



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIMYCOBACTERIAL ACTIVITY OF ETHANOLIC ROOT EXTRACTS FROM THREE TANZANIAN MEDICINAL PLANTS

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ABSTRACT

This study, screened crude extracts of *Croton dichogamus*, *Antidesma membranaceum* and *Crassocephalum manii* against two fast growing non-pathogenic *Mycobacterium* species namely *Mycobacterium madagascariense* DSM 44641 and *Mycobacterium indicus pranii* DSM 45239. These organisms were used as markers to determine anti-TB activity of extracts. The two folds micro-dilution method was used to determine minimum inhibitory concentration (MIC) of extracts. The ethanolic extract from *C. dichogamus* had activity against *Mycobacterium indicus pranii* (MIC 0.3125 mg/ml). Likewise, *C. manii* against *Mycobacterium madagascariensis* (MIC 0.625) as compared to standard isoniazid with no activity but less active compared to standard ciprofloxacin (<0.05mg/ml). Crude extract of *A. membranaceum* exhibited moderate activity (MIC 1.25mg/ml). Generally, extracts of the selected plants showed activity against mycobacteria and this substantiate their use by traditional health practitioners for management of tuberculosis patients, and suggests further research for possible development of anti-tuberculosis drugs.

Keywords: Antimycobacterial Activity, Medicinal Plants, Tuberculosis, Tanzania

INTRODUCTION

Tuberculosis (TB) cost millions of lives in the world. In African especially East Africa, TB is responsible for 1500000 deaths. In developing countries such as Tanzania, TB is one of the major communicable disease responsible for about 11% of deaths

countrywide [1]. For many years efforts have been made to combat tuberculosis using anti-TB drugs such as rifampicin and isoniazid. Unfortunately, the emergence of Multi-drug resistant strains and HIV infection has fuelled the spread of the infections [2]. This is because some strains do not respond to the current anti-TB drugs rifampicin and isoniazid [3-4] while individuals who are immuno-compromised by HIV/AIDS becomes more susceptible to Tuberculosis infection. Due to these reasons, there is higher need for discovery of new therapy for combating this infection. This project aimed at investigating the antimycobacterial activity of medicinal plants that are used in Rorya and Musoma Rural Districts in Tanzania by traditional Health Practitioners (THPs) to manage patients suffering from health conditions related to TB by THPs in the study area. Some of the high ranked medicinal plants in this ethnobotanical survey were selected for bioassay test namely *Croton dichogamus Pax.*, *Antidesma membranaceum Müll.Arg.* and *Crassocephalum manii* (Hook.f.) Milne-Redh.

METHODOLOGY

Collection and Extraction of Plant Materials

The whole roots of *A. membranaceum*, *C. dichogamus* and *C. manii* were collected from

Rorya and Musoma Rural districts, in Mara region Tanzania between June and August 2012. At such months, most plants in the study area have dropped their foliage and the secondary metabolites are concentrated in the storage organs including the roots. Identification of plants was done in the field and plant voucher specimen deposited in the Herbarium of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences. Plant materials were dried under shade for two weeks and thereafter macerated into powdery form. About 300 g of powdered plant materials for each plant species was extracted using ethanol. Extraction process was done three times for 24 hours before concentrating the extracts in *vacuo* using rotary evaporator.

Chemicals and Growth Media

Solvents were purchased from Carlo Erba (France), Middlebrook 7H9 broth base was obtained from HIMEDIA (India), Glycerol (AR) obtained from Lab Equip Ltd (Tanzania), iodinitrotetrazolium (INT) chloride, Ciprofloxacin (R&D) and Isoniazid (R&D) were purchased from Sigma (UK), Cyclophosphamide was purchased from Sigma Aldrich (South Africa). 96 wells microtitre plates supplied by KAS Medics (Tanzania).

