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Cytotoxic and Antimicrobial Activities of the Constituents of Ten Plant Species from Tanzania

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A b s t r a c t

This paper reports on the antimicrobial and cytotoxicity activities of crude extracts from the plant species *Clemaptosis scabiosifolia* (Ranunculaceae), *Diospyros mafiensis* (Ebenaceae), *Dolichos kilimandscharicus* (Leguminosae), *Gnidia kraussiana* (Thymelaeaceae), *Hugonia castaneifolia* (Linaceae), *Neorautanenia mitis* (Pappilionaceae), *Tagetes minuta* (Asteraceae), *Uvaria acuminata* (Annonaceae), *Vernonia amygdalina* (Asteraceae) and *Zanha africana* (Sapindaceae). All crude extracts were screened for their cytotoxicities in the brine shrimp lethality test (BST). Fractions and pure compounds from *Hugonia castaneifolia*, *Diospyros mafiensis* and *Uvaria acuminata* were tested for their cytotoxicity and antimicrobial activities against ten microorganisms, namely *Candida albicans*, *Bacillus anthracis*, *Escherichia coli*, *Klebsiela pneumoniae*, *Proteus* sp., *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella boydii*, *Staphyloccus aureus*, and *Vibrio cholerae*. Of all the tested crude samples, extracts from *Neorautanenia mitis* had the highest cytotoxicity, having LC_{50} values of 0.12, 0.65, 1.54 and 2.33 µg/mL for pet ether, dichloromethane, ethanol and methanol/chloroform (1:1 v/v) extracts, respectively. The mixture of uvaretin (**2**) and diuvaretin (**3**) isolated from *Uvaria acuminata* had the highest cytotoxicity (LC_{50} = 3.59 µg/mL). Furthermore, the mixture of uvaretin (**2**) and diuvaretin (**3**) was active against the Gram-positive bacteria *S. aureus* and *B. anthracis*, being 65% and 57% as active as the standard antibacterial drug, gentamycin, against the two bacteria species, respectively.

Keywords: *Artemia salina*, *Candida albicans*, Gram-Negative Bacteria, Gram-Positive Bacteria.

Introduction

Plant products, in the form of extracts or powdered materials, have been used as medicines as well as insecticides even before the time of ancient Romans. Plants are considered as rich sources of medicines and insecticides because they produce metabolites that have intrinsic biological activities. Several of such compounds are metabolised as part of the strategies of the plants to defend themselves against diseases or plant predators (War et al. 2012).

The diversity of microbes which cause infectious diseases in both humans and plants is quite large. Similarly, the development of resistant strains of pathogenic bacteria, fungi and protozoa that cause parasitic diseases like malaria has prompted the need to establish alternative chemotherapeutic agents (Adil et al. 2018). As such, efforts to search for naturally occurring insecticides and therapeutic drugs from plants through bioprospecting ventures have resulted in the discovery of a vast array of chemical entities that offer structural diversity far unmatched to other strategies involved in drug discovery (Dias et al. 2012). Thus, identifying constituents of plants which control diseases in crop plants and humans is a growing field of natural products chemistry. Therefore, investigations reported in this paper form part of the endeavours to search for antimicrobial

and cytotoxic agents from plants, as possible candidates for the development of alternative agents for control of diseases.

Materials and Methods

Collection and identification of plant materials

The plant materials were collected from Iringa, Mbeya, Rukwa, and Pwani regions in Tanzania from 2000 to 2002. Selection of the localities where the plant species were collected was based on ethnobotanical information on the traditional use of the investigated plant species or the families to which the species belong being sources of medicinal plants. Field identification of the plant species was carried out by Mr. F. M. Mbago and Mr. L.B. Mwasumbi of the Herbarium of the Botany Department at the University of Dar es Salaam, where voucher specimens are deposited. The plant species chosen for the investigations reported in this paper, *viz*. *Clemaptosis scabiosifolia* (Ranunculaceae), *Diospyros mafiensis* (Ebenaceae), *Dolichos kilimandcharicus* (Leguminosae), *Gnidia kraussiana* (Thymelaeaceae), *Hugonia castaneifolia* (Linaceae), *Neorautanenia mitis* (Pappilionaceae), *Tagetes minuta* (Asteraceae), *Uvaria acuminata* (Annonaceae), *Vernonia amygdalina* (Asteraceae), and *Zanha africana* (Sapindaceae) are either used traditionally for medicinal purposes or belong to genera known for their medicinal values.

