Vol. 12(23), pp. 346-352, 10 September, 2018
DOI: 10.5897/JMPR2018.6645
Article Number: A5BD8BD58441
ISSN: 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Evaluation of anti-*Mycobacterium tuberculosis* activity of fractions from selected medicinal plants used traditionally for treating cough and respiratory disorders in South West of Nigeria

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Received 13 July 2018; Accepted 6 August 2018

Tuberculosis is a contagious airborne infection that mostly affects the lungs. The causative agent of tuberculosis in human is *Mycobacterium tuberculosis*. The emergence and dissemination of *M. tuberculosis* isolates that are resistant to multiple antimicrobial drugs represent a growing public health threat. Fractions from *Alafia barteri*, *Chasmanthera dependence*, *Chrysophyllum albidum*, *Emilia coccinea*, *Mezoneuron benthamianum*, *Phyllanthus muellerianus*, *Secamoni afzeli*, *Senna alata*, *Xylopia aethiopica* and *Acalypha fimbriata* were screened for activity against drug susceptible *M. tuberculosis* H_{37}Rv and the local isolates using proportion and nitrate reduction methods. The organisms used were *M. tuberculosis* H37Rv strain and the local isolates from TB patients. The standard antitubercular drugs used were isoniazid and rifampicin. No fractions from *A. barterii*, *C. dependens*, *E. coccinea*, *S. afzeli*, *S. alata* and *X. aethiopica* showed sensitivity against the *M. tuberculosis* strains. The hexane fraction of *C. albidum*, butanol fraction of *M. benthamianum*, ethyl acetate fraction of *P. muellerianus* and ethyl acetate fraction of *A. fimbriata* showed sensitivity with minimum inhibition concentration of 0.5 mg/ml. The ethylacetate and hexane fractions of *M. benthamianum* together with hexane fraction of *P. muellerianus* showed sensitivity with MIC value of 1.25 mg/ml. The highest MIC value of 2.5 mg/ml was obtained from hexane fraction of *A. fimbriata*. Thus, *C. albidum*, *M. benthamianum*, *P. muellerianus* and *A. fimbriata* possessed antimycobacterium tuberculosis activity and further research work would be required to assess possible antitubercular agents present in the four medicinal plants.

Key words: Tuberculosis, anti-mycobacterium, fractions, sensitivity and inhibition.

INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. It is transmitted from an in active tuberculosis patient by exposure to tubercle bacilli air-borne droplets from coughing or sneezing.

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Tuberculosis spreads easily in overcrowded settings and in conditions of malnutrition and poverty. In 1993, the World Health Organization (WHO) declared tuberculosis a global emergency because it killed more adults each year than any other infectious disease (Kandel et al., 2008).

Tuberculosis still constitutes a major health problem in Nigeria. According to the WHO, the estimated incidence of TB in Nigeria is 322 per 100,000 population with only 15% of the total burden of the disease in the country being notified in 2015 (Onyedum et al., 2017). Over 80 % of TB cases in Nigeria were still undetected while it claimed over 1.5 million lives annually in the country (Olokor, 2017).

Mycobacterium tuberculosis is successful in surviving the presence of toxic compounds because they produce effective permeability barriers comprising the outer membrane and the mycolate-containing cell wall on the surface (Liu et al., 2016). The ability of the organism to remain dormant or persistent within host cells for many years with the potential to be activated allows the bacterium to escape the immune system of the host (Meena and Rajini, 2010). Survival mechanisms of the bacterium include prevention of phago-lysosome fusion (Pieters, 2008), prevention of cell acidification (Queval et al., 2017) and protection against reactive nitrogen intermediates (RNI) (Rousseau et al., 2004).

