European Journal of Medicinal Plants
2(2): 125-131, 2012

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Anti-Mycobacterial and Toxicity Activities of Some Priority Medicinal Plants from Lake Victoria Basin, Tanzania

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Research Article

Received 9th August 2011
Accepted 18th September 2011
Online Ready 25th February 2012

ABSTRACT

Aims: This study has evaluated ethanol extracts from five medicinal plants selected through ethnobotanical study from Lake Victoria basin, Tanzania for their *in vitro* anti-mycobacterial activity against two *Mycobacterium species* and cytotoxicity against brine shrimp larvae.
Study Design: Laboratory experimental tests.
Place and Duration of Study: Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania, between July 2010 and July 2011.
Methodology: Five medicinal plants were selected from the priority list obtained from Lake Victoria basin, Tanzanian side. Collection, processing and drying of plant samples were done in the field with the assistance of a botanist while extraction and concentration of plant samples to obtain crude extracts were done in the laboratory following standard procedures. The plants included in this study are *Antidesma membranaceum*, *Crassocephalum manii, Entada abyssinica*, *Croton dichogamus* and *Rubia cordifolia*. The two fold microdilution method was used to determine the MIC values of extracts against

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two *Mycobacterium* marker strains (*Mycobacterium indicus pranii* and *Mycobacterium madagascariense)*. The cytotoxicity of plant extract was evaluated against brine shrimp larvae. Furthermore, the extracts were screened phytochemicaly to establish the group of compounds responsible for the activity.
Results: Among the tested extracts, the stem bark of *A. membranaceum* and *C. manii* showed moderate to mild activity against *M. indicus pranii* (MIC = 0.3125 mg/ml) and *M. madagascariense* (MIC = 0.625 mg/ml) respectively. Furthermore, *A. membranaceum* exhibited significant toxicity activity with LC_{50} value of 36.134 µg/ml against brine shrimp larvae. Other plants were moderately active when tested *in vitro* against the above organisms. Phytochemical screening of extracts indicated the presence of different classes of compounds.
Conclusion: This study has shown the potential of the priority medicinal plant extracts to be the source of possible lead compounds and anti-TB drug candidates needed for the management of Tuberculosis. Isolation of active principles from active fractions will be further undertaken.

Keywords: Extracts; phytochemical screening; anti-mycobacterial; cytotoxicity; Lake Victoria basin; Tanzania.

1. INTRODUCTION

Tuberculosis (TB) is the global health disaster which have not only killed but disabled millions of people in their prime productive life. Tuberculosis is caused by a micro-organism of the genus *Mycobacterium*, with its effect being compounded by the development of resistance to available antibiotics and by the emergence of HIV/AIDS and the associated opportunistic infections. It is believed that, one person is infected with tuberculosis in every second. The WHO report estimated to have 90 million new cases with 30 million deaths by the year 2010 mainly from sub-Saharan Africa (WHO, 1999). Further estimates by WHO indicated that two billion peoples or one third of the world's population are infected with *M. tuberculosis* (*M. tb*), the bacillus that causes the disease (USAID, 2010).

Problem of TB has become serious as *M. tuberculosis* developed resistance against both the first line and the second line drugs. Due to this, there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* all over the world (Singh, 2007). The spread of multi-drug resistant tuberculosis necessitates the discovery of new classes of antibacterials and compounds that inhibit macromolecules involved in these resistant mechanisms (Chimponda et al., 2010). Medicinal plants offer a hope for developing alternate medicines for the treatment of TB (Gupta et al., 2010). Several plants are used locally to treat TB-related disease (Green et al., 2010). Inventories of medicinal plants used to treat TB exist in the literature (Hutchings et al., 1996). In the East African region, many lists of plants used to treat TB exist. This includes work by Tabuti et al. (2003). However until now no systematic studies have been undertaken to identify priority species, practices and technologies for the treatment and control of TB. The main objective of this study was to determine the anti-mycobacterial and cytotoxic efficacy of ethanolic extracts of the priority medicinal plants from Lake Victoria basin, Tanzania.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The plant materials were collected from different parts in the Lake Victoria basin, Tanzania and identified by the ethnobotanist, Dr. Joseph N. Otieno of the Institute of Traditional Medicine. They include *A. membranaceum* Müll.-Arg*, E. abyssinica,* Steud. ex A. Rich. (Leguminosae), *C. dichogamus* Pax (Euphorbiaceae), *R. cordifolia* Linn. (Rubiaceae) and *C. manii* Hook.f. (Asteraceae). The voucher specimens (*A. membranaceum*- ITMOT 1*), E. abyssinica,* (ITMOT 2), *C. dichogamus* (ITMOT 3), *R. cordifolia* Linn. (ITMOT 4) and *C. manii* (ITMOT 5) were deposited in the Herbarium at the Institute of Traditional Medicine, Muhimbili University of Health and Health Sciences, Tanzania.

2.2 Preparation of Crude Extracts

Twelve grams (12 g) of the root barks of each dried plant material were soaked in ethanol (300 ml) for 48 hours at room temperature. The ethanol extracts were filtered and evaporated under vacuum on a rotary evaporator (BÜCHI Labortechnik AG-Switzerland). The crude extracts (20 mg) were dissolved in DMSO (5 ml) for bioassay.