Extraction and isolation of compounds

The plant parts (i.e., leaves, tubers, stem and root barks) were air dried, pulverized and thereafter extracted consecutively using pet ether, dichloromethane and methanol. In some cases, extraction was done using a 1:1 (v/v) mixture of methanol and chloroform. The extracts were stored at –20 ºC until needed for analyses. Prior to isolation of the individual constituents, the crude extracts were first screened for activity in the brine shrimp test (BST). This was followed by fractionation of the crude extracts using vacuum liquid chromatography (VLC) in which extracts were adsorbed on silica gel and eluted using increasing polarities of petroleum ether and ethyl acetate (100% petroleum ether – 100% ethylacetate v/v). The obtained fractions were subjected to bioassay. Fractions from *Uvaria acuminata* were further purified by repeated gravitational column chromatography using silica gel by eluting with a mixture of petroleum ether and ethylacetate and by gel chromatography on Sephadex LH-20 eluting with a mixture of 1:1 methanol/dichloromethane (v/v). The isolated compounds were identified by spectroscopic techniques which involved Nuclear Magnetic Resonance (NMR) and Mass spectrometry (MS).

Brine shrimp lethality test

The Brine Shrimp Test (BST) was carried out at the Department of Chemistry at the University of Dar es Salaam, following standard procedures (Meyer et al. 1982). Brine shrimp (*Artemia salina* Leach) nauplii were used as indicator organisms for preliminary assays of cytotoxic activity of the crude extracts, VLC fractions and pure compounds. Artificial sea water was prepared by dissolving sea salt (38 g) in distilled water (1000 mL), and then filtered. The salt solution was filled in a tank divided into two compartments by a perforated polythene wall. Shrimp eggs were sprinkled into the covered part of the tank and a lamp illuminated the uncovered part of the tank to attract the hatched shrimps. The mature nauplii were collected after 48 h of hatching. Each sample was tested at sample concentrations of 240, 120, 80, 40, 24 and 8 µg/mL dissolved in DMSO (dimethyl sulfoxide) in triplicate vials each containing 10 brine shrimp nauplii. An additional fourth vial containing only the solvent DMSO and 10 shrimp larvae acted as the control. The number of survivors was analysed to determine the LC_{50} (the concentration required to kill 50% of the shrimp larvae) using the Kaleidagraph computer program, whereby the LC_{50} values

were obtained from the graph of percentage mortality against the logarithm of the concentrations of the tested samples.

Antifungal tests

Only *Candida albicans* (strain HG 392) was used as the test organism for the evaluation of antifungal activity. Clinical isolates of this organism were obtained from the Department of Microbiology and Immunology at the then Muhimbili University College of Health Sciences (MUCHAS), University of Dar es Salaam. The assays were conducted using the disc method (Malele et al. 1998, Van de Berge and Vlientick 1991). Thus, filter paper discs (Whatman No.1), 5 mm in diameter, were impregnated with crude extracts (5 mg/disc), VLC fractions (0.2 mg/disc) and pure compounds or mixtures of compounds (0.2 mg/disc). Miconazole (20 µg/disc) was used as the standard antifungal drug. A sterilised Saboraud's dextrose agar (SDA) was aseptically aliquoted at volumes of 20 mL to petri dishes and left to congeal. A colony forming unit of *C. albicans* was sampled from an overnight culture and dispersed in sterile distilled water (5 mL) using an inoculating loop to make an equivalent of a 0.5 McFarland solution. A cotton swab was dipped in the suspension to soak, then streaked on the surface of SDA. Discs previously impregnated with test samples were placed almost equidistant onto the surface of the seeded agar using sterile forceps. One disc impregnated with miconazole was placed at the centre of the medium. Another disc previously impregnated with only solvent was used as the negative control. The petri dish with its contents was incubated at 37 ºC and the zones of inhibition (mm) were recorded after 24 h whereby miconazole was used as the standard antifungal agent.

