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In vitro Antimycobacterial Activity of *Sterculia quinqueloba* (Garcke) K. Schumand *Canthium crassum* Hiern

Elihuruma Wilson[1*], Musa Chacha[1] and Justin Omolo[2]

[1]Department of Biodiversity and Sustainable Agriculture, Nelson Mandela African Institution of Science and Technology, P.O.Box 447, Arusha, Tanzania.
[2]Department of Traditional Medicine Research and Environmental Science, National Institute for Medical Research, P.O.Box 9653, Dares Salaam, Tanzania.

Authors' contributions

This work was carried out in collaboration between all authors. All the authors have cordially supported the work and preparation of the manuscript. Author EW designed and supervised the study and prepared the first draft of the manuscript. Authors MC and JO advised and guided the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To screen for the anti-mycobacterial activity of *Canthium crassum* and *Sterculia quinqueloba* using two mycobacteria species the *Mycobacteria madagascariense* and *Mycobacteria indicuspranii.*
Study Design: *In vitro* assay of anti-mycobacterial assay was done using 96-well micro-dilution method.
Place and Duration of Study: School of Life Science and Bioengineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania, from April 2014 to June 2014.
Methodology: 96-well-microtitre serial micro-dilution method was used to determine anti-

**Corresponding author: E-mail: wilsonkilango@yahoo.com;*

mycobacteria activity to plant extracts.
Results: All extracts exhibited anti-mycobacterial activity to both mycobacteria tested. The minimum inhibition concentration (MIC) ranged from 0.39 – 12.5 mg/mL, with ethyl acetate leaf extract of *S. quinqueloba* being the most active extracts with MIC value of 0.39 mg/mL against *Mycobacteria madagascariense* (MM) and 0.78 mg/mL against *Mycobacteria indicuspranii* (MIP). Petroleum ether and ethyl acetate leaf extract of *C. crassum* also gave MIC value of 0.78 mg/mL against MM and MIP.
Conclusion: Findings from the present study showed that both plants exhibited activity against mycobacterium species tested. These plants may therefore serve as a source for new ant-mycobacterium drugs worth further studies including isolation and identification of the active compounds.

Keywords: Anti-mycobacterial; Canthium crassum; Sterculia quinqueloba.

1. INTRODUCTION

According to the World Health Organization (WHO), tuberculosis (TB) caused by micro-organism of genus *Mycobacterium* is a second to HIV/AIDS as the greatest killer worldwide and resulted in deaths of approximately 1.3 million patients in 2012 alone. It is a leading killer of people living with HIV and over 95% of the deaths occur in the developing countries [1]. The most available treatment requires a long lasting (at least six months) multi-drug scheme which causes difficulties to patients. Similarly, the recorded multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *Mycobacterium tuberculosis* [2], increases the hurtful to a patient's health due to repeated use of the drugs and increased levels of multi-drug toxicity to the patient's body [3]. Recent records showed that in 2012 alone, about 450,000 people developed multidrug-resistant tuberculosis; most of them were from developing countries [1].

Africa which is the poorest continent in the world, faces the worst tuberculosis epidemic ever since time immemorial [4]. It is a home of approximately 13% of the world population but faces approximately 30% of the global burden of all the reported TB cases [4,5]. This is also trickle down to many poorest African countries like East African countries. For instance, Tanzania is among the 22 countries with highTB-burden in the world with approximately more than 70,000 new cases annually and in 2007 alone with approximately 292 incidences per 100,000 population [6,7]. This growth of tuberculosis cases in Tanzania and other parts in Africa is attributed by many factors including ineffective treatment programme for TB control, HIV epidemic and continued transmission due to low level of population precaution [6,8,9], this factors have also contributed much into the emergence and spread of MDR and XDR strains [7,8].
This emergence and spread of MDR and XDR strains and the recorded high human death rate due to TB infections, necessitate the discovery of new classes of antibacterial and compounds that can inhibit the growth of these micro-organisms [10,11]. It is also important to ensure that the discovery process of the new anti-TB agents is seriously undertaken, since most of the affected individuals in developing countries like Tanzania are in productive age class, thus put development activities to these countries in jeopardy [12]. Medicinal plants offer a great hope for developing alternative medicines for the treatment of TB due to their phytochemical diversity [2,3]. Since ancient times, several plants have been used locally to treat various ailments including TB-related diseases [13,14], these include plants from East Africa [15,16]. The genus *Sterculia* and *Canthium* are well represented in East Africa and known to comprise antimicrobial activities; some of which have demonstrated anti-TB activity. For instance, Babalola et al. [17] and Yang et al. [18] observed that *Sterculia setigera* and *Canthium horridum* respectively had promising antimicrobial activity with *S. setigera* exhibiting promising anti-TB activity.