2.3 Anti-Mycobacterial Test

2.3.1 Sub-culturing of *Mycobacterium species*

The strains of *Mycobacterium* were sub-cultured in Middlebrook 7H9 broth base supplemented with glycerol. This was followed by suspending about 1.2 g of Middlebrook 7H9 broth base in 240 ml of distilled water in a Scotch bottle (500 ml) followed by addition of 1 ml of glycerol. The mixture was heated and later autoclaved at 122°C for 15 minutes. The mixture was left to cool to 31 and 35°C under lamina flow hood, before separately being inoculated with *M. madagascariense* (MM) and *M. indicus pranii* (MIP) respectively. Hence MM was incubated at 31°C while MIP was incubated at 37°C. The optimal growth of the bacteria cultures was observed after 5 days.

2.3.2 Determination of minimum inhibition concentration (MIC) of extracts

The two fold microdilution method was used to determine the MIC values of extracts against two *Mycobacterium* marker strains by adopting the method by Eloff (1998), in the sterile flat-bottomed 96 - well polystyrene microtiter plates. Bacterial inoculums were prepared from five days grown cultures in middlebrook 7H9 broth base containing 0.1% tween 80 and the turbidity was adjusted to the equivalent of 0.5 McFarland units to “approximately 1.2 x108 CFU/ml”. The concentration of stock solution of all test extracts before serial dilutions was 20 mg/ml. The extracts were serially diluted two folds with a broth base containing 0.1% tween 80. The serial dilution was performed by addition of 50 µl of extracts into the first well which had 50 µl of broth base, and thereafter mixed well and transferred 50 µl of the first well sample-broth base mixture to next and subsequent wells of each row. The remaining 50 µl of the mixture was discarded from the last well of the row. This was followed by the separate inoculation of 50 µl of mycobacteria cultures in each well, to complete a twofold broth microdilution. Two additional wells were used as growth controls, where no drugs were added as negative control, and while a row with inoculums and control drugs were used as positive control. The inoculated microtiter plates were incubated at 31°C for MM and 37°C

for MIP for 24 hours. To determine the MIC values of extracts, 40 µl (0.2 mg/ml) iodonitrotetrazolium (INT) chloride salt was added into each well and plates incubated at 31 and 37°C for 1 hour. The minimal inhibitory concentration (MIC) value of each extract was read at the concentration where a marked reduction in color formation due to bacterial growth inhibition was noted. Positive control used in this study was Ciprofloxacin.

2.4 Brine Shrimps Lethality Test

The brine shrimp lethality test (BST) was used to predict the presence of toxicity in the extracts. The experiment was set according to Meyer et al. (1982). Briefly, stock solutions "(40 mg/mL) of all extracts were prepared in DMSO". Different levels of concentrations (240, 120, 80, 40, 24 and 8 µg/mL) were prepared by drawing different volumes from the stock solutions and then added into vials, each containing ten brine shrimps larvae. The volume was then adjusted to 5 mL with artificial sea water prepared by dissolving 3.8 g of sea salt in 1 L of distilled water. Each level of concentration was tested in trplicate. The negative control contained brine shrimp, artificial sea water and DMSO (0.6%) only. The vials were incubated under light for 24 h. The dead larvae were counted and mean was subjected to analysis using Fig P computer program (Biosoft Inc, USA).

2.5 Phytochemical Screening for the Crude Extracts

The methods of Trease and Evans (1983); Harbourne (1983) to test for alkaloids, tannins, flavonoids, steroids and saponins were used.

An amount 0.3 g of the extract was dissolved in 3 ml of methanol and heated. 1.0 g of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the presence of flavonoids or any other phenolic compounds.

About 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl and warmed on steam bath. The filtrate (1 ml) was mixed with 3 drops of Dragendorff's reagent. Reddish orange precipitation was considered as indicative of the presence of alkaloids.

The extract (1 g) was dissolved in 20 ml of distilled water and filtered. Three drops of 10% of $FeCl_3$ were added to 2 ml of the filtrate. The appearance of blackish-blue or blackish green colouration was indicative of tannins. Some 2 ml of the filtrate was added into 1 ml of bromine water and a precipitate was taken as positive for tannins.

The 7% blood agar medium was used. The extract in methanol was applied with distilled water and methanol used as negative control while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35°C for 6 hours. A total haemolysis of the blood around the extract was indicative of saponins.

About 0.5 g of the extract was dissolved in 3 ml of $CHCl_3$ and filtered. Concentrated H_2SO_4 was added to the filtrate. A reddish brown colour was taken as positive for steroid ring.

2.6 Data Analysis

The mean results of the percentage mortality were plotted against the logarithms of concentrations using the Fig P computer program. Regression equations obtained from the

graphs were used to obtain LC_{16}, LC_{50}, LC_{84} and the 95% CI values. An LC_{50} value greater than 100 µg/mL is considered to represent an inactive compound or extract.