A person infected with *M. tuberculosis* incurs 10% risk of developing active TB (WHO, 2007). Major risk factors for TB activation include HIV infection, recent contact with an infectious patient, initiation of an anti-tumor necrosis factor (TNF) treatment, receiving dialysis, receiving an organ or hematologic transplantation, silicosis, being in prison, being an immigrant from high TB burden countries, being a homeless person and being an illicit drug users (WHO, 2018). Alcohol consumption, particularly heavy consumption, is an important risk factor for tuberculosis (Lonroth et al., 2008; Rehm et al., 2009).

Adverse effects of antituberculosis drugs, drug interactions, high cost of drugs, shortage of drugs and a complex long time therapeutic regimen still make TB one of the major health challenges in the world (Arbex et al., 2010; Sotgiu et al., 2015). The emergence of Multi-Drug Resistant Tuberculosis (MDR-TB) and Extended-Drug Resistant Tuberculosis (XDR-TB) strains has threatened the efficacy of many existing antibiotics (Calligaro et al., 2014; Prasad et al., 2017).

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug leads because of the unmatched chemical diversity (Mahalingam et al, 2011). Herbal drugs whether extract or decoction used against any pathogen will not cause the problem of drug resistance (Shashidhar et al., 2015).

Pure drugs or synthezised drugs are expensive and sometimes are not available in remote areas (Ammal and Bar, 2013). The search for new plant chemicals as antimicrobial agents becomes paramount because of an increase in antimicrobial resistance by pathogens and the emergence of new drug-resistant pathogens. Among the 11 currently used nature-derived TB drugs, seven of them were either isolated from microbes or semi-synthesized from microbial natural products (Liu et al., 2016) for example, streptomycin and kanamycin from *Streptomyces griseus* and capreomycin isolated from *S. capreolus* (Copp, 2003; Shu, 1998).

Rifampicin is a semi-synthetic drug that has been derived from Rifamycin, a product of *A. mediterranei* (Tribuddharat and Fennewald, 1999). Thus, plant kingdom can be looked at as an important source of new drugs for the treatment of TB because of its enormous chemical diversity (Gautam et al., 2007). The new drugs may not necessarily be new antibiotics but rather other drugs that prevent persistence within the host and leave the vegetative cells susceptible to treatment.

Anti-mycobacterium studies of some Nigeria medicinal plants demonstrate that they could be good sources of compounds with anti-mycobacterium activities worth of investigation (Mann et al., 2008; Ibekwe and Ameh, 2014). Some medicinal plants have been reported to possess anti-mycobacterium tuberculosis activity in Nigeria (Adeleye et al., 2008; Faleyimu et al., 2009). The selection of the ten medicinal plants is based on their usage by traditional practitioners in treating tuberculosis, cough or respiratory disorders.

The aim of this research work was to evaluate the anti-mycobacterium tuberculosis activity of the selected medicinal plants.

MATERIALS AND METHODS

Plant materials

The medicinal plants were obtained from Olokemeji Forest Reserve in Oyo state, Iberekodo market in Ogun state and Mushin market in Lagos state. The plants were identified by Mr T. K. Odewo of the Forestry Research Institute of Nigeria, (FRIN), Ibadan.

Preparation of extracts

80% ethanol solutions were added to the dried powdered samples of the plants. The mixtures were kept at room temperature for 72 h with gentle and intermittent shaking and thereafter filtered. Filtrates were dried at 42.5°C. Sequential extraction with hexane, ethyl acetate and butanol solvents were carried out. Table 1 shows the ten plants evaluated for anti-mycobacterium tuberculosis. Only *A. fimbriata* and *P. muellerianus* are of the same family, Euphorbiaceae, while others belong to different families. The Table 1 also shows the part of the plants used in the research work. The local name of the plant represents the name in Yoruba language. Table 2 shows the yield in both the 80% ethanol extraction and in the fractionation. No hexane fraction was obtained for *A. barteri*, *C. dependens*, *E. coccinea*, *S. afzelii, S. alata* and *X. aethiopica*. *A. fimbriata* was restricted to only hexane and ethylacetate partitioning.