Antibacterial tests

Crude extracts were assayed against ten bacteria *viz. Bacillus anthracis*, *Escherichia coli*, *Klebsiela pneumoniae*, *Proteus* sp., *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella boydii*, *Staphyloccus aureus*, and *Vibrio cholerae*. Filter paper discs (Whatman No.1), 5 mm in diameter, were impregnated with crude extracts (5 mg/disc), VLC fractions (0.2 mg/disc) and pure compounds (0.2 mg/disc). For reference purpose, gentamycin (20 µg/disc) and ampicillin (20 µg/disc) were used as the standard antibacterial drugs. Clinical isolates of the organisms were obtained from the Department of Microbiology and Immunology at MUCHAS. *S. aureus* and *B. anthracis* represented the Gram-positive bacteria while the rest represented Gram- negative ones. Tryptone soya agar (TSA) was aseptically aliquoted at volumes of 20 mL to petri dishes and allowed to congeal. A colony forming unit of a particular organism was sampled from an overnight culture and dispersed in sterile distilled water (5 mL) using an inoculating loop to make an equivalent of a 0.5 McFarland solution (approximately 10^6 bacteria/mL). A cotton swab was dipped in the suspension to soak, then streaked on the surface of TSA. Discs previously impregnated with test samples were placed almost equidistant onto the surface of the seeded agar using sterile forceps. One disc impregnated with a standard drug was placed at the centre of the medium. Another disc previously impregnated with only solvent was used as the negative control. The petri dish with its contents was incubated at 37 ºC and the Inhibition Zones **(IZ)** were recorded (in mm) after 24 h and this was therefore considered evidence that the organism was susceptible to the antibacterial agent (Van de Berge and Vlientick 1991, Malele et al. 1998).

Determination of activity index (AI)

The Activity Index (**AI**) was calculated as the ratio of the inhibition zone of the test sample to that of the standard drug (Singh et al. 2002):

$$\mathbf{AI = IZ_T/IZ_D}$$

Where: $\mathbf{IZ_T}$ is the diameter of the inhibition zone of the test sample at a given

concentration and $\mathbf{IZ_D}$ is the diameter of the zone of inhibition of a standard drug at a known concentration.

Results and Discussions

The brine shrimp test (BST)

Fifty two crude extracts obtained from ten plant species belonging to nine different families were assayed in the brine shrimp test. Pet ether, dichloromethane, ethanol, and in some cases water extracts of the plant samples and those obtained by soaking in a mixture of methanol and chloroform (1:1 v/v) were tested (Table 1). For these tests, the concentration of each test sample ranged from 8 to 240 µg/mL, where the sample demonstrated higher mortalities and the available amounts of samples were reasonable, the concentration was lowered. The results for the lethal concentrations at which 50% of the larvae were killed were found to be interesting. Thus, pet ether extracts demonstrated the highest activities as compared to other extracts indicating that less polar components possessed high cytotoxic activity. A similar trend was previously observed for the antimalarial activity of crude plant extracts (Weenen et al. 1990). Of all the tested samples, extracts from *Neorautanenia mitis* had the highest cytotoxicity, having LC_{50} values of 0.12, 0.65, 1.54 and 2.33 µg/mL for the pet ether, dichloromethane, ethanol and methanol/chloroform (1:1 v/v) extracts, respectively. In the previous study on this particular plant species, crude extracts demonstrated high activity against the malaria transmitting mosquitoes, *Anopheles gambiae* (Joseph et al. 2004). The study reported isolation of six compounds from *N. mitis* namely, pachyrrhizine, neotenone, neorautanone, neoduline, 4-methoxyneoduline and nepseudin that were all active against *A. gambiae* adult mosquitoes at different concentrations (Joseph et al. 2004).

