plant metabolites could vary with seasons and environment.

86 ETMs were collected initially when the compositions of the recipes were unknown, 73 were recollected after ensuring compatibility with the first samples and 43 of these had specimens of component plants available for inspection and identification.

3.2 Biological activities of the ethnomedicines, ETMs

Preliminary screening of the 86 plant recipes showed the various percentage activities of these recipes against BCG and *M. tb.* in different ranges of concentration (Figs 2 and 3). The activities of the herbal recipes (ETMs) were classified using the MICs into highly active, ***blue*** coded in figures (MIC ≤ 500 μg/ml) moderately active, ***red*** coded in figures (500μg/ml < MIC ≤ 1 mg/ml) weakly active, ***green*** coded in figures (1mg/ml < MIC ≤ 2.5mg/ml), and little or no activity, **purple** coded in figures (≥ 2.5 mg/mL). The individual ETMs and plants making up 43 of these recipes (ETMs) and the MICs of these recipes are shown in Table 2.

3.2.1 BCG and *M. tb.* test results—Extracts of 72 ETMs were tested against BCG. Of these 16 (22%) were highly active, 13 (18%) were moderately active, 21 (29%) were weakly active and 22 (31%) had little or no activity. The distribution of activities in MIC ranges against BCG is presented in fig.2.

Extracts of 78 ETMs were tested against *M. tb.*. Of these 12 (15%) were highly active, 17 (22%) were moderately active, 21 (27%) were weakly active and 28 (36%) had little or no activity. The distribution of activities in MIC ranges against *M. tb.* is presented in Fig. 3.

Extracts of 66 ETMs were each individually tested against BCG as well as *M. tb.* Of these, 51 (77%) had highly active to weakly active results for both organisms. 8 (12%) were active against BCG but inactive against *M. tb.* 7 (11%) were active against *M. tb.* and inactive against BCG and 13 (20%) were inactive against both organisms.

In this study, there was 77% agreement in the anti-*Mycobacterium* activities obtained using either BCG or *M. tb.* strain H_{37}Rv as test organisms.

3.3 Phytochemistry and pharmacology of selected plants listed in the ETMs

The phytochemistry and pharmacology of most of the plants claimed by the TMPs to have been used in formulating their ETMs have been published in various details. The publications have not necessarily been in connection with their anti-*Mycobacterium* activities. Twelve of the plants stand out owing to the highly active nature of their extracts. Thus the known chemistry and biological activities of all such plants which have been given as the sole components of ETMs and in some cases used in conjunction with other plants are hereby discussed.

Abrus precatorius is the only given component for ETM 18. An active component from the plant extract has been isolated and characterized as the isoflavanoid quinone, abruquinone which was shown (Limmatvapirat *et al.*, 2004) to have an MIC of 12.5μg/mL against the *M. tb.* strain H_{37}Rv.

Anogeissus leocarpus is listed as a component in 10 of the 43 ETMs whose formulations were divulged by the TMPs and in two of these ETMs, 18 and 40, it is the single component. *A. leocarpus* is thus the most commonly used plant among the TMPs visited. The chemistry and the antimicrobial activity of the extracts of the Genus *Anogeissus* have been reviewed (Mann *et al* 2009a) .The leaves and or stem bark are the parts usually utilized. Our MIC results of 266μg/mL and 988μg/mL respectively for the extracts of ETMs 18 and 40 against BCG are in rough agreement with the MICs, 312μg/mL for the hexane fraction and 1250μg/mL for the methanol fraction (Mann *et al.*, 2008) when their initial 70% aqueous methanol extract was partitioned into hexane and methanol. *A. leocarpus* extracts contain polyphenols and triterpenoids (Adigun et al, 2000; Chaabi et al, 2008; Mann *et al.*, 2009a). There seems to be clear cases of synergism between the extracts of the plants combined with *A. leocarpus* in ETMs 38 and 48 while the opposite is the case for ETMs 45, 49 and 60 where *A. leocarpus* is combined with other plants. These combinations need to be further studied.