Table 1. Plants for the anti-mycobacterium tuberculosis evaluation.

S/N	Name of plant	Family	Local name	Part of plant used
1	*Alafia barteri*	Apocynaceae	Agbari etu	Leaves
2	*Chasmanthera dependens*	Menispermaceae	Ato	Stem
3	*Chrysophyllum albidum*	Sapotaceae	Agbalumo	Cotyledon of seeds
4	*Emilia coccinea*	Compositae	Odundun'do	Leaves
5	*Mezoneuron benthamianum*	Leguminoseae	Jeninfinran	Leaves
6	*Phyllanthus muellerianus*	Euphorbiaceae	Egungun eja	Leaves
7	*Secamoni afzelii*	Asclepiadaceae	Ailu	Leaves
8	*Senna alata*	Caesalpiniaceae	Asunwon oyinbo	Leaves
9	*Xylopia aethiopica*	Annonaceae	Eru awonrika	Pods
10	*Acalypha fimbriata*	Euphorbiaceae	Jinwinni	Leaves

Table 2. Weight of extracts and fractions from the medicinal plants.

S/N	Medicinal plant	Weight of dried powdered sample (g)	Weight of ethanol extract (g)	Weight of butanol fraction (g)	Weight of ethylacetate fraction (g)	Weight of Hexane fraction (g)
1	*Alafia barteri*	50	0.95	0.57	0.28	-
2	*Chasmanthera dependens*	50	0.8	0.14	0.29	-
3	*Chrysophyllum albidum*	60	2.9	1.94	0.48	0.01
4	*Emilia coccinea*	50	0.8	0.13	0.25	-
5	*Mezoneuron benthamianum*	120	3.9	0.85	0.93	0.15
6	*Phyllanthus muellerianus*	50	1.2	0.16	0.34	0.01
7	*Secamoni afzelii*	40	1.7	0.05	0.13	-
8	*Senna alata*	34	1.2	0.16	0.25	-
9	*Xylopia aethiopica*	60	3.2	0.17	0.82	-
10	*Acalypha fimbriata*	60	1.2	-	0.21	0.05

Preparation of samples of roasted seeds of *C. albidum*

In line with the folklore usage of the seeds of *C. albidum* in treating tuberculosis infection, 100 sun dried seeds of the plant were put in a closed crucible and heated for 30 min at 50, 100 and 120°C separately. The seeds were removed from their shells after heating and were ground to powder for hexane extraction at room temperature. The yields were 0.13, 0.15 and 0.16 g of hexane extracts respectively. Various concentrations of the 50°C and the 120°C were subjected to anti-*M*. *tuberculosis* test as described above.

The test organisms

The reference *M. tuberculosis* strain $H_{37}R_V$ labelled PT_{12} and the local isolates labelled PT_{10} were used. The local isolates were isolated from TB patients using standard methods. The organisms were sub-cultured in Middle Brook 7H9 broth supplemented with OADC at 37°C for 21-28 days and were confirmed acid fast gram positive bacillus using Ziehl Nelson stain.

Anti-*M. tuberculosis* test

The anti-*M. tuberculosis* test was done using proportion method. 5 ml of the filtered extract solutions (DMSO as solvent) was added to 15 ml of the homogenized egg LJ media to arrive at various concentrations ranging from 0.5 to 0.5 mg/ml. Each 20 ml medium was divided into 10 ml in universal containers. Standard drugs, isoniazid and rifampicin, at 0.2 and 0.4 µg/ml respectively, were added to LJ media accordingly. The media were slanted to form slopes. The LJ slopes without extracts and drugs were used as control. The slopes were insipissated (the slopes were thickened) at 85° C for 45 min, cooled and stored in a refrigerator at 4°C. Sterility and viability check were carried out before inoculation.