3. RESULTS AND DISCUSSION

3.1 Anti-Mycobacterial Activity

Two non-pathogenic *Mycobacterium species* namely, *M. madagascariense* (MM) and *M. indicus pranii* (MIP) were used to determine the anti-mycobacterial potential of ethanolic extracts of the priority medicinal plants. Among the tested extracts, the root bark of *A. membranaceum* exhibited higher anti-mycobacterial activity against *M. indicus pranii* while the root bark of *C. manii* showed moderately high activity against *M. madagascariense*. The *A. membranaceum* extract had MIC value of 0.3125 mg/ml against *M. indicus pranii* and the ethanol extract of *C. manii* showed the MIC value of 0.625 mg/ml against *M. madagascariense* (Table 1). The rest of the tested extracts showed mild activity with MIC ranging from 1.25-2.5 mg/ml against the organisms under this study (Table 1).

Table 1. Antimycobacterial activity of the ethanolic extracts of priority medicinal plants from Lake Victoria basin

Sl. No.	Plant extract	Minimum Inhibition Concentration (MIC) in mg/ml	
		M. madagascariense	***M. indicus pranii***
1	*A. membranaceum*	1.25	0.3125
2	*E. abyssinica*	2.5	2.5
3	*C. dichogamus*	1.25	1.25
4	*R. cordifolia*	2.5	1.25
5	*C. manii*	0.625	1.25
	Ciprofloxacin	<0.05	<0.0

3.2 Brine Shrimp Lethality Test

The brine shrimp lethality test (BST) was used to predict cytotoxicity properties of plant extracts under investigations. Results revealed that all extracts were less toxic to brine shrimp larvae (Table 2). The ethanolic extract of *A. membranaceum* indicated to be toxic to shrimp larvae (LC_{50} value of 36.13 µg/ml) than any other tested extracts (Table 2). All test samples were far less toxic to shrimps compared to a standard anticancer drug cyclophosphamide which had LC_{50} value of 16.3 µg/ml.

Table 2. Cytotoxicity activity (BST) of ethanol extract of priority plants

Sl. No.	Plant extract	LC_{50} (µg/ml)	95% Confidence interval (µg/ml)
1	*A. membranaceum*	36.13	26.8– 48.60
2	*E. abyssinica*	41.70	32.992 – 52.7
3	*C. dichogamus*	40.70	31.138 – 53.192
4	*R. cordifolia*	3839.90	1368.0 – 10778.8
5	*C. manii*	57.2	40.53 – 80.8
	Cyclophosphamide	16.3	10.60 – 25.15

3.3 Phytochemical Screening

All plant extracts were subjected to phytochemical screening methods that revealed the presence of different classes of compounds, namely alkaloids,phenolics, terpenoids, saponins, tannins and steroids (Table 3).

Table 3. Phytochemical screening of extracts from priority medicinal plants roots from Lake Victoria basin

Plant name	Class of compounds tested*					
	Tannins	Saponins	Phenolics	Terpenoids	Steroids	Alkaloids
A. membranaceum	-	-	+	++	+	++
E. abyssinica	-	-	-	++	+	-
C. dichogamus	-	-	-	++	+	-
R. cordifolia	-	-	++	++	-	-
C. manii	+	+	+	++	+	-

* *- = Absent; + = present (small amount), ++ = Present (large amount)*

The present study has shown that some crude extracts from the six priority medicinal plant species collected from Lake Victoria basin, Tanzania have both cytotoxic and anti-mycobacterial activities. Out of the six extracts tested in an anti-mycobacterial assay, two extracts (stem barks of *A. membranaceum and C. manii*) showed significant activities against *M. indicus pranii and M. madagascariense* respectively. Phytochemical screening indicated *A. membranaceum* to contain mainly triterpenoids and alkaloids while *C. manii* contains mainly triterpenoids. The literature indicated that these classes of compounds are widely reported from these genera displaying different biological activities (Bringmann et al., 2000; Chhabra et al., 1984; Hegazy et al., 2008; Steenkamp et al., 2009). Comparing the activities of extracts six medicinal plants under this investigation and the standard antibiotics, Ciprofloxacin had much higher activities than all plant extracts. Since there is no any reported anti-mycobacterial activity for the active plants, there is a great need to isolate the compounds responsible for the noted activity.

The cytotoxicity on the brine shrimps revealed that all extracts were less toxic to brine shrimps (Table 2) compared to the standard anticancer drug cyclophosphamide which had LC_{50} value of 16.3 µg/ml.

4. CONCLUSION

The significant anti-mycobacterial and cytotoxicity activities of crude extracts of six priority medicinal plants from Lake Victoria basin, provides a good opportunity for drug development in this area of study. In particular, *A. membranaceum and C. manii* showed high activities, hence the continuation of study on these two plant species is crucial to isolate, characterize and identify the bioactive compounds responsible for the observed pharmacological activities.

ACKNOWLEDGEMENTS

We are grateful for the support from the generous funding from VicRes in Kampala, Uganda. We also appreciate a collaboration we get from Traditional Practitioners in Lake Victoria region, Tanzania side and the efforts made by other respondents and research assistants.

The assistance of the host institution, Muhimbili University of Health and Allied Sciences is highly acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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