Sterculia quinqueloba (Garcke) K. Schum (Malvaceae) and *Canthium crassum* Hiern (Rubiaceae) are tree plant species found in eastern and southern Africa countries and which are medicinally used to some of the communities to treat various ailments including diarrhea, skin diseases, earache and venereal diseases [19,20]. The anti-bacterial and anti-fungal activities of extracts from dried leaves, stem and root barks of these plants against common microbes such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*

pneumonia, Staphylococcus aureus, Vibrio cholera, *Shigella flexineri, Proteus vulgaris, Salmonella typhi, Klebsiella oxytoca, Cryptococcus neofarmans* and *Candidas albicans* have been reported [21]. In this respect, the current study was undertaken to evaluate the anti- mycobacterium activity of extracts from the two plants, the *S. quinqueloba* and *C. crassum*.

2. MATERIALS AND METHODS

2.1 Solvents, Reagents and Growth Media

Methanol was bought from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) and Dimethyl sulfoxide (DMSO) was purchased from RFCL Limited, Hayana, India whereas petroleum ether and ethyl acetate were purchased from Loba Chemie Pvt Ltd, Mumbai, INDIA. Middle Brook 7H9 Broth was purchased from Sigma-Aldrich® Co whereas standard TB drugs; Isoniazid, Rifampicin and kanamycin were supplied by Macleods Pharmaceuticals LTD-Atlanta Arcade, Marol Church Road, Andheri (E), Mumbai-400-059, India.

2.2 Collection of Plant Materials and Extraction

Mr. Haji Seleman, a botanist from University of Dar es Salaam identified leave, stem and root barks of *S. quinqueloba* and *C. crassum* which were collected inKigoma region atGombe National Park. The specimens were kept at Nelson Mandela Africa Institution of Science and Technology (NM-AIST) with voucher specimen numbers 2141 and 2142 for *S. quinqueloba* and *C. crassum* respectively. The samples were dried under the shade and then powdered into the fine particles. The powdered leave of *S. quinqueloba* and *C. crassum*, stem bark of *S. quinqueloba* and root bark of *C. crassum* were sequentially extracted through maceration using petroleum ether, ethyl acetate and methanol for 24 hours. The extracts were then filtered through muslin cloth on a plug of glass wool in a glass column and solvents were separated from extract using rotary evaporator and pure extract were stored in a refrigerator at 4℃.

2.3 Sub-culturing of the Mycobacteria Strains

The *S. quinqueloba* and *C. crassum* extracts were evaluated against *Mycobacteria madagascariense* (DSM 44641) and *Mycobacteria indicuspranii* (DSM 45239) supplied by DSMZ - The Germany Resource Centre for Biological Materials, Braunschweig, Germany. These strains were used as a marker for determination of potential anti-tuberculosis efficacy of extracts. The sub-culturing activity was conducted as explained by Mwembela et al. [22], by simply sub-culturing the strains in liquid media and Middle brook 7H9 broth base. 0.64g of Middlebrook 7H9 broth base was suspended in 115 mL of distilled water in two separate Scotch bottles of 250 ml each. Thereafter, 0.5 ml of glycerol (AR) was added into each scotch bottle and the mixture was thoroughly shaken to dissolve the broth completely and then autoclaved at 121℃ for 15 minutes. Thereafter, the mixture was left to cool to 31 and 37℃ under lamina flow before being inoculated with *Mycobacteria madagascariense (MM)* and *Mycobacteria indicuspranii (MIP)* respectively. Later on, *MM* and *MIP* were incubated at 31℃ and 37℃ respectively.