Cassia siberiana is the sole component given for ETMs 19 and 20. The leaves and roots extracts of the plant have been shown to possess antibacterial activity (Ndukwe *et al*, 2004). Anthraquinone and polyphenolic flavonoids 1-epicatechol and leucopelargonidol have been identified in its extracts (Duquenois and Anton, 1968; Ndukwe et al 2004; Paris and Etchepare, 1967) and these compounds could contribute to the anti*Mycobacterium* activity observed in this work.

Combretum molle, the only component in ETM 31 stem bark acetone extract reportedly gave tannin, punicalgin which gave an MIC higher than 600μg/mL against *M. tb.* strain ATCC 27294 and a clinical isolate (Asres *et al.*, 2001). Extracts of *C. mole* had MIC of 0.5mg/mL against *M. tb.* strain H_{37}Rv and inhibited the resistant *M. tb.* CCK02869V (Lall and Meyer, 1999). A hydroxycycloartenol glycoside, mollic acid has been isolated from the leaf extract of *C. molle* (Rogers and Thevan, 1996).

Erythrina senegalensis is the lone plant for ETM 16. On a chemotaxonomic basis *E. Senegalensis* extracts could be expected to contain isoflavonoidsand coumarin derivatives just like *E. gibbosa* and *E. indica* respectively. The isoflavonoids pasellidin and erythobissin with MICs respectively lying between 8μg/mL and 25μg/mL against *M.tb.* have been isolated as active principles from *E. gibbosa* extracts (Mitscher and Baker, 1998) while a 3-phenyl coumarin derivative indicanine with MIC of 18.5μg/mL against *M. smegmatis* has been isolated from *E. indica* extracts (Waffo *et al.*, 2000).

Garcinia kola is the only component given for ETM 28 and is in ETMs 48 and 75. Its extracts are known to contain anthraquinones, biflavonoids, saponins and xanthones. Four of the bioflavonoids and a xanthone which have antibacterial, antihepatoxic and antidiabetic

activities have been identified (Christopher *et al.*, 2007; Iwu *et al.*, 1987, 1990; Oluronke *et al*, 1999).

Khaya grandifolia, the only component given for ETM 63 is well known to have limonoids (bitter principles) as the main components of its extracts. The antimalarial activity and the effects of its extracts on biochemical and haematological parameters in mice have been reported (Agbedahunsi and Elujoba, 1998a; Bumah, et al., 2005).The limonoids - grandifolilenone, methyl 6-acetoxy angolensate and grandifolin - were isolated from the plant extracts (Agbedahunsi and Elujoba, 1998b; Connolly and McCrindle, 1967). Grandifolin came from the antimalarial active chromatographic fraction of the extract of the stem bark. Adesogan and Taylor (1967) reported the isolation of a steroid hormone from the plant extract. We are aware from direct personal experience of the use of stem bark tannin-containing aqueous decoction in the treatment of dysentery. Tannins are in general antimicrobial and hence could contribute to the observed antimycobacterial activities of the plant extract.

Pentaclethra macrophylla (ETM 95) seed and root bark extracts gave a phenolic and steroidal glycosides but with no reports of anti*Mycobacterium* screening (Folefoc *et al.*, 2005).

Pterocarpus osun (ETM 72) extracts have been shown to have antimicrobial activities and to contain glycosides, saponins, steroids and tannins (Ebi and Ofoefule, 2001) which could be responsible for the observed anti *Mycobacterium activity*.

Securidaca longepedunculata methanol and hexane extracts yielded hydroxybenzoic acids and xanthones which had MICs of 312μg/mL and 1250μg/mL respectively against BCG (Green *et al.*, 2010; Lannang *et al.*, 2006; Mann *et al.*, 2009b). The ethanolic extract of the plant was shown to be active at a concentration of 0.050g/mL against *M. tb.* strain $H_{37}Rv$ and a clinical isolate (Adeleye *et al.*, 2008)

Tapinanthus sessifolia extract is highly active as the sole component in ETM 21 but inactive also as the sole component given for ETM 30. This inconsistency could arise from incorrect information but more likely from the taxonomic problems associated with the genus in Nigeria which has now been resolved (Ibrahim and Ayodele, 2011). The plant, mistletoe, being a parasite might also exhibit metabolite differences depending on its hosts.

