Antibacterial Activities and Phytochemical Screening of Crude Extracts from Kenyan Macaranga Species Towards MDR Phenotypes Expressing Efflux Pumps

Article *in* Pharmacognosy Communications · April 2021

DOI: 10.5530/pc.2021.2.22

CITATIONS
5

READS
212

8 authors, including:

Ibrahim Hashim
University of Nairobi
7 PUBLICATIONS **35** CITATIONS
SEE PROFILE

Leonidah Kerubo Omosa
University of Nairobi
64 PUBLICATIONS **641** CITATIONS
SEE PROFILE

Vaderament-Alexe Nchiozem-Ngnitedem
Universität Potsdam
18 PUBLICATIONS **69** CITATIONS
SEE PROFILE

John Mmari Onyari
University of Nairobi
64 PUBLICATIONS **1,018** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

antimicrobial activity of natural compounds View project

Antimicrobial activity View project

Pharmacogn. Commn. 2021;11(2):119-126.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Antibacterial Activities and Phytochemical Screening of Crude Extracts from Kenyan *Macaranga* Species Towards MDR Phenotypes Expressing Efflux Pumps

Ibrahim Hashim[1,2], Leonidah Kerubo Omosa[1,*], Vaderament-Alexe Nchiozem-Ngnitedem[1], John Mmari Onyari[1], Shital Mahindra Maru[3], Michel-Gael Fofack Guefack[4], Armelle Tsafack Mbaveng[4], Victor Kuete[4]
[1]Department of Chemistry, University of Nairobi, Nairobi, KENYA.
[2]Department of Chemistry, Federal University Lafia, Nasarawa State, NIGERIA.
[3]Department of Pharmaceutics and Pharmacy Practice, University of Nairobi, Nairobi, KENYA.
[4]Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, CAMEROON.

ABSTRACT

Introduction: *Macaranga* species are traditionally used for the treatment and management of coughing, fungal infection, and wounds. In this study, the phytochemical screening and antibacterial activities of nine crude extracts from *Macaranga conglomerata*, *Macaranga kilimandscharica* and *Macaranga capensis* were determined against 13 bacterial strains expressing multi-drug resistance (MDR) phenotypes. **Methods:** Phytochemical screening of the extracts were carried out according to the standard methods, while the iodonitrotetrazolium chloride (INT) colorimetric assay was used to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the plants extracts. **Results:** Flavonoids, terpenoids, saponins and coumarins were the major secondary metabolites found in all the plant extracts. The results of antibacterial studies revealed that all the plant extracts displayed good activities with MIC values ranging from 4 – 128 µg/mL against the tested micro-organisms. Most of the extracts exhibited a bactericidal effect against *E. coli, E. aerogenes, K. pneumoniae, P. stuartii, P. aeruginosa*, and *S. aureus* with MBC/MIC ratio ≤ 4. In the presence of efflux pump inhibitor (PaβN), the inhibition potency of all the crude extracts against the tested bacterial strains were substantially enhanced. It is worth noting that the activities of MKL, MCL, and MCR towards *P. stuartii* (NEA16), *E. aerogenes* (ATCC13048), and *K. pneumoniae* (KP55), respectively were improved by more than 8-fold in the presence of PAβN. **Conclusion:** The findings of this study indicated the possibility of using all the tested plant extracts as a source of therapeutic agents in the fight against multi-drug resistant bacteria.

Key words: *Macaranga capensis, Macaranga kilimandscharica, Macaranga conglomerata*, Euphorbiaceae, Pathogenic microbes, Multidrug resistance.

Correspondence:
Prof. Leonidah Kerubo Omosa
Department of Chemistry, University of Nairobi, 30197-00100, Nairobi, KENYA.
Phone no: +254721797175
E-mail: lkerubo@uonbi.ac.ke
DOI: 10.5530/pc.2021.2.22

INTRODUCTION

The emergence and spread of multi-drug resistant (MDR) micro-organisms (bacteria, fungi, viruses and protozoans) have compromised the management and/or treatment of common infections such as malaria, pneumonia, tuberculosis, measles and HIV/AIDS.[1,2] As a result, the costs of treatment, hospitalization time, morbidity and mortality rate are all in the rise. A high poverty index, limited access to modern health care facilities, clean water and affordable medicines as well as the gross misuse and overuse of antimicrobials, particularly in developing nations, are the contributing factors accelerating the development and spread of multi-drug resistant micro-organisms.[3]

The prevalence of multi-drug resistant bacteria constitutes a very big burden to both the developed and developing nations with respect to public health. These bacteria cause different classes of antibiotics to lose their effectiveness in the treatment of infectious diseases.[4-8] thereby, resulting in high morbidity and mortality rate, in addition to the negative impact on the World's economy.[9,10]

Due to the presence of diverse phytochemicals with multiple pharmacological potentials, medicinal plant extracts present a very good prospect in combating effectively the multi-drug resistant bacteria and potentially restore the efficacy in the management of infectious diseases using antibiotics.[11] There is an urgent need therefore, to continue to search for better antimicrobial agents especially of natural origin, which are not only available, but also affordable.