2.4 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) of the extracts against two standard *Mycobacterium* were determined by micro dilution method [23] done in triplicate using 96 well microtitre plates. Firstly the plates were loaded with 50 µL of the broth media followed by addition of 50 µL of the extract (100 mg/mL) into first wells of each row tested to make a total volume of 100 µL in each first wells. After thorough mixing of first row of each plate, 50 µL were taken from each of the first row wells and added into the next row wells. This process was repeated down the columns to the last wells at the bottom at which 50 µL taken from the last rows of each column were discarded. Thereafter, 50 µL of approximately 0.5 MacFarhland standard turbidity of both MM's and MIP's suspension were added into each well to make the final of 100 µL in each well. Additionally, the rows containing DMSO were used as negative control and the rows with broth and bacteria only were used to monitor bacterial growth whereas the rows containing isoniazid, rifampicin and kanamycin were used as a standard positive control drugs. Thereafter, the plates were incubated at 32℃ and 37℃ for MM and MIP respectively for 24 hours. The minimum inhibitory concentrations were then determined by addition of 20 µL of 0.02% *p*-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation for 1 hour at 32℃ and 37℃. Change to pink color was taken as an

indication of mycobacterium growth and the rows with no color change marks the activity of extracts and MICs

3. RESULTS

The minimum inhibition concentration (MIC) of *S. quinqueloba* and *C. crassum* extracts were evaluated for their anti-mycobacterial activity against two very fast growing *Mycobacteria* strains which are *Mycobacteria madagascariense* (MM) and *Mycobacteria indicuspranii* (MIP). Results observed are summarized in Table 1. The anti-MIP and anti-MM activity of the both plants extracts displayed different MIC range values, 0.39 mg/mL – 12 mg/mL for MM and 0.78 mg/mL – 12.5 mg/mL for MIP. All leaf extracts of the two plants and ethyl acetate root bark extracts of *C. crassum* exhibited a higher activity MIC range of 0.39 – 6.25 mg/mL against the tested strains when compared with isoniazid which is the first line anti-mycobacterial drug which gave MIC value of 12.5 mg/mL against both strains tested. Previous studies had reported the observed mycobacterial resistance cases against isoniazid [24,25] and indeed they are in line with the current results.

The trend of activity displayed by both plants indicated thatethyl acetate leaf extracts of *S. quinqueloba* hada higher activity compared to other plant parts (MIC value range of 0.39 – 0.78 mg/ml), followed by ethyl acetate leaf extracts of *C. crassum* (0.78 mg/mL), methanolic leaf extracts of *S. quinqueloba* and petroleum ether leaf extracts of *C. crassum* (0.78 mg/mL – 1.56 mg/mL), petroleum ether leaf extracts of *S. quinqueloba* (1.56 mg/mL) and methanolic leaf extracts of *C. crassum* (1.56 mg/mL – 3.12 mg/mL). Save for ethyl acetate root bark extracts of *C. crassum* which had MIC value of 0.78 mg/mL against MM, all other stem and root bark extracts of both plants against the 2 strains tested gave activity that ranged from 3.12 – 12.5 mg/mL (Table 1).

4. DISCUSSION

There are many researches on anti-mycobacterial activities of plants but based on our investigation, there was no any published study of anti-mycobacterial evaluation on *S. quinqueloba* and *C. crassum*. Results from the present study indicated that *S. quinqueloba* and *C. crassum* have anti-mycobacterial activity against both strains tested with leaf extracts being most active compared to other parts. The ethyl acetate leaf extracts of *S. quinqueloba* exhibited a higher activity range than other parts indicating the anti-mycobacterial potential of middle polar compounds (secondary metabolites) existing in the leaves of *S. quinqueloba.* Although Babalola et al. [17] performed ant-mycobacterium test using only leaves extract of *S. setigera* against virulent *Mycobacterium tuberculosis* ($H_{37}R_v$) strain, it is evident that, leaves extracts of the both plants have promising therapeutic potency against mycobacterium strains, with middle polar compounds being significantly active comparable to other extracts (non-polar and polar extracts). Yang et al. [18] on the other hand had observed that, the stem extract of *C. horridium* had remarkable activity against various bacteria strains. Although *C. horridium* extracts were not tested for anti-mycobacterial activity, the syringic acid and coumarins isolated from this plant had being identified to possess ant mycobacterial activity [26,27]. Generally genus *Sterculia* and *Canthium* have shown a number of anti-bacterial activities which gave an indication that, some of these species might have anti-mycobacterial potency particularly on the aerial parts [28]. The leaf extracts of the both plants, were very active than other parts tested, and they had activity 16 times higher than isoniazid, the first line anti-TB drug.