Terminalia avicennioides is the only component given for ETM 25. Its extracts have been reported (Mann *et al.*, 2008, 2011) to yield arjunolic acid and friedelin which respectively had MICs against BCG of 156 g/mL and 4.9 g/mL. Our observations and the claims of the TMPs are in accord with these reports.

Tetrapleura tetraptera is the only component given for ETM 93. Oleanane type saponins and sulphates have been reported in the plant extracts and shown to be highly toxic to *Mollusca (*Aladesanmi, 2007*).* The same compounds may be the principles responsible for the anti*Mycobacterium* activities observed and reported in this work.

4.0 Conclusion

4.1 Efficacy of Nigerian herbal ethnomedices

Our study clearly showed that Nigerian herbalists have recipes that have likely been effective to some extent for the management of tuberculosis among the rural population of the Country. The recipes need to be fully analyzed for the purpose of potentially identifying new antituberculosis drug scaffolds and in the process, assist in the standardization of the local antituberculosis herbal recipes. The case has been made for applying 'omics' technologies to phytomedicines and traditional recipes which have historically been used over decades or centuries for the treatment of tuberculosis symptoms as a starting point for the discovery of new drugs and drug scaffolds (Shyur and Yang, 2008; Wells, 2011; Ngo *et al*, 2013). We anticipate that using 'omics' technologies in systems biology approaches combined with chemical informatics of various scaffolds characterized in active at least partially purified extracts, could make studies initiated around plants and indigenous herbal recipes relatively efficient in the rapid identification of new drug leads for tuberculosis (Boshoff and Lun, 2010; Cho *et al.*, 2006; Sundramurthia *et al.*, 2012; Tang and Marshall, 2011).

4.2 BCG as test organism for potential anti-*M. tb.* principles

On the basis of the fact that there is 77% qualitative agreement between the MIC results observed using BCG in place of *M. tb.* we conclude that usable information can be obtained using BCG to assess potential sources of anti-*M. tb.* principles where no facilities suitable for handling the more dangerous pathogenic *M. tb.* exist.

4.3 Prioritization criteria for selection of plants for detailed analysis

The following criteria are recommended for the prioritization of the plants for further studies: (i) potency of the extract based on the MIC values, (ii) published work on the biology and chemistry of the plants, (iii) novelty of information of the plant's use as anti-TB remedy and (iv) the frequency of occurrence of the plants in the collected recipes. Using these criteria, the following plants are recommended for the initial further studies: *Ficus sur*, *Pavetta crassipes*, *Combretum molle*, *Waltheria indica* and *Crotolaria lachnosema*, *Anogiessus leocarpus*, *Calliandra portoricensis*, *Cassia sieberiana*, *Abrus precatorius* and *Cussonia arborea*.

Acknowledgments

We thank the NIAID-NIH for support and for the award of postgraduate fellowships to N. Ibekwe and J. Nvau. Engineer B. Dogonyaro and M. Tsado and members of the NIAID-NIH IT team gave instrument and information technology supports. Bitrus Pam drove the vehicle that took the team safely to various parts of Nigeria to visit the TMPs. We appreciate his contributions to the success of the project. We thank Dr. Jemilat Ibrahim and Grace Ugbabe of the Herbarium, National Institute For Pharmaceutical Research And Development, Abuja, Nigeria who assisted in the identification of plant specimen samples. We acknowledge the administrative and logistics support enthusiastically given to the project by the then Director General of the National Institute for Pharmaceutical Research and Development, Dr. Uford S. Inyang. We thank the management and staff of NIPRD for their support. We are grateful to Katherine M. Perry who was the project manager and a source of strength and encouragement to the team.