Extracts from *Gnidia krausiana* that was collected from Sumbawanga area in Rukwa Region, Tanzania were significantly active up to the concentration of 8 µg/mL. It should be noted that human deaths have been reported following ingestion of herbal drugs prepared from *Gnidia kraussiana*, including one case of a woman who died a few hours after taking a teaspoonful of the powdered tuber for stomach pains. The plant is reported to be highly poisonous and rapidly fatal to livestock (Hutchings et al. 1996). The plant is also said to cause irritation when in contact with the skin (personal communication with researchers at ARI-Uyole, Tanzania). The activity of this plant species is explained by the possible presence of diterpenoids, similar to those that have been isolated from other *Gnidia* species including *G. kraussiana* (Borris and Cordell 1984, Seigler 1998). Accordingly, the cytotoxicity of *G. kraussiana* extract as demonstrated in these investigations would be assumed to be due to such constituents.

Table 2 shows that VLC fractions and compounds or mixtures of compounds exhibited activity against the brine shrimp larvae at different levels. Repeated chromatography of the dichloromethane extract of *U. acuminata* led to isolation of the previously known compounds namely, chamuvaritin (**1**, Mbaveng et al. 2014; Makangara et al. 2002), the mixture of uvaretin (**2,** Makangara et al. 2002) and diuvaretin (**3,** Makangara et al. 2002), mixture of triuvaretin (**4,** Makangara et al. 2002) and isotriuvaretin (**5,** Makangara et al. 2002) as well as the mixture of diuvaretin (**3**), triuvaretin (**4**) and angoluvarin (**6,** Nkunya 2005). The isolated compounds showed higher cytotoxicity than the corresponding fractions. Thus, chamuvaritin (**1**), the mixture of uvaretin (**2**) and diuvaretin (**3**), as well as the mixture of triuvaretin (**4**) and isotriuvaretin (**5**), exhibited LC_{50} values that were lower than the standard drug, cyclophosphamide which had an LC_{50} value of 17.78 µg/mL. Cyclophosphamide is a known chemotherapeutic drug that is used for the treatment of certain types of cancer and leukaemia, including lymphomas, multiple myeloma, mycosis fungoides, neuroblastoma, ovarian carcinoma, retinoblastoma and breast

cancer (Bagley Jr. et al. 1973). It is also given to children before and after organ transplants. Indeed, some of the LC_{50} values are better than those observed for the standard drug. Hence, such constituents may be promising candidates for the development of antitumor or pesticidal agents provided that there is ample selectivity in the cytotoxicity between tumorous and healthy cells or pests and non-pest insects.

Table 1: Toxicity of crude extracts to brine shrimp larvae (LC_{50} values in µg/mL)