Like wise, some extracts from other parts of the two plants tested had the activity higher than isoniazid (activity range 0.78 – 6.25 mg/ml). These results is an indication that the two plants have a number of secondary metabolites that can be worth further researched for the effort to discover new classes of compounds responsible for ant-mycobacterial activity. This effort should be intensified because despite the intense hard work on the ant-mycobacterial screening from plants, none of the current ant-TB agents from plant origin are used as first or even second line drugs, while TB infections is still remains as one of the global development and health problems [3,27]. The emergence and spread of MDR and XDR strains further expected to destabilize the very effort undertaken to control this disease in many parts of the world including developing country like Tanzania. Among other things this country (Tanzania) is limited to funding, experts, facilities, medication supplies and laboratory capabilities which due to the current existence of MDR and XDR strains cases, fighting against TB become even more serious problem to contain [7].

Table 1. Antimycobacterial Activity of *Sterculia quinqueloba* and *Canthium crassum* against MM and MIP

Minimum inhibitory concentration (MIC) in mg/Ml		
Plant extracts	***Mycobacteria madagascariense* (MM)**	***Mycobacteria indicuspranii* (MIP)**
SQLP	1.56	1.56
SQLE	0.39	0.78
SQLM	0.78	1.56
SQSP	12.5	3.12
SQSE	3.12	3.12
SQSM	12.5	6.25
CCLP	1.56	0.78
CCLE	0.78	0.78
CCLM	1.56	3.12
CCRP	3.12	6.25
CCRE	0.78	12.5
CCRM	3.12	12.5
Isoniazid	12.5	12.5
Rifampicin	0.19	0.19
DMSO	12.5	12.5

Key: SQLP= S. quinqueloba leaf PE extract, SQLE= S. quinqueloba leaf EtOAc extract, SQLM= S. quinqueloba MeOH leaf extract, SQSP =S. quinqueloba stem PE extract, SQSE= S. quinqueloba stem EtOAc extract, SQSM= S. quinqueloba stem MeOH extract, CCLP= C. crassum leaf PE extract, CCLE= C. crassum leaf EtOAc extract, CCLM= C. crassum leaf MeOH extract, CCRP= C. crassum root PE extract, CCRE= C. crassum root EtOAc extract, CCRM= C. crassum root MeOH extract

It is therefore our interest to report the anti-mycobacterial activity of these two plants which are medicinally used to treat various ailments in Tanzania including bacterial infections. The anti-mycobacterial activity observed from these two plants might be due tothe presence of alkaloids, flavonoids, tannins, saponins, long chain fatty acid, quinone, tarpenoids, phenols and steroids reported to exist in many *Sterculia* and *Canthium* species [17, 29-32], which according to Celis et al. [33], Meenakshi et al. [34] and Abd-Alrahman et al. [35] the mentioned compounds had shown the antimicrobial activity. Likewise, Brine shrimp lethality test which is used as preliminary test of extract cytotoxicity conducted by Wilson et al. [21] indicated that, ethyl acetate, petroleum ether and methanolic leaf extracts of the two plants are not toxic to brine shrimp cell. In this regard,it was regarded that, the no toxicity to brine shrimp cell observed is a good and promising indication that, the compounds available in the mentioned extracts might be not toxic to human and/or give more chances for easily addressing the toxicity problem if ever existed, when further studies are carried out.

4. CONCLUSION

Sterculia quinqueloba and *Canthium crassum* extracts have shown anti-mycobacterial activity with leaf extracts being more active than other parts. All leaf extracts showed a higher activity than first line anti-mycobacterial drug and the isoniazid for at least 16 times. Therefore, leaf extracts may be a good source of anti-mycobacterial compounds worth further development. It is also recommended that, further toxicity study is needed to verify the safety of these two plants since the preliminary toxicity study conducted on the leaf extracts showed no toxicity effects tobrine shrimp cell.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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