Test Organisms

The two test organisms namely *Mycobacterium madagascariense* (MM) DSM 44641 and *Mycobacterium indicus pranii* (MIP) DSM 45239 were supplied by DSMZ – the Germany Resource Centre for Biological Materials, Braunschweig, Germany. The two fast growing mycobacteria strains were used as markers for determination of a potential anti-TB efficacy of extracts.

Sub-Culturing of *Mycobacterium* Species

The *Mycobacterium* strains were sub-cultured in Middlebrook 7H9 broth base supplemented with glycerol. 1.18 g of Middlebrook 7H9 broth base was suspended in 230 ml of distilled water in a Scotch bottle (500 ml) followed by addition of 1 ml of glycerol (AR). The mixture was heated to dissolve the broth base completely, thereafter autoclaved at 121°C for 15 minutes. The mixture was left to cool to 31 and 35°C under lamina flow, before separately being inoculated with MM and MIP respectively. Thereafter 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, and thus ready for antimycobacterial screening.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of extracts against two *Mycobacterium* strains was determined by two fold microdilution method [5]. 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 (approximately 1.2×10^8 CFU/ml). The concentration of stock solution of all test extracts and compounds before serial dilutions were 20 mg/ml and 6.4 mg/ml respectively. 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 50µl 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 two fold 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 *M.*

madagascariense and 37°C for *M. indicus pranii* for 24 hours.

To determine the MIC values of extracts 40µl (0.2 mg/ml) iodinitrotetrazolium (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. Isoniazid and Ciprofloxacin were used as positive controls, negative control was broth with DMSO and

solvent control was DMSO both in the culture media except the sterility control.

RESULTS

The ethanolic root extract of *C. dichogamus* exhibited highest activity against *Mycobacterium indicus pranii*, while, the *C. manii* extract showed high activity against *Mycobacterium madagascariense* (Table 1).

Isoniazid showed no activity to MIC value 0.05mg/ml that was the cutoff point and also its reported MIC value. Ciprofloxacin showed activity to 0.05mg/ml that was the cutoff point.

Table 1: MIC Results of Ethanolic Root Extracts from *A. Antidesma membranaceum*, *Crassocephalum manii* and *Croton dichogamus*

Extract	Minimum inhibitory concentration (mg/ml)	
	<i>Mycobacterium madagascariense</i>	<i>Mycobacterium indicus pranii</i>
<i>Croton dichogamus</i>	1.25	0.312
<i>Antidesma membranaceum</i>	1.25	1.25
<i>Crassocephalum manii</i>	0.62	1.25
Isoniazid	NA*	NA
Ciprofloxacin	< 0.05	< 0.05

*NA = Not Active

DISCUSSION

The ethanolic root extracts from all plant species exhibited significant activity against tested Mycobacterial strains used in the study. Previous reports show that *A. membranaceum* is rich in terpenoids that possess antimycobacterial activity [6, 7]. Furthermore, *C. dichogamus* has also been reported to possess terpenoids and alkaloids [8]. These natural products may be responsible for the exhibited activity against tested microbes. The extracts from *C. manii* exhibited a

significant activity against *M. madagascariense* and *M. indicus pranii*. This indicated that the extracts are good candidate for further anti-TB investigation.

Further observation revealed that isoniazid (INH) a standard anti-TB drug lacked efficacy against the two mycobacteria strains used. This observation corroborate with the reported findings on the resistance of some *Mycobacterium* species against first line anti-TB chemotherapies [9]. Because the organisms were not sensitive to INH while

showing sensitivity against extracts from the roots of studied plant species, is indicative of a promising activity worthy for further anti-TB investigation.

CONCLUSION

The observed antimycobacterial efficacy of the extracts of this plant species supports the ethnomedical claims that the plant species possess anti-TB activity. However more work involving *in-vivo* experiments on extracts and structure elucidation of a pure compounds needs to be done in a different research for more confirmation of the observed activities.

ACKNOWLEDGEMENT

Authors appreciate cooperation of traditional health practitioners in Lake Victoria region for availing the information on plants used for management of respiratory infections. Support by the host institution the Muhimbili University of Health and Allied Sciences is highly appreciated.

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