Macaranga genus consist of over 300 species mainly found in tropical Asia and New Guinea.[12] It belongs to the Euphorbiaceae family and it's a soft-wooded tree that rapidly grows to about 15 – 20 m tall.[13,14] Seven species of *Macaranga* were reported to be native of East African forest of which *M. kilimandscharica*, *M. capensis, M. schweinfurthii* and *Macaranga conglomerata* are found in Kenya.[12-14]

The species in this genus are used traditionally in the treatments of several ailments in different parts of the world. For instance, the roots and leaves decoction of *M. kilimandscharica* are used, in Kenya for the treatment of bilharzia and cough, as well as stomach problems.[15] *M. tanarius* root decoctions are used for fever relief and to suppress coughing;[16] leaf extract is used for healing of wounds and relieve inflammation;[17] dried root is used as an emetic agent.[18] Stem and leaf decoctions of *M. denticulate* are used in the prevention of infections after childbirth.[19] Red gum of *M. indica*, leaves of *M. deheiculata*, and young shoot of *M. gigantean* are used for healing wounds,[20] treating jaundice,[21] and treating fungal infection.[22] Besides the traditional uses, crude extracts obtained from *Macaranga* species have been reported for diverse biological activities including anticancer,[23] antibacterial,[16] antiplasmodial,[24] antifungal,[25] and anti-inflammatory activity.[26] Phytochemical studies indicated prenylated flavonoids and stilbenes as the main secondary metabolites found in the genus.[14,27] Other phytochemicals including diterpenes and tannins were also reported from the genus, although few (< 10%) of the 300 species in the genus have been investigated phytochemically.[14]

Despite the wide-range of ethnomedicinal applications and potential pharmacological activities of *Macaranga* species reported in the

literature, neither the phytochemical studies nor the antimicrobial efficacy of the crude extracts from *M. conglomerata*, *M. kilimandscharica*, and *M. capensis* have been investigated. Based on this, the current study focuses on the phytochemical screening and antibacterial activities of nine (9) crude extracts from the aforementioned *Macaranga* species against a panel of multi-drug resistant (MDR) phenotypes.

MATERIALS AND METHODS

Sample collection and extraction

M. conglomerata and *M. capensis* were collected from Ngangao forest, while *M. kilimandscharica* was harvested from Kieni forest in Kenya. Each of the plant material was identified by a taxonomist from the School of Biological Sciences (SBS), University of Nairobi, where voucher specimens of each sample HIUON 2019/001, HIUON 2020/003, and HIUON 2020/002, respectively, were deposited. Samples (leaves, stem bark, and root) of each plant were air-dried under shade, powdered, weighed and stored for subsequent use. The powdered plant materials were extracted exhaustively with 50% methanol (MeOH) in dichloromethane (CH_2Cl_2) (obtained from Kobian Kenya Ltd. in Nairobi) at room temperature and the solvent concentrated *in vacuo* using a rotatory evaporator.

Phytochemical analysis

Phytochemical screening of all the extracts for the presence of flavonoids, terpenoids, saponins, anthraquinones, glycosides, tannins, alkaloids, phlobatannins, carbohydrates and coumarins were performed following reported protocols.[28-30] Change in colour and/or formation of precipitate were considered as confirmation for the presence or absence of a particular active phytochemical.

Antibacterial assay

Chemicals

The reference antibiotics (RA) ciprofloxacin and *β*-naphtylamide arginine phenylalanine (PA*β*N) were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Iodonitrotetrazolium chloride (INT) (Sigma-Aldrich) was used as bacterial growth revelator; dimethylsulfoxide (DMSO) (Sigma-Aldrich) was used to dissolve the plant extracts and compounds.

Culture media and microbial strains

The studied micro-organisms were cultured overnight on Mueller Hinton Agar 24 h prior to assaying. Mueller Hinton Broth (MHB) was used as liquid culture medium for susceptibility assays. A panel of six pathogenic microbes including sensitive and multidrug resistant Gram-negative (*Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Providencia stuartii*) and Gram-positive (*Staphylococcus aureus*) bacterial strains expressing efflux pumps were provided by the American Type Culture Collection (ATCC) were used. Their bacterial features are depicted in Table 1.