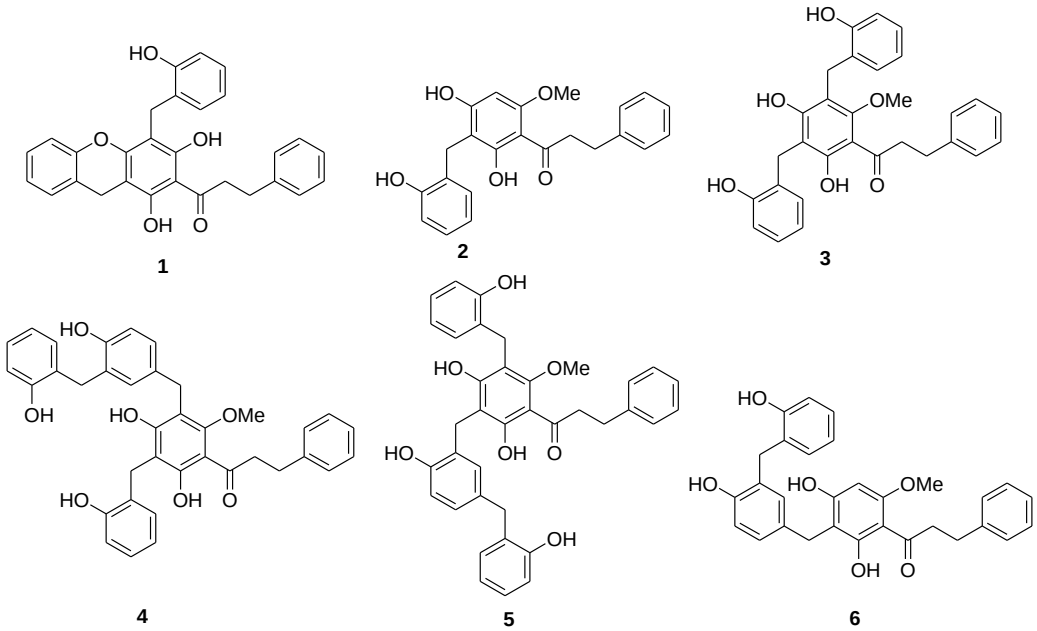
Plant species	Collection locality	Plant part	Pet ether extract	Dichloro -methane extract	Ethanol extract	MeOH/ $CHCl_3$ extract	Water extract
N. mitis	Iringa	Tuber	0.12	0.65	1.54	> 240	19.52
N. mitis	Mbeya	Tuber	0.22	< 8	1.92	3.01	NT
D. kilimandscharicus	Iringa	Tuber	< 8	0.63	58.06	2.33	45.39
D. kilimandscharicus	Mbeya	Tuber	11.08	7.74	< 8	35.94	NT
G. kraussiana	Sumbawanga	Tuber	< 8	9.12	< 8	< 8	10.52
G. kraussiana	Iringa	Tuber	12.91	0.66	< 8	9.14	NT
T. minuta	Iringa	Leaves	< 8	10.31	56.96	14.31	NT
D. mafiensis	Coast	Root bark	25.12	69.18	120.23	NT	NT
V. amygdalina	Iringa	Leaves	33.11	65.73	224.45	85.77	37.84
H. castaneifolia	Coast	Root bark	39	> 240	NT	NT	NT
C. scabiosifolia	Iringa	Root bark	45.94	< 8	32.77	16.51	33.11
Z. africana	Mbeya	Root bark	> 240	45.01	153.7	41.05	72.78
U. acuminata	Coast	Root bark	NT	< 8	NT	NT	NT
Cyclophosphamide	-		17.78	17.78	17.78	17.78	17.78

Key: NT = Not tested and MeOH/$CHCl_3$ = Methanol/chloroform (1:1 v/v) extract

Table 2: Cytotoxic activity of VLC fractions and compounds from *Hugonia castaneifolia*, *Uvaria acuminata* and *Diospyros mafiensis*

Sample	LC_{50} (µg/mL)			
	HCRD	UARD	DMRP	DMRD
VLC 3	43.2	-	45.71	-
VLC 4	NT	64.57	<8	-
VLC 5	54.08	-	-	5.08
VLC 6	>240	>240	11.22	-
VLC 7	-	>240	-	-
Chamuvaritin (**1**)	-	7.38	-	-
Uvaretin (**2**) & Diuvaretin (**3**)	-	3.59	-	-
Triuvaretin (**4**) & Isotriuvaretin (**5**)	-	27.35	-	-
Cyclophosphamide	17.78	17.78	17.78	17.78

Key: VLC 3 = Vacuum liquid chromatography fraction 3; VLC 4 = Vacuum liquid chromatography fraction 4; VLC 5 = Vacuum liquid chromatography fraction 5; VLC 6 = Vacuum liquid chromatography fraction 6; VLC 7 = Vacuum liquid chromatography fraction 7; HCRD = *Hugonia casteneifolia* root dichloromethane extract; UARD = *Uvaria accuminata* root dichloromethane extract; DMRP = *Diospyros mafiensis* root petroleum ether extract; DMRD = *Diospyros mafiensis* root dichlromethane extract.