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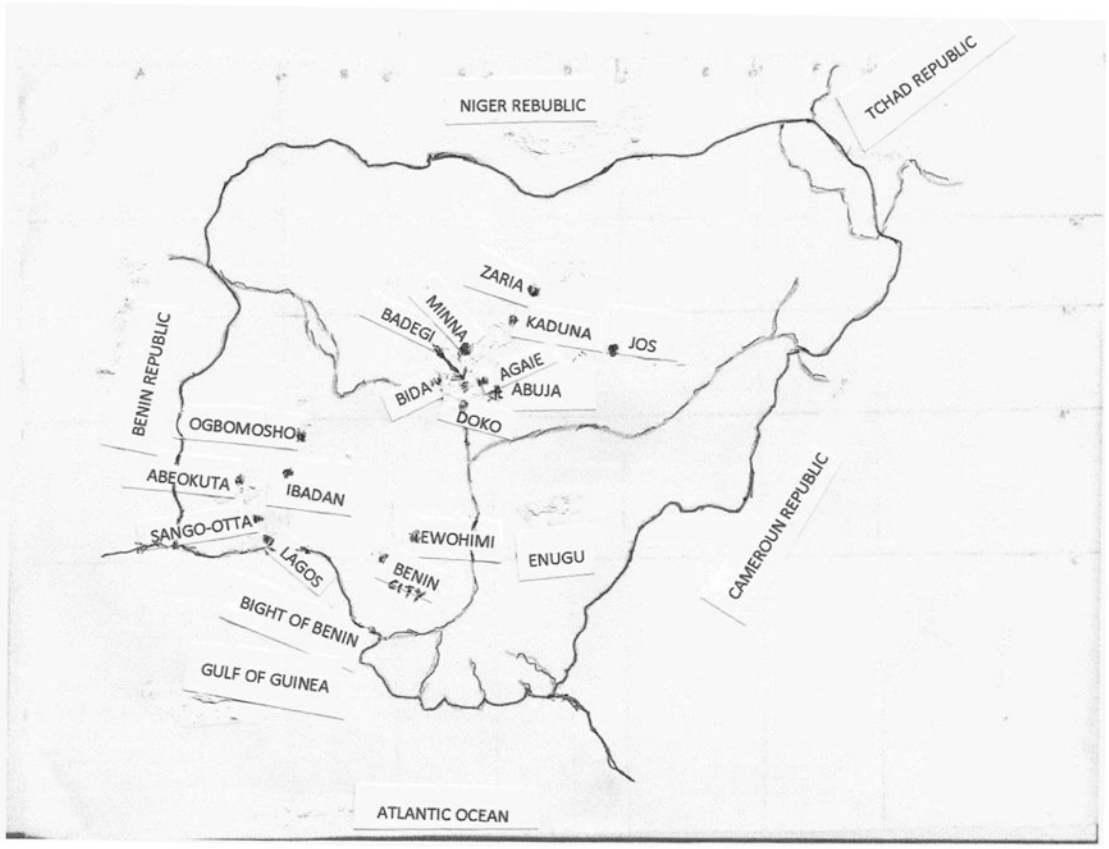

