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Antimicrobial and cytotoxic activities of extracts from *Sterculia quinqueloba* (Garcke) K. Schum and *Canthium crassum* Hiern

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Abstract
Sterculia quinqueloba (Garcke) K. Schum and *Canthium crassum* Hiern extracts were evaluated for antimicrobial activity using 96-well-microtitre serial micro-dilution method against *Vibrio cholera, Klebsiella oxytoca, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus* and *Candida albicans*. All extracts exhibited antimicrobial activity with minimum inhibition concentration range of 0.39 – 12.5 mg/mL. The *S. quinqueloba* leaf methanolic extract had the highest activity with minimum inhibitory concentration range of 0.78 – 1.56 mg/mL and 0.78 – 1.50 mg/mL against bacterial and fungal species respectively. The lethality test against brine shrimp larvae revealed that *S. quinqueloba* and *C. crassum* leaf extracts with LC_{50} value range of 113.439 – 840.537 µg/mL is non-cytotoxic. The antimicrobial activity and lethality results indicate therapeutic potency of *Sterculia quinqueloba* and *Canthium crassum*.

Keywords: Antimicrobial, *Sterculia quinqueloba*, *Canthium crassum*, Brine shrimps and Cytotoxic

1. Introduction
Plant derived medicine has made largest contribution to human health and well-being all over the world. Around 1900, 80% of the drug was derived from plants [1] but in the years that followed, the development of synthetic drugs caused a remarkable decline on the dependence of plant origin drugs. However, recent trend of increased drug resistance has necessitated search for new classes of drug leads with unique mode of actions [2-3]. The resurgence in the use of herbal medicines globally in the last 50 years has also fueled the interest in screening therapeutic agents from plants [4]. Today, about 80% of the world's population depends on traditional medicine practices for the management of various diseases, which make it necessary to evaluate and establish their safety and efficacy [5].
Medicinal plants have shown significant activity to control various infectious diseases including bacterial and fungal infections [6]. These diseases account for approximately one half of all the deaths in most developing countries of the world [7]. This problem has increased to such recorded level, partly due to the observed resistance cases to most of the currently in use antimicrobial drugs [8]. Therefore, searching for new antimicrobial agents from different sources including plants to address drug resistant cases is very important particularly to developing countries.
Sterculia quinqueloba (Garcke) K. Schum (Malvaceae) and *Canthium crassum* Hiern (Rubiaceae) are medicinal plants widely used in Tanzania and other eastern African countries to treat various diseases including stomach ache, diarrhea, skin diseases, earache and venereal diseases [9 - 10]. They are native tree to Albertine rift valley and Southern African countries [10]. Till to date, there is little or no available information in the scientific literature validating the use of *S. quinqueloba* and *C. crassum* in the Tanzania folkloric medicine. Therefore this study was aimed to evaluate the antimicrobial bio-prospecting potential of the species and finally investigating the cytotoxicity potential of the plant on brine shrimp (*Artemia salina*) larvae. This paper reports for the first time, the antimicrobial and cytotoxicity activities of the leaf, stem bark and root bark extracts of *S. quinqueloba* and *C. crassum*.

2. Materials and Methods
2.1 Preparation of Plant Materials
Stem barks, root barks and leaves of *S. quinqueloba* and *C. crassum* were collected from

Kigoma region, around Gombe National Park and identified by Mr. Haji Seleman, a botanist from the department of botany; University of Dar es Salaam. *S. quinqueloba* and *C. crassum* specimens were kept at Nelson Mandela Africa Institution of Science and Technology with voucher specimen numbers 2141 and 2142 respectively. Plant samples were dried under the shade at room temperature and dried plant materials were pulverized to get powders from which the extracts were prepared. Leaves (800 g), stem bark (800 g) and root bark (800 g) from each plant were independently extracted by maceration using petroleum ether (PE), ethyl acetate (EtOAc) and methanol (MeOH). Solvents were evaporated using vacuum evaporator.

2.2 Solvents, Reagents and Culture Media

Methanol (absolute) was bought from FlukaChemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) and Dimethyl sulfoxide (DMSO) was purchased from RFCL Limited, Hayana, India. Petroleum ether, Ethyl acetate and Chloroform were purchased from Loba Chemie Pvt Ltd, Mumbai, India while Nutrient broth, Nutrient agar, Saboraud Dextrose broth and Saboraud's Dextrose agar were bought from HIMEDIA®, India. Strains namely; *Staphylococcus aureus* (NCTC 25923), *Streptococcus pyogenes* (clinical isolate), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC29953), *Vibrio cholera* (clinical isolate), *Shigella flexneri* (clinical isolate), *Salmonella kisarawe* (clinical isolate), *Klebsiella oxytoca* (clinical isolate), *Proteus vulgaris* (clinical isolate), *Klebsiella pneumonia* (ATCC 700603) and two fungi; *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (clinical isolate) were obtained from the department of Microbiology and Immunology, School of Medicine at Muhimbili Institute of Health and Allied Sciences. Iodonitrotetrazolium chloride was bought from SIGMA® (Sigma - Aldrich®, St Louis, USA). The Brine Shrimp eggs were purchased from Aquaculture Innovations (Grahamstown 6140, South Africa) and sea salt was prepared locally by evaporating water collected from Indian Ocean, along the Dar es Salaam coast.