Determination of bacterial susceptibility

INT colorimetric assay[38,39] was performed to assess the minimal inhibitory concentrations (MICs) of crude extracts and ciprofloxacin against a panel of 13 Gram-negative and Gram-positive bacteria. Briefly, each crude extract was first dissolved in DMSO/MHB mixture. The solution obtained was then added to MHB and serially diluted two-fold (in a 96-well microplate). One hundred microlitres (100 µL) of inoculum (1.5×106 CFU/mL) prepared in MHB was then added. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a shaker and incubated at 37°C for 18 h. The final concentration of DMSO was lower than 2.5% and does not affect the microbial growth. Wells containing MHB, 100 µL of inoculum, and DMSO at a final concentration of 2.5% served as a negative control. Ciprofloxacin was used as a reference antibiotic. The MICs of crude extracts were determined after 18 h of incubation at 37°C, following addition of (40 µL) of 0.2 mg/mL INT and incubation at 37°C for 30 min.[40] Viable bacteria reduced the colourless dye to pink. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of microbial growth. All assays were performed in triplicate and repeated thrice. For the minimal bactericidal concentration (MBCs) determination, a volume of 150 µL of MHB has been introduced in a new 96-well microplate, following addition of 50 µL of the previous well microplate contents where no microbial growth was observed and which did not receive an INT (during the reading of MICs). After 48 h incubation, at 37°C, the MBC of each crude extracts were determined and defined by adding 40 µL of 0.2 mg/mL INT as previously described. It is important to note that crude extracts were tested alone, and then in combination with PA*β*N (an efflux pumps inhibitor) at 30 mg/L final concentration. In this last case, the activity

Table 1: Characteristics of bacterial strains and features.

Bacterial Species	Types	Relevant features
Escherichia coli		
	ATTC 10536	Reference strain[31]
	AG102	AG 100 over-expression of pumps *Acr AB*[32]
Enterobacter aerogenes		
	ATCC 13048	Reference strain[31]
	EA27	Clinical strain present efflux energy-dependant of chloramphenicol norfloxacin and KAN^r, AMP^r, NAL^r, STR^r, TET^r [33,34]
Klebsiella pneumoniae		
	ATCC 11296	Reference strain[31]
	Kp55	Clinical MDR isolate: TET^r, AMP^r, ATM^r, CEF^r [31]
Providencia stuartii		
	PS2636	*AcrAB-TolcC* associate of porines of types OMPF *et* OMPC[31]
	NEA16	Clinical isolate of *P. stuartii AcrAB-TolC*[31]
Pseudomonas aeruginosa		
	PA01	Reference strain[31]
	PA124	Clinical strain multi-resistant *MexAB-OprM*[35]
Staphylococcus aureus		
	ATCC 25923	Reference strain
	MRSA3	Clinical isolate: $Ofxa^r$, Kan^r, Tet^r, Erm^r[36]
	MRSA6	Clinical isolate: $Ofxa^r$, Flx^r, Kan^r, Tet^r, Cyp^r, IM/Cs^r, Chl^r, Gen^r, Nis^r, Amp^r[36,37]

AMP^r, ATM^r, CEF^r, CHL^r, KAN^r, NAL^r, NOR^r, STR^r and TET^r : resistant (r) to ampicillin, aztreonam, cefepime, chloramphenicol, kanamycin, nalidixic acid, norfloxin, streptomycin and tetracycline, respectivement; *AcrAB-TolC, MexAB-OprM :* Efflux pump; $Ofxa^r$, Kan^r, Tet^r, Flx^r, Cyp^r, IM/Cs^r, Chl^r, Gen^r, Nis^r, Amp^r and Erm^r : resistant (r) to Ofloxacine, Kanamycin, Tetracyclin, Flomoxef, Cyprofloxacin, Imipenem/Cilastatin sodium, chloramphenicol, Gentamicin, Ampicillin, Nisin, and Erythromycin, respectively.

improvement factors (AIFs) were determined to qualify the potentiation level of sample activity by this inhibitor, using the MIC_{sample} alone/ MIC_{sample}-PAβN combination ratio.

RESULTS

Phytochemical screening of plant extracts

Phytochemical screening of the classes of compounds in the different plant extract is reported in Table 2. The results showed that, the major classes of secondary metabolites found in all plant extracts were mainly flavonoids, terpenoids, saponins, and coumarins, while anthraquinones, alkaloids, phlobatannins were not detected in all plant extrats under investigation.