Fig.1. Sketch Map of Nigeria: TB Ethnomedicine Samples Collection Sites

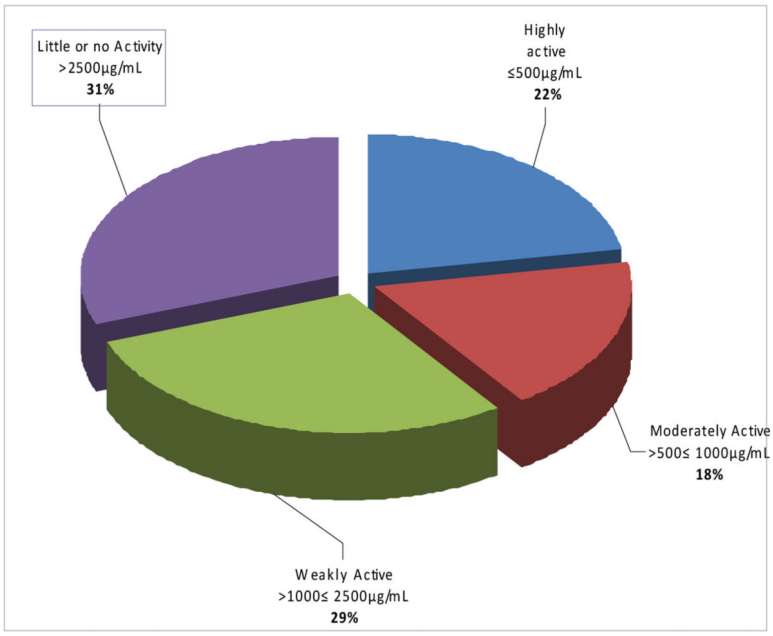

Fig 2. Activity percentages of ETMs against BCG

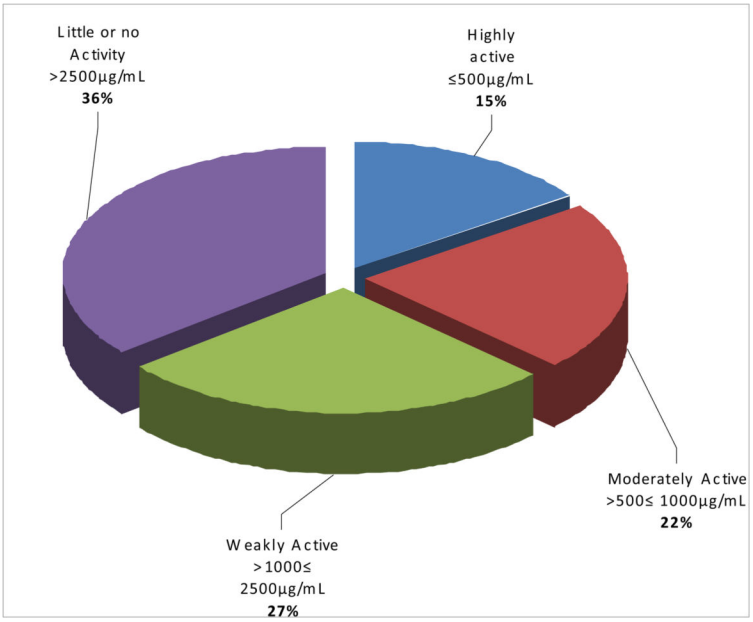

Fig 3. Activity percentages of ETMs against MTB

Table 1

Herbalists and their addresses

Extract designation ETM numbers	Town	State	Name of traditional healer
15	Minna	Niger	Alhassan Alhaji Baba
16	Badegi		Alhaji Amuda Soma
17	Agaie		Ladan Moh'd Ekogi
18	Zuba	Abuja	Market purchase: ETM 19 and 20 were purchased from different sellers.
19			
20		Niger	
21	Doko		Mohammed Isa
22	Badegi		Mohammed Kudu
23	Doko		Mohammed Abdullahi
24	Agaie		Shehu Ekogi
25	Bida		Alhassan Alhaji Baba
26			Records not available
27			Records not available
28	Abuja	FCT	Oladele Rahoof
29	not given	FCT/South East Nigeria	Ndulaka Ngozi Ezeji
30	Minna		Mohammed Aliyu Alhaji
31	Doko	Niger	Jibrilu Abubakar
32	Zaria		Abubakar Mohammed
33	Likoro		Alhassan Umaru
34	Zaria		Idris Bala
35		Kaduna	Sanusi Abdullahi Maimagani
36			Dan Makera Adamu
37	Kaduna		Mama Shehu Mai Magani
38	Zaria		Isa N. Mai Magani
39	Zaria		Rabiu Sale
40	Kaduna		Jibo Haruna
41			Jabbi Aliyu Danfulani
42	Zaria	Kaduna	Alhaji Dr. Audu Maimasaki
43	Gwakura		Mal Tanko Maiyasin
44	Tarfa		Idi Abdulahi
45	Jos	Plateau	Azijah Oyhu
46			Dachester Iliya Dung
47			Bazanfara Mohammed Jibril
48			Mailafiaya Audu
49			Maidori Ahmadu
50			Labaran Mohammed