It was further demonstrated that percentage mortality increased with increasing purity of the plant components (Table 2). Thus, the lowered activity of the crude extract can be attributed to the large number of compounds present in the extract that may be acting antagonistically against the active principle, or presence of other junky components and hence lowering the activity of the latter when present in the extract. Upon purification, the activity of the active principle is correspondingly enhanced.

Antifungal activities

The antifungal activities of crude extracts, VLC fractions and compounds was investigated for *Uvaria acuminata* and *Diospyros mafiensis*. Generally, the crude extracts, VLC fractions and the compounds obtained from *Uvaria acuminata* were inactive against the yeast like fungus *Candida albicans* but the extracts of *Diospyros mafiensis* showed remarkable activity against the fungus. Thus, the VLC fraction DMRP 6, the crude extract DMRD and VLC fraction DMRD 5 were more than 50% as active against the fungus as the standard antifungal drug, miconazole (Table 3). No antifungal activities were observed for the compound chamuvaritin (**1**) as well as the mixture of three compounds, diuvaretin (**3**), triuvaretin (**4**) and angoluvarin (**6**) from *Uvaria acuminata*. The mixture of uvaretin (**2**) and diuvaretin (**3**) mildly inhibited the growth of *C. albicans* by exhibiting a 6 mm zone of inhibition with an AI of 0.3.

Table 3: Antifungal activities of crude extracts and VLC fractions from U*varia acuminata* and *Diospyros mafiensis*

Sample	*C. albicans*	
	IZ (mm)	AI
DMRP	7	0.24
DMRD	20	0.69
UARD	0	0
DMRP VLC 4	6	0.21
UARD VLC 3	7	0.24
UARD VLC 4	0	0
DMRD VLC 5	29	1.0
DMRD VLC 7	19	0.66
DMRP VLC 6	19	0.66
UARD VLC 6	6	0.21
Miconazole	29	1.0

Antibacterial activities

Antibacterial activities of crude extracts and VLC fractions from *Diospyros mafiensis* and *Uvaria acuminata* against ten different species of both Gram-positive and Gram-negative bacteria were investigated. Activities of the test samples were observed for both Gram-positive and Gram-negative bacteria. Of all the crude extracts, the dichloromethane extract of *U. acuminata* showed good activities against both Gram-positive and Gram-negative bacteria, with the activity index ranging from 0.29 to 0.88. The pet ether extract exhibited a moderate activity against all bacterial species. Interestingly, the VLC fractions DMRP 4 and 6 exhibited higher activities against the bacteria, having an activity index ranging from 0.43 to 0.82 with fraction DMRP VLC 6 being more active than DMRP VLC 4 (Table 4). The dichloromethane extract from *Diospyros mafiensis* was only active against the Gram-positive bacteria species *S. aureus* and *B. anthracis*, having an activity index of 0.48 and 0.6 for the two species, respectively. Chamuvaritin(**1**) and the mixtures of dihydrochalcones isolated from *U. acuminata* were inactive against the Gram-negative bacteria species *Escherichia coli*, *Klebsiela pneumoniae*, *Salmonella typhimurium*, *Shigella boydii* and *Vibrio cholerae*, except for the mixture of diuvaretin (**3**), triuvaretin (**4**), and angoluvarin (**6**) which had a considerable activity against *Proteus* sp., a Gram-negative bacterium. However, some activity was observed against the Gram-positive bacteria species, namely *S. aureus* and *B. anthracis* (Table 5). All extracts, VLC fractions and compounds isolated from *U. acuminata* and *D. mafiensis* were inactive against the Gram-negative bacteria species, *Pseudomonas aeruginosa*. These results therefore indicate that the two plant species are not possible sources of broad spectrum antibacterial agents.