Antibacterial activity of the plant extracts

The antibacterial activity of *M. capensis* (leaves, stem, and root), *M. kilimandscharica* (leaves, stem, and root), and *M. conglomerata* (leaves, stem, and root) towards 13 micro-organisms including drug-sensitive and multidrug-resistante are reported in Table 3. All plant extracts displayed good activities with MIC values ranging from 4 to 128 µg/mL. Crude extracts from *M. capensis* showed potent activity against 13/13 bacteria tested. All extracts obtained from *M. kilimandscharica* and *M. conglomerata* showed a large spectrum of activities against MDR phenotypes except MKL, MCL, and MCR. Their inhibition potencies being observed against 12/13 (92.3%) of the bacterial strains. Ciprofloxacin, a standard antibiotic, was effective against all bacterial strains with MICs values as low as 1 to 4 µg/mL and exhibited a bactericidal effect on all bacterial strains except *E. aerogenes* (ATCC 13048), *P. aeruginosa* (PA124), and *S. aureus* (MRSA6). It is noteworthy that the majority of the extracts showed bactericidal effects against *E. coli, E. aerogenes, K. pneumoniae, P. stuartii, P. aeruginosa, S. aureus,* with MBC/MIC ratio ≤ 4.

Role of efflux pumps in susceptibility of tested micro-organisms

In the current study, all crude extracts which displayed antibacterial potencies against most study bacterial strains were associated with the efflux pump inhibitor, phenylalanine beta naphthylamide, PAβN, in order to evaluate the contribution of efflux in the activities of these extracts. The overall results showed that in the presence of PAβN, the activity of plants extracts against tested bacteria were substantially enhanced. Table 4 showed that, the observed MICs (in absence of PAβN) of *M. kilimandscharica* leaves, *M. conglomerata* leaves, and *M. conglomerata* root towards *P. stuartii* (NEA16), *E. aerogenes* (ATCC13048), and *K. pneumoniae* (KP55), respectively were > 512 µg/mL. However, in the presence of efflux pump inhibitor, their activities improved by more than 8-fold, displaying MICs ranging from 16 to 64 µg/mL.

DISCUSSION

When referring to crude extracts derived from plants, many authors defined the antibacterial activity to be strong when MIC is less than 100 µg/mL, moderate when MIC between 100 and 625 µg/mL, and low when MIC more than 625 µg/mL.[41,42] Based on this cutoff point, all crude extracts displayed strong antibacterial activities against most bacterial strains, with the lowest MIC value being recorded at 4 µg/mL. It is important to note that the activity of these plant extracts against bacterial strains were more or less the same. This led to the conclusion that the chemical composition of all the plant materials may be similar. The classes of phytochemical compositions found in the tested plant extracts are in agreement with the previously reported isolated metabolites from other *Macaranga* species.[14] The pronounced antibacterial activities of all the tested crude extracts could be attributed to the presence of flavonoids, terpenoids, saponins and coumarins found in all the extracts. These phytochemicals may be acting synergistically or additively to exert the noted strong antibacterial activities.

Gram-negative bacteria of the species *E. coli* (ATTC10536 and AG102) and *P. aeruginosa* (PA01 and PA124) known for their multi-resistance to drugs, were less resistant to all crude extracts (MIC ≤ 128 µg/mL). A clear look on the MBC/MIC ratio value of each studied sample in most cases against *E. coli* and *P. aeruginosa* indicate bactericidal effects (MBC/MIC ≤ 4).[43] Previous reports identify *Macaranga* species as rich sources of prenylated flavonoids and stilbenes, many of which have biological activities (including antibacterial properties) that encompasses almost the entire area of pharmacological sciences.[14,27] Marked antibacterial activity of all plant extracts recorded towards *S. aureus* (ATCC25923, MRSA3 and MRSA6) was in conformity with similar research carried out by Putri and collaborators which showed strong inhibitory activity against *S. aureus.*[44] The low minimum inhibitory concentration (MIC) and minimum bacteriostatic concentration (MBC) values against Gram-positive bacterial strains exhibited by all plant crude extracts might be owing to their high concentrations in prenylated phenolic contents. Prenylation increases the lipophilicity of phenolic

Table 2: Parts used, and phytochemical composition of each *Macaranga* species.