Extract designation ETM numbers	Town	State	Name of traditional healer
51			Suraj Abdul Azeez
52			Iliya Rahila
53			Yakubu Hassan
54			Ibakwe F.C.
55			Zamani Imil
56			Bomo John
57			Shuaib Abubakar Dlakwa
58			Akaso Adigwu
59			Sankachi Abdullahi
60			Haruna Mary
61	Enugu	Enugu	Nnebe Olisa Isaac
62	Akajiofor	Anambra	Okeakpu Ifeoma
63	Enugu	Enugu	Igwe Sosmus Ozonnamalu
64			Obi D.C.N. Digbo
65			P.C. Uzonze Nkalebe
66			Echieteka Enyi
67			Agu Matthew
68			Obi D.C.N. Digbo
69			Nwabueze Chukwu
70		Abia	Akwakrija U. Alele
71	Enugu	Enugu	Ezeakor Chimaeze
72	Sango-Otta		
73		Ogun	Oloyede Akinhanmi Adesola (
74	Abeokuta		Oladehinde Fakemi
75		Lagos	A. Immanuel Uwa
76	Sango-Otta		Alani Oloyede
77			Alani Oloyede
78			Fadipe Rafiu
79		Ogun	Adeyinka Nureni
80	Abeokuta		Lawal Sakiru (Sako)
81			Salako Ganiyu (Alhaji)
82			Lawal Jimoh
83			Late Bamgboye Emmanuel Akanni
84		Ogun	Oloyede Omoleso
85	Sango-Otta		Oloyede Omoleso
86	Agbado		Oyewolu Hakeem Abore
87	Abeokuta		Global Herbs (Taro' Olu Adeola)
88			Bamgboye Morounfoluwa
89			Bamgboye Morounfoluwa

Extract designation ETM numbers	Town	State	Name of traditional healer
90	Ogbomosho	Oyo	Lawal Suleiman
91			Oluwofin Alhaja Bintu
92			Awodeji Thomas Agberi
93	Ewohimi	Edo	Innocent Enobakhale
94			
95			
96			Francis Agbon
97			
98, 99, 100	Bwari	Abuja	Adamu Muhammed

Table 2

Recipes and the MICs

ETM* nos.	Plant Composition in Recipe	Physical state of recipe	MIC in μg/mL (MTB)	MIC in μg/mL (BCG)
15	*Abrus precatorius* L. (Fabaceae)	Solid	1838	500
16	*Erythrina senegalensis* DC. (Fabaceae)	Solid	168	1200
17	*Ficus exasperata* Vahl. (Moraceae)	Solid	650	1000
18	*Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae)	Solid	925	266
19	*Cassia siberiana* DC. (Caesalpiniaceae)	Solid	150	2500
20	*Cassia siberiana* DC. (Caesalpiniaceae)	Solid	416	1625
21	*Tapinanthus sessilifolia* Polh. & Wiens.(Loranthaceae)	Solid	128	1000
22	*Securidaca longepedunculata* Fres. (Polygalaceae)	Solid	>2500	2125
23	*Guiera senegalensis* J. F. Gmel (Combretaceae)	Solid	919	1000
24	**a.** *Commiphora kerstingii* Engl. (Burseraceae), **b.** *Vernonia amagdalina* Del. (Asteraceae), **c.** *Abrus precatorius* L. (Fabaceae)	Solid	ND	2250
25	*Terminalia avicennioides* Guill & Perr (Combretaceae)	Solid	1200	2000
26		Solid	200	>2500
27	*Ximenia americana* Linn. (Olacaceae)	Solid	>2500	875
28	*Garcinia kola* Heckel (Guttiferae)	Solid	ND	2000
29	*Calliandra portoricensis* (Jacq.) Benth (Mimosaceae)	Solid	ND	563
30	*Tapinanthus sessilifolia* Polh. & Wiens. (Loranthaceae)	Solid	ND	>2500
31	*Combretum molle* R. Br ex G. Don (Combretaceae)	Solid	2000	250
32		Solid	>2500	>2500
33		Solid	2100	1000
34	**a.** *Cassia mimosoides* Linn (Caesalpiniaceae), **b.** *Waltheria indica* Linn (Sterculiaceae), **c.** *Ficus thonningii* Blume (Moraceae)	Solid	850	1125
35	**a.** *Ficus platyphylla* Del (Moraceae), **b.** *Nigella sativa* Linn (Ranunculaceae),	Solid	>2500	>2500