2.3 Determination of antibacterial and antifungal activity

Minimum inhibitory concentrations (MICs) for both bacteria and fungi were determined by serial micro-dilution method in triplicate using 96-well-microtitre plates. Initially 50 µL of the nutrient broth and saboraud's dextrose broth media for bacteria and fungi respectively, were added in each well followed by addition of 50 µL of the extract making 100 mg/mL into each wells of the first row to make a total volume of 100 µL in the first row. After careful mixing of the first row of each plate, 50 µL were drawn from each of the first row wells and added into the subsequent row wells. The process was repeated down the columns to the last wells at the bottom whereas 50 µL was discarded. Thereafter, 50 µL of the bacterial and fungal suspensions approximately 0.5 Mac Farhland standard turbidity was added to each well to make the final volume of 100 µL in each well. The rows containing gentamycin and fluconazole were used as standard positive control drugs. DMSO was used as a negative control while the rows with nutrient broth and Saboraud's dextrose broth only were used to monitor bacterial and fungal growth respectively. The plates were then incubated at 32 °C for 24 hrs. For each extract, MICs for both bacteria and fungi were determined by adding 20 µL of 0.02% p-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation for 1hr at 32 °C. The growth of bacteria and fungi was indicated by the change in color from colorless to pinkish. The lowest concentrations which showed no growth were considered as MICs.

3. Results

3.1 Antimicrobial activity of *S. quinqueloba* and *C. crassum* leaf, root bark and stem bark extracts

The minimum inhibitory concentrations (MIC) of *S. quinqueloba* and *C. crassum* extracts were evaluated against human pathogenic bacteria and results are summarized in Table 1 and 2.

Table 1: Antimicrobial activity of *S. quinqueloba* leaf, root bark and stem bark extracts

Microorganisms	Minimum Inhibitory Concentrations, MIC(mg/ml)										
	SQLP	SQLE	SQLM	SQSP	SQSE	SQSM	SQRP	SQRE	SQRM	Gent	Fluc
K. pneumonia	1.56	1.56	0.78	1.56	3.12	12.5	12.5	12.5	12.5	0.00033	NT
K. oxytoca	1.56	0.78	1.56	3.12	3.12	12.5	NA	NA	NA	0.00033	NT
S. pyogenes	3.12	0.78	1.56	1.56	1.56	3.12	3.12	3.12	3.12	0.00125	NT
V. cholera	3.12	3.12	0.78	1.56	3.12	3.12	6.25	6.25	6.25	0.00156	NT
P. vulgaris	6.25	3.12	0.78	1.56	12.5	12.5	NA	NA	NA	NT	NT
S. aureus	6.25	0.78	1.56	6.25	6.25	6.25	6.25	6.25	6.25	0.000781	NT
S. flexineri	6.25	3.12	1.50	6.25	1.56	3.12	6.25	6.25	6.25	0.00125	NT
E. coli	6.25	3.12	0.78	NA	NA	NA	NA	NA	NA	0.00156	NT
C. neoformans	3.12	0.78	0.78	1.56	1.56	12.5	3.12	3.12	12.5	NT	0.00313
C. albicans	6.25	0.78	1.50	3.12	3.12	3.12	6.25	6.25	6.25	NT	0.00625

Key: SQLP = *S. quinqueloba* leaf PE extract, SQLE = *S. quinqueloba* leaf EtOAc extract, SQLM = *S. quinqueloba* leaf MeOH extract, SQSP = *S. quinqueloba* stem bark PE extract, SQSE = *S. quinqueloba* stem bark EtOAc extract, SQSM = *S. quinqueloba* stem bark MeOH extract, SQRP = *S. quinqueloba* root bark PE extract, SQRE = *S. quinqueloba* root bark EtOAc extract, SQRM = *S. quinqueloba* root bark MeOH extract, NA = not active, NT = not tested,

The antibacterial and antifungal activity displayed MIC range values of 0.39 – 12.5 mg/mL and 0.78 – 12.5 mg/mL respectively. *S. quinqueloba* and *C. crassum* leaf extracts demonstrated higher activity compared to stem bark and root

bark extracts with MIC value range of 0.39 – 6.25 mg/mL except against *Escherichia coli* (12.5 mg/mL). The stem bark extracts exhibited higher activity than root bark extracts (Table 1 and 2).