Inoculation of slopes with the bacteria

Bacterial dilutions 10^{-5} and 10^{-3} mg/ml were prepared for inoculation. 0.1 ml of the chosen bacterial dilutions was inoculated into all the labelled LJ slopes (Adeleye et al., 2008). The universal containers were loosely closed with caps to allow evaporation and were incubated at 37°C. The specimens were checked on the 7^{th}, 14^{th}, and 21^{st} days to ensure no contaminations. Readings were done on the 28^{th} day.

Nitrate reduction test

Nitrate reduction test was performed on all the slopes after 28 days. This involved addition of 2 ml Nitrate Substrate Broth, incubation at 37°C for 2 h, addition of 1 drop of 50% hydrochloric acid, 2 drops of AFB Nitrate Reagent A (sulfanilamide 0.2%), 2 drops of AFB

Table 3. Results of anti-mycobacterium tuberculosis test.

S/N	Medicinal plants	Fractions	Weight (mg/ml)	*Mycobacterium tuberculosis*	
				PT_{12}	PT_{10}
1	*Alafia barteri*	Butanol	25	R	R
		Ethylacetate	10	R	R
2	*Chasmanthera dependens*	Butanol	5	R	R
		Ethylacetate	10	R	R
3	*Chrysophyllum albidum*	Butanol	50	R	R
		Ethylacetate	10	R	R
		Hexane	0.5	S	S
4	*Emilia coccinea*	Butanol	5	R	R
		Ethylacetate	10	R	R
5	*Mezoneuron benthamianum*	Butanol	25	S	S
		Ethylacetate	25	S	S
		Hexane	5	S	S
6	*Phyllanthus muellerianus*	Butanol	5	R	R
		Ethylacetate	10	S	S
		Hexane	0.5	S	S
7	*Secamoni afzelii*	Butanol	5	R	R
		Ethylacetate	5	R	R
8	*Senna alata*	Butanol	5	R	R
		Ethylacetate	10	R	R
9	*Xylopia aethiopica*	Butanol	5	R	R
		Ethylacetate	25	R	R
10	*Acalypha fimbriata*	Ethylacetate	10	S	S
		Hexane	2.5	S	S
11	Isoniazid	-	0.2 µg	S	S
12	Rifampicin	-	0.4 µg	S	S

PT_{12}, The standard strain of $H_{37}Rv$; PT_{10}, the local isolate strain, R, resistance (no inhibition of bacteria growth), S, sensitive (bacteria growth was inhibited).

Nitrate Reagent B (naphthylethylenediamine dihydrochloride, 0.1 %) and a pinch of Nitrate Reagent C (Zinc dust). Colour change was examined for resistance while no colour changes were examined for sensitive.

Determination of minimum inhibition concentration (MIC)

Minimum inhibition concentration of the eight anti-*M. tuberculosis* fractions from the four medicinal plants was determined. The stock solution for each fraction contained 0.2 g of sample in 20 ml of DMSO. Various dilutions were made to arrive at 2.5, 1.25, 0.75, 0.5 and 0.25 mg/ml. The LJ media were duplicated to serve both the standard Mtb strain, PT_{12} and the local strain, PT_{10} to obtain 80 LJ slopes of samples. The anti-mycobacterium tuberculosis test procedure described above was used.

RESULTS AND DISCUSSION

Fractions from ethanolic extracts of the ten medicinal plants were evaluated for anti-mycobacterium tuberculosis activities. Table 3 shows the results of the anti-mycobacterium tuberculosis test. Butanol, ethylacetate and hexane fractions from *M. benthamianum*,