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ETM* nos.	Plant Composition in Recipe	Physical state of recipe	MIC in µg/mL (MTB)	MIC in µg/mL (BCG)
	c. *Ficus ingens* Miq. (Moraceae)			
36	*Ficus sur* Forrsk. (Moraceae)	Solid	750	813
37	**a.** *Antidesma venosum* E. Mey. ex Tul. (Euphorbiaceae), **b.** *Capiscum frutescens* Linn. (Solanaceace), **c.** *Piper guineense* Schum & Thonn. (Piperaceae), **d.** *Citrus aurantifolia* Swing. (Rutaceae)	Liquid	>2500	>2500
38	**a.** Solanum spp. **b.** *Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae), **c.** *Pavetta crassipes* K.Schum (Rubiaceae), **d.** *Jusiaea suffruticosa* Linn. (Onagraceae), **e.** *Tamarindus indica* Linn. (Fabaceae), **f.** *Zingiber officinale* Rosc (Zingiberaceae) **g.** *Butyrospermum parkii* Kotschy (Sapotaceae)	Solid	220	33
39	**a.** *Pavetta crassipes* K.Schum (Rubiaceae), **b.** *Azadirachta indica* A. Juss (Meliaceae)	Solid	198	1475
40	*Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae)	Solid	1013	988
41		Solid	894	2200
42	**a.** *Cussonia barteri* Hochst (Araliaceae), **b.** *Butyrospermum parkii* Kotschy (Sapotaceae), **c.** *Psidium guajava* Linn. (Myrtaceae), **d.** *Evolvulus alsinoides* Linn. (Convolvulaceae)	Solid	154	466
43	**a.** *Waltheria indica* Linn (Sterculiaceae), **b.** *Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae)	Solid	1488	156
44		Solid	250	200
45	**a.** *Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae), **b.** *Ficus sur* Forrsk. (Moraceae), **c.** *Securidaca longepedunculata* Fres. (Polygalaceae),	Solid	1344	>2500

ETM* nos.	Plant Composition in Recipe	Physical state of recipe	MIC in µg/mL (MTB)	MIC in µg/mL (BCG)
	d. *Pavetta crassipes* K.Schum (Rubiaceae)			
46		Solid	>2500	>2500
47		Liquid	>2500	ND
48	**a.** *Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae), **b.** *Acacia nilotica* Linn. (Fabaceae), **c.** *Garcinia kola* Heckel (Guttiferae), **d.** *Pilostigma thoningii* (Schum.) Milne-Redh (Fabaceae)	Solid	660	313
49	**a.** *Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae), **b.** *Citrus aurantifolia* Swing.(Rutaceae), **c.** *Zingiber officinale* Rosc (Zingiberaceae), **d.** *Allium sativum* Linn. (Liliaceae)	Solid	>2500	313
50	*Crotolaria lachnosema* Staph (Fabaceae)	Solid	1800	>2500
51	**a.** *Pavetta crassipes* K.Schum (Rubiaceae), **b.** *Securidaca longepedunculata* Fres. (Polygalaceae)	Solid	>2500	ND
52		Solid	1363	ND
53	*Cassytha filiformis* Linn. (Lauraceae)	Solid	950	>2500
54	**a.** *Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae), **b.** *Zingiber officinale* Rosc (Zingiberaceae)	Solid	875	1500
55		Solid	1813	ND
56		Solid	>2500	>2500
57	*Crotolaria lachnosema* Staph (Fabaceae)	Solid	869	ND
58		Solid	1406	1250
59		Solid	675	300
60	**a.** *Anogeisus leocarpus* (DC.) Guill & Perr (Combretaceae), **b.** *Securidaca longepedunculata* Fres. (Polygalaceae), **c.** *Pavetta crassipes* K.Schum (Rubiaceae)	Solid	2213	488
61		Liquid	>2500	1400