The trend of the activity displayed by *S. quinqueloba* leaf extracts indicated that, leaf methanolic extract had higher activity with MIC value range of 0.78 – 1.56 mg/mL followed by ethyl acetate extract (0.78 – 3.12 mg/mL) and petroleum ether extract (1.56 – 6.25 mg/mL). The trend was different in the case of *C. crassum* leaf extracts and in case, ethyl acetate extract was the most active with MIC value range of 0.39 – 6.25 mg/mL) while methanolic and ethyl acetate extracts had the same activity with MIC value range of 0.78 – 12.5 mg/mL.

Canthium crassum leaf ethyl acetate exhibited much higher activity against *Vibrio cholera* with MIC value of 0.39 mg/mL suggesting the presence of secondary metabolite(s) effective against cholera causing bacteria. *Escherichia coli* were the least susceptible pathogen against the tested extracts with MIC value range of 3.12 – 12.5 mg/mL with exception of *S. quinqueloba* leaf methanolic extract which demonstrated relatively higher activity with MIC value of 0.78 mg/mL.

Table 2: Antimicrobial activity of *C. crassum* leaf, root bark and stem bark extracts

Microorganisms	Minimum inhibitory concentrations (mg/ml)							
	CCLP	**CCLE**	**CCLM**	**CCRP**	**CCRE**	**CCRM**	**Gent**	**Fluc**
K. Oxytoca	1.50	0.39	0.78	6.25	6.25	6.25	0.00033	NT
K. pneumonia	6.25	3.12	3.12	6.25	1.56	12.5	0.00033	NT
S. pyogenes	6.25	1.50	12.5	6.25	12.5	12.5	0.00125	NT
V. cholera	0.78	0.39	1.56	3.12	1.56	6.25	0.00156	NT
P. vulgaris	3.12	1.50	6.25	6.25	6.25	6.25	NT	NT
S. aureus	3.12	0.39	3.12	3.12	0.78	12.5	0.000781	NT
S. flexineri	1.50	0.78	6.25	6.25	3.12	6.25	0.00125	NT
S. typhi	6.25	3.12	6.25	6.25	1.56	6.25	0.00125	NT
E. coli	12.5	6.25	12.5	12.5	6.25	12.5	0.00156	NT
C. neoformans	3.12	1.56	3.12	6.25	1.50	3.12	NT	0.00313
C. albicans	1.56	0.78	0.78	6.25	6.25	12.5	NT	0.00625

Key: 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* leaf EtOAc extract, CCRM = *C. crassum* leaf MeOH

3.2 Brine shrimp activity of *S. quinqueloba* and *C. crassum* leaf, root bark and stem bark extracts

S. quinqueloba and *C. crassum* stem bark, leaves and root bark extracts were evaluated for lethality activity against the brine shrimp and results are summarized in Table 3.

Table 3: Brine shrimp activity of extracts from *S. quinqueloba* and *C. crissum*

Plant extracts	Regression equation	LC_{50}(µg/ml)	95% Confidence interval (CI)	R. coefficient
SQLP	y = 75.342x - 104.81	113.439	87.227 - 147.528	0.93
SQLE	y = 43.909x –42.496	127.802	81.454 – 200.521	0.96
SQLM	y = 71.391x – 114.65	202.440	153.364 -267.221	0.98
SQSP	y = 49.597x – 41.695	70.600	47.351 – 105.265	0.91
SQSE	y = 74.520x - 81.37	57.927	44.423 – 75.537	0.93
SQSM	y = 53.912x - 104.32	728.522	487.241 – 1089.286	0.97
SQRP	y = 80.130x - 106.77	90.458	70.670 – 115.786	0.93
SQRE	y = 46.283x - 37.385	77.278	50.377 – 118.544	0.91
SQRM	y = 113.60x - 214.26	211.950	178.109 – 252.221	0.99
CCLP	y = 39.195x – 64.628	840.537	507.264 – 1392.770	0.83
CCLE	y = 48.547x – 80.369	484.639	322.448 – 728.412	0.78
CCLM	y = 34.066x – 30.684	233.593	130.645 – 417.664	0.91
CCRP	y = 65.098x – 58.685	46.728	34.486 – 63.316	0.98
CCRE	y = 73.153x – 76.612	53.8	41.047 – 70.532	0.93
CCRM	y = 69.679x - 77.856	68.38	51.530 – 90.740	0.91