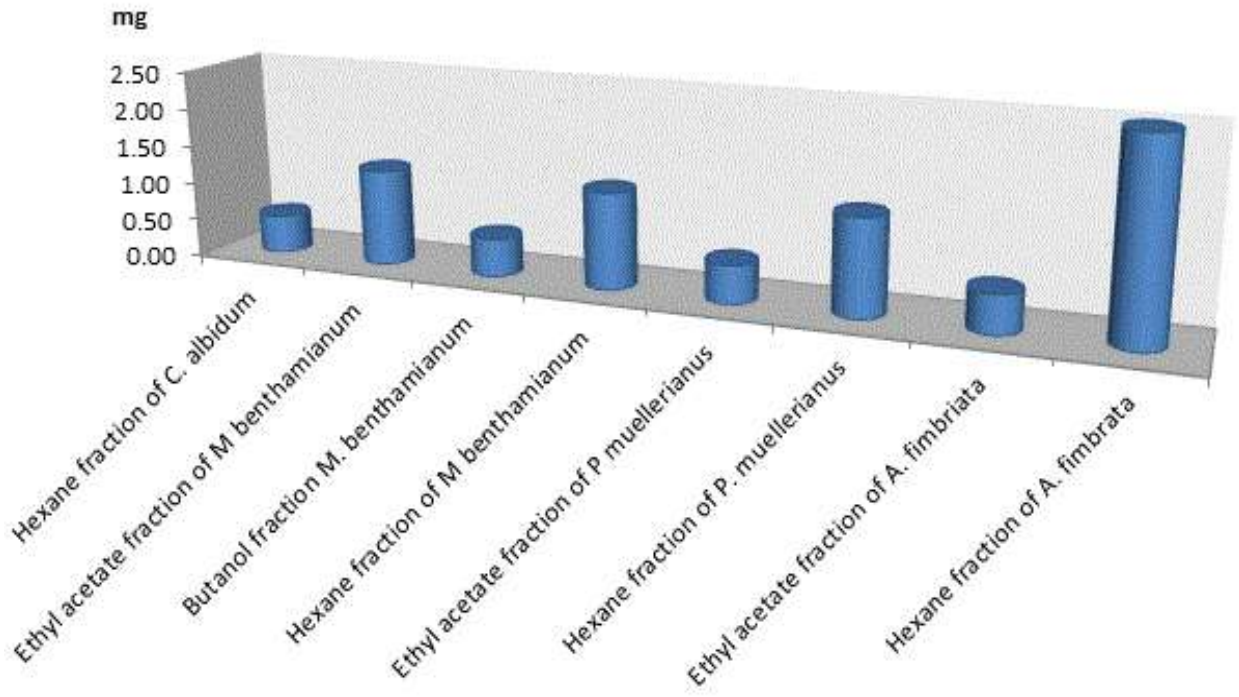

Figure 1. Minimum inhibition concentration of the anti-mycobacteruim fractions.

ethylacetate and hexane fractions from *P. muellerianus*, hexane fraction from *C. albidum* together with hexane and ethylacetate fractions from *A. fimbriata* inhibited the growth of the *M. tuberculosis* strains. No fractions from *A. barterii, C. dependens*, *E. coccinea*, *S. afzelii, S. alata* and *X. aethiopica* inhibited the growth of the bacteria. The medicinal plants were selected based on their antimicrobial activities and their traditional use in treating respiratory diseases.

The negative result obtained for *X. aethiopica* as shown in Table 3 is consistent with the earlier report by Adeleye et al. (2008) and Ogu (2011), of the ineffectiveness of the plant in inhibiting the growth of the *M. tuberculosis*. *X. aethiopica* had been reported to be antihypertensive (Gbadamosi and Kalejaye, 2017) and the association between hypertension and tuberculosis had been reported (Seegert et al., 2017). Thus, the use of *X. aethiopica* in combination therapy with other antitubercular medicinal plants by traditional practitioners of Southwestern Nigeria could have the advantage of limiting hypertension of the TB patients during the treatment period.