Extracts	*M. capensis*			*M. kilimandscharica*			*M. conglomerata*		
Parts used	Leaves	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root
Flavonoids	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-
Glycosides	+	-	-	+	-	-	+	-	+
Tannins	+	+	+	+	+	-	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-
Steroids	+	+	-	+	+	-	+	+	+
Carbohydrates	-	-	-	-	-	-	-	-	-

+ = Presence; - = Absent.

Table 3: MIC and MBC (in µg/mL) of crude extracts from selected plants and ciprofloxacin against a panel of 13 bacterial strains.

Bacterial strains	MCPL		MCPS		MCPR		MKL		MKS		MKR		MCL		MCS		MCR		Ciprofloxacin	
	MIC (MBC)	R	MIC (MBC)	R	MIC / (MBC)	R	MIC (MBC)	R	MIC (MBC)	R	MIC (MBC)	R	MIC (MBC)	R	MIC (MBC)	R	MIC (MBC)	R	MIC (MBC)	R
E. coli																				
ATTC10536	16 (64)	4	16 (64)	2	4 (16)	4	32 (64)	2	32 (64)	2	16 (64)	4	128 (512)	4	8 (16)	2	16 (64)	4	1 (4)	4
AG102	32 (128)	4	32 (64)	2	4 (8)	2	128 (512)	4	16 (32)	2	16 (32)	2	16 (128)	8	8 (16)	2	64 (128)	2	1 (2)	2
E. aerogenes																				
ATCC13048	32 (128)	4	8 (64)	8	16 (32)	2	32 (64)	2	32 (128)	4	8 (32)	4	-	nd	32 (64)	2	64 (128)	2	1 (8)	8
EA27	128 (256)	2	8 (32)	4	4 (16)	4	64 (128)	2	32 (64)	2	16 (256)	16	128 (256)	2	8 (32)	4	64 (128)	2	1 (4)	4
K. pneumoniae																				
ATCC11296	32 (128)	2	32 (64)	2	8 (16)	2	16 (32)	2	8 (32)	4	8 (32)	4	32 (64)	2	8 (32)	4	128 (256)	2	2 (4)	2
KP55	32 (64)	2	16 (32)	2	8 (16)	2	64 (128)	2	32 (64)	2	8 (16)	2	16 (64)	4	16 (32)	2	-	nd	1 (1)	1
P. stuartii																				
PS2636	16 (32)	2	8 (32)	4	32 (64)	2	64 (128)	2	32 (64)	2	8 (64)	8	32 (128)	4	32 (64)	2	32 (64)	2	2 (8)	4
NEA16	16 (32)	2	16 (64)	4	8 (32)	4	-	nd	32 (64)	2	32 (64)	2	32 (128)	4	16 (64)	4	64 (128)	2	1 (4)	4
P. aeruginosa																				
PA01	32 (64)	2	32 (128)	4	32 (64)	2	128 (256)	2	16 (64)	4	16 (64)	4	128 (512)	4	32 (64)	2	64 (128)	2	4 (16)	4
PA124	64 (128)	2	32 (128)	4	32 (64)	2	128 (256)	2	32 (64)	2	16 (32)	2	32 (128)	4	16 (64)	4	64 (256)	4	2 (16)	8
S. aureus																				
ATCC25923	8 (32)	4	4 (16)	4	8 (16)	2	16 (32)	2	8 (16)	2	4 (32)	8	8 (32)	4	8 (32)	4	8 (16)	2	1 (1)	1
MRSA3	4 (16)	4	8 (32)	4	8 (64)	8	8 (16)	2	8 (16)	2	16 (32)	2	16 (64)	4	16 (32)	2	16 (32)	2	1 (4)	4
MRSA6	4 (16)	4	4 (16)	4	4 (16)	4	16 (32)	2	8 (16)	2	8 (32)	4	16 (64)	4	8 (32)	4	16 (32)	2	2 (16)	8

MCPL: *Macaranga capensis* leaves; MCPS: *Macaranga capensis* stem; MCPR: *Macaranga capensis* root; MKL: *Macaranga kilimandscharica* leaves; MKS: *Macaranga kilimandscharica* stem; MKR: *Macaranga kilimandscharica* root; MCL: *Macaranga conglomerata* leaves; MCS: *Macaranga conglomerata* stem; MCR: *Macaranga conglomerata* root; MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; R: MBC/MIC ratio (a sample is considered as bacteriostatic or bactericidal when R >4 or ≤4 respectively); (-): MIC or MBC >512 µg/mL for crude extract; nd: not determined (as no MIC and MBC values were not observed till 512 µg/mL)

Table 4: MIC (in µg/mL) of crude extracts and ciprofloxacin in combination of PAβN against a panel of 