Table 4: Antibacterial activities of crude extracts and VLC fractions from *U. acuminata* and *D. mafiensis*

Sample	*E. coli*		*S. typhi*		*S. boydii*		*K. pneum.*		*S. aureus*		*V. cholerae*		*Proteus sp.*		*B. anthracis*	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
DMRP	7	0.3	0	0	8	0.36	6	0.29	7	0.28	7	0.33	0	0	9	0.45
DMRD	0	0	0	0	-	-	0	0	12	0.48	NT	-	0	0	12	0.6
UARD	12	0.51	13	0.62	16	0.73	6	0.29	13	0.52	10	0.48	15	0.88	13	0.65
DMRP VLC 4	10	0.43	10	0.48	11	0.5	0	0	11	0.44	9	0.43	14	0.82	15	0.75
UARD VLC 3	6	0.26	0	0	0	0	0	0	6	0.24	6	0.29	6	0.35	9	0.45
UARD VLC 4	6	0.26	0	0	7	0.32	0	0	6	0.24	6	0.29	6	0.35	9	0.45
DMRD VLC 5	12	0.51	10	0.48	10	0.45	13	0.62	13	0.52	0	0	0	0	17	0.85
DMRD VLC 7	11	0.48	10	0.48	12	0.55	12	0.57	12	0.48	0	0	0	0	12	0.6
DMRP VLC 6	15	0.65	15	0.71	16	0.73	14	0.67	14	0.56	0	0	0	0	14	0.7
UARD VLC 6	14	0.61	13	0.62	14	0.64	13	0.62	14	0.56	0	0	0	0	13	0.65
Gentamycin	23	1.0	21	1.0	22	1.0	21	1.0	25	1.0	21	1.0	17	1.0	20	1.0
Ampicillin	26	-	23	-	24	-	18	-	29	1.0	24	1.0	23	1.0	-	-

IZ = inhibition zone in mm; AI = activity index

Table 5: Antibacterial activity of compounds from *U. acuminata* and *D. mafiensis*

Sample	*S. aureus*		*Proteus* sp.		*B. anthracis*	
	IZ	AI	IZ	AI	IZ	AI
Chamuvaritin (**1**)	0	0	0	0	6	0.29
Uvaretin (**2**) & Diuvaretin (**3**)	13	0.65	0	0	12	0.57
Diuvaretin (**3**), Triuvaretin (**4**) & Angoluvarin (**6**)	7	0.35	10	0.59	8	0.38
Gentamycin	20	1.0	17	1.0	21	1.0

Conclusions

Fifty two crude extracts obtained from ten plant species belonging to the families Annonaceae, Asteraceae, Ebenaceae, Leguminosae, Linaceae, Pappilionaceae, Thymelaeaceae, Rununculaceae and Sapindaceae were screened for their cytotoxic activity against brine shrimp larvae. Most of the extracts demonstrated very low LC_{50} values, thus indicating high cytotoxic activities. Fractions of three plant species *Uvaria acuminata, Diospyros mafiensis and Hugonia castaneifolia* belonging to Annonaceae, Linaceae and Ebenaceae families, respectively were evaluated for their cytotoxicities whereby fractions from *Diospyros mafiensis* exhibited higher cytotoxicities. Furthermore, fractions from *U. acuminata* and *D. mafiensis* were evaluated for their antifungal and antibacterial properties. It was observed that *D. mafiensis* was more active than *U. acuminata* in antifungal tests. However, higher activities were generally observed for *U. acuminata* in antibacterial tests. Only compounds isolated from *U. acuminata* were investigated and demonstrated activities in the BST and antimicrobial tests whereby the mixture of uvaretin (**2**) and diuvaretin (**3**) were active against the Gram-positive bacteria *S. aureus* and *B. anthracis*, displaying inhibition zones of 13 and 12 mm, respectively against the two bacterial species. The mixture was 65% and 57% as active as the standard antibacterial drug, gentamycin, against the two bacteria species, respectively. In the BST the mixture of uvaretin (**2**) and diuvaretin (**3**) had an LC_{50} value of 3.59 µg/mL, being five times more cytotoxic than the standard anticancer drug, cyclophosphamide. In general, the results obtained in these investigations have contributed knowledge on the potential of the plant species as sources of therapeutic agents.