Figure 1 shows the results of the MIC. Hexane fraction of *C. albidum*, butanol fraction of *M. benthamianum*, ethylacetate fractions of both *P. muellerianus* and *A. fimbriata* had MIC value of 0.5 mg/ml. The ethylacetate and hexane fractions of *M. benthamianum* together with the hexane fraction of *P. muellerianus* showed minimum inhibition concentration of 1.25 mg/ml. The highest MIC value obtained was 2.5 mg/ml for hexane fraction of *A. fimbriata*. Extracts of *A. fimbriata* are used in the treatment of asthma and respiratory tract inflammation (Essiett and Okoko 2013). The anti-tuberculosis activity of an *Acalypha* specie, *Acalypha indica*, against multi-drug resistant *M. tuberculosis* isolates had been reported (Gupta et al., 2010).

Three fractions obtained from *M. benthamianum* inhibited the growth of *M. tuberculosis*. Gallic acid and its derivatives had been isolated from *M. benthamianum* (Tchinda et al., 2016). Gallic acid derivative isolated from another benthamianum species, *Disthemonanthus benthamianum*, had demonstrated antitubercular activity (Evina et al., 2017). Synthesized derivatives of gallic acid showed anti-tuberculosis activity (Ilango and Arunkumar, 2010).

The ethylacetate and hexane fractions from *P. muellerianus* showed inhibition of the bacteria. Its butanol fraction was not sensitive as shown in Table 3. The leaves extract of *P. muellerianus* was reported to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Doughari and Sunday, 2008). The leaves extract of *P. muellerianus* possessed anti-inflammatory property and the main constituent isolated from the plant, geraniin, had been shown to be anticarcinogenic, antihyperglycemic and antihypertensive (Boakye et al., 2016, Elendran et al., 2015). Only the hexane fraction of *C. albidum* inhibited the growth of *M. tuberculosis*. The traditional practitioners in Abeokuta, Ogun state part of

Table 4. Results of anti-mycobacterium tuberculosis test of the extracts of roasted seeds of *Chrysophyllum albidum*.

S/N	Samples tested	Concentrations	Mycobacterium tuberculosis	
			PT_{12}	PT_{10}
1	Hexane extract (50°C)	2 mg/ml	S	S
		1.5 mg/l	S	S
		0.4 mg/ml	S	S
2.	Hexane extract (120°C)	2 mg/ml	S	S
		1.5 mg/ml	S	S
		0.4 mg/ml	S	S
3	Rifampicin	0.4 µg/ml	S	S
4	Positive control	Agar inoculated only	R	R
5	Negative Control	Agar not inoculated	-	-

Nigeria, put roasted and powdered cotyledon of the seeds of *C. albidum* in honey for TB patients to lick for a period of one month. Table 4 shows the results of anti-*M. tuberculosis* test of the roasted seeds of *C. albidum*. There was no bacteria growth on the agar without inoculation (negative control) while there was growth on the agar inoculated (positive control). Both the hexane extracts of the 50 and 120°C roasted seeds showed sensitivity at 0.4 mg/ml. The fruit and the leaves extracts of *C. albidum* had been reported to possess high antimicrobial activity (George et al., 2018; Olasehinde et al., 2015).

Conclusion

There were no fractions from *A. barterii*, *C.dependens*, *E. coccinea*, *S. afzeli*, *S. alata* and *X. aethiopica* that showed sensitivity against the drug susceptible *M. tuberculosis* strains. Hexane fraction from *C. albidum*, butanol, ethylacetate and hexane fractions from *M. benthamianum*, ethylacetate and hexane fractions from *P. muellerianus* and *A. fimbriata* showed sensitivity against *M. tuberculosis* H_{37}RV and the local isolate from TB patients. The hexane extracts of the roasted seeds of *C. albidum* were also sensitive to the *M. tuberculosis* strains. The active fractions would be investigated for the presence of anti-mycobacterium tuberculosis agents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors sincerely acknowledge the Nigerian Institute of Medical Research, NIMR, Yaba, for granting the permission to use their tuberculosis laboratory facilities. And also sincere gratitude goes to Mr Nshiogu Michael, a member of staff of NIMR, for his technical assistance.

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