ETM* nos.	Plant Composition in Recipe	Physical state of recipe	MIC in μg/mL (MTB)	MIC in μg/mL (BCG)
62		Liquid	>2500	400
63	*Khaya grandifolia* C. Dc. (Meliaceae)	Solid	663	2000
64		Liquid	>2500	>2500
65		Liquid	>2500	>2500
66		Liquid	>2500	1000
67		Liquid	ND	ND
68		Solid	ND	ND
69		Liquid	625	>2500
70		Liquid	>2500	ND
71	*Ocimum gratissimum* Linn. (Lamiaceae)	Liquid	2400	>2500
72	*Pterocarpus osun* Craib (Fabaceae)	Liquid	1225	1100
73		Solid	388	ND
74		Solid	>2500	>2500
75	**a.** *Alstonia boonei* De Wild (Apocynaceae), **b.** *Ageratum conyzoides* Linn. (Asteraceae), **c.** *Garcinia kola* Heckel (Guttiferae)	Liquid	>2500	1125
76		Liquid	>2500	ND
77		Solid	506	2000
78		Solid	>2500	>2500
79		Liquid	>2500	>2500
80		Liquid	ND	300
81		Liquid	>2500	ND
82		Solid	188	350
83		Liquid	>2500	>2500
84		Liquid	>2500	2000
85		Solid	600	1000
86		Liquid	>2500	ND
87		Solid	>2500	ND
88		Solid	1425	>2500

ETM* nos.	Plant Composition in Recipe	Physical state of recipe	MIC in µg/mL (MTB)	MIC in µg/mL (BCG)
89	**a.** *Securidaca longepedunculata* Fres. (Polygalaceae), **b.** *Piper guineense* Schum & Thonn. (Piperaceae), **c.** *Eugenia aromaticum* (L.) Merr. & Perr. (Myrtaceae), **d.** *Capsicum frutescens* Linn. (Solanaceace), **e.** *Gladiolus daleni* van Geel (Iridaceae), **f.** *Allium cepa* L. var. aggregatum G. Don (Liliaceae)	Solid	1513	1900
90		Solid	>2500	>2500
91		Solid	1175	1125
92		Solid	775	ND
93	*Tetrapleura tetraptera* (Schum & Thonn.) Taub (Fabaceae)	Solid	277	1125
94		Solid	1875	1000
95	*Pentaclethra macrophylla* Benth. (Fabaceae)	Solid	1625	975
96	*Calliandria portoricensis* (Jacq.) Benth (Mimosaceae)	Solid	1175	228
97		Solid	1738	144
98		Liquid	ND	>2500
99	**a.** *Anogeisus leocarpus leocarpus* (DC.) Guill & Perr (Combretaceae) **b.** *Parkia biglobosa* (Jacq.) R. Br. ex G, Don f. (Fabaceae)	Solid	638	875
100		Solid	>2500	>2500

ETM = Recipe. ETMs 1-14 belonged to earlier investigation. Please note that the MIC values are not absolute but are results obtained using our screening procedure that may not have been appropriate for some of the ETMs especially for the ETMs that were not active in our experiments.