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Declaration of conflict interest

The author declares no competing or conflicting interest.

References

Adil M, Khan R and Rupasinghe HPV 2018 Application of medicinal plants as a source for therapeutic agents against *Streptococcus pyogenes* infections. *Curr. Drug Metab.* 19: 695-703.

Bagley Jr. CM, Bostick FW and DeVita Jr. VT 1973 Clinical pharmacology of cyclophosphamide. *Cancer Res*. 33: 226-233.

Borris RP and Cordell GA 1984 Studies of the Thymelaeaceae II. Antineoplastic

principles of *Gnidia kraussiana*. *J. Nat. Prod*. 47: 270-278.

Dias DA, Urban S and Roessner U 2012 A historical overview of natural products in drug discovery. *Metabolites* 2: 303-336.

Hutchings A, Scott AH, Lewis G and Cunningham AB 1996 *Zulu Medicinal Plants*; *An Inventory*: University of Natal Press, Pietermaritzburg.

Joseph CC, Ndoile M, Malima RC and Nkunya MHH 2004 Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from *Neorautanenia mitis*. *Trans. Royal Soc. Trop. Med. Hyg.* 98: 451-455.

Makangara JJ, Jonker SA and Nkunya MHH 2002 A novel phenanthrenolide and C-benzyldihydrochalcones from *Uvaria puguensis*. *Nat. Prod. Lett*. 16: 267-272.

Malele RS, Moshi MJ, Mloka DAS, Melekzedeck S and Mbwambo ZH 1998 In vitro antimicrobial susceptibility test of some Tanzanian plants. *Tanz. Med. J.* 3: 31-34.

Mbaveng AT, Zhao Q, Kuete V 2014 Harmful and protective effects of phenolic compounds from African medicinal plants. In *Toxicological Survey of African Medicinal Plants* pp. 577-609, Elsevier, Amsterdam.

Meyer BN, Ferrigin NR, Putnam JE, Jacobsen LB, Nicholas DE and McLaughlin JL 1982 Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med.* 45: 31-34.

Nkunya MHH 2005 Unusual metabolites from some Tanzanian indigenous plant species. *Pure Appl. Chem.* 77: 1943-1955.

Seigler DS 1998 Diterpenes and Sesterterpenes. In: *Plant Secondary Metabolism* (pp. 398-426). Springer, Boston, MA.

Singh B, Sahu PM and Sharma MK 2002 Anti-inflammatory and antimicrobial activities of triterpenoids from *Strobilanthes callosus* nees. *Phytomed*. 9: 355-359.

Van de Berghe DA and Vlietinck AJ 1991 Screening Methods for Antibacterial and Antiviral Agents from Higher Plants. In Hostettmann K (Ed) *Methods in Plant Biochemistry: Assays for Bioactivity*. Vol. 6, Academic Press, London. pp 47-69.

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S and Sharma HC 2012 Mechanisms of plant defense against insect herbivores. *Plant Signal Behav*. 7: 1306-1320.

Weenen H, Nkunya MHH, Bray DH, Mwasumbi LB, Kinabo LS and Kilimali VAEB 1990 Antimalarial activity of Tanzanian medicinal plants. *Planta Med.* 56: 368-370.