Key: SQLP = *S. quinqueloba* leaf PE extract, **SQLE** = *S. quinqueloba* leaf EtOAc extract, **SQLM** = *S. quinqueloba* leaf MeOH extract, **SQSP** = *S. quinqueloba* stem PE extract, **SQSE** = *S. quinqueloba* stem EtOAc extract, **SQSM** = *S. quinqueloba* stem MeOH extract, **SQRP** = *S. quinqueloba* root PE extract, **SQRE** = *S. quinqueloba* root EtOAc extracts, **SQRM** = *S. quinqueloba* root 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

The interpretation of the results was according to criteria developed by [11] in which LC_{50} value less than 100 µg/mL was considered toxic and LC_{50} value of greater than 100 µg/mL non-toxic. Thus, *S. quinqueloba* leaf petroleum ether extract (113.439 µg/mL), ethyl acetate extract (127.802 µg/mL) & methanolic extract (202.440 µg/mL); *S. quinqueloba* stem bark methanolic extract (728.520 µg/mL); *S. quinqueloba* root methanolic extract (211.950 µg/mL); *C. crassum* leaf petroleum ether extract (840.537 µg/mL), petroleum ether extract (484.639 µg/mL) and methanolic extract (233.593 µg/mL) are considered non-toxic. The leaf extracts of both plants are therefore non-toxic and their use in traditional medicine in Africa is substantiated. The level of toxicity of stem bark and root bark *S. quinqueloba* and *C. crassum* is depending on the solvents used for extraction and thus types of secondary metabolites extracted. The *C. crassum* root bark petroleum ether, ethyl acetate and methanolic extracts displayed LC_{50} value of 46.728, 53.8 and 68.38 µg/mL indicating high level of cytotoxicity of this part.

4. Discussion

The antimicrobial screening of traditional medicinal plants has been the source of innumerable therapeutic agents [5]. The readily availability of medicinal plants with various biological activities including antimicrobial agents to most communities particularly in developing countries like Tanzania has fueled the recent increased popularity of alternative and complementary medicine [2]. The effort to validate the effectiveness of the therapeutic properties of these plants both *in vitro* and *in vivo* has also increased in many parts of the world, and hence provide a platform for further research and development of new therapeutic agents.

S. quinqueloba and *C. crassum* traditionally used for treatment of bacterial and fungal infections in Tanzania were evaluated for antibacterial and antifungal activities. They demonstrated activity at varying degree. Leaves of both plants poses high activity against bacterial and fungal species tested compared to stem bark and root bark extracts. Brine shrimp lethality test as a measure of extracts cytotoxicity has revealed low potency of leaf extracts and thus regarded to pose no toxicity to humans. It is therefore suggested that secondary metabolites present in the leaves of both plants are candidates for the development of antimicrobial drugs with low toxicity to human cells.

Phytochemical investigations conducted on the leaves of *Sterculia setigera* revealed the presence of steroids, terpenoids, fatty acids, cardiac glycosides and alkaloids [12]. Since plant species of the same genus are known to pose similar classes of secondary metabolites, it is likely that *Sterculia quinqueloba* contains steroids, terpenoids, fatty acids, cardiac glycosides and alkaloids. Likewise, previous phytochemical reports from the leaves of *Canthium parviflorum* indicated the presence of steroids, saponins, terpenoids and quinines [13]. The activity displayed with C. *crassum* leaf extracts might be due to the presence of these compounds.

Pathogenic organisms evaluated in this study are of medical importance in Africa especially in sub-Saharan Africa. For instance, typhoid and cholera have been associated with low income individuals of which 75% lives in Sub-Saharan Africa [14]. With HIV-AIDS epidemic, oral candidiasis and other forms of candidiasis caused by *Candida albicans* has been a serious health problem affecting HIV-AIDS patients of which Sub-Saharan Africa has the most serious epidemic in the world [15, 16]. Poor sanitation which accelerates the cholera infection and hospital acquired infections caused by *Klebsiella oxytoca* and *Staphylococcus aureus* affects mostly poor population of the world [17, 18, 19]. Shigella species causative agents of shigellosis which causes around 600,000 deaths per year, two-thirds of which occurred in children under 10 years of age affect mostly poor people in the developing countries [20, 21, 22, 23].

5. Conclusion

Sterculia quinqueloba and *Canthium crassum* extracts have demonstrated antimicrobial activity against various pathogenic organisms affecting Sub-Sahara Africa region. The leaf extracts of both plants showed higher antimicrobial activity than the other parts i.e. stem bark and root bark; therefore leaf extract may be good source of antimicrobial compounds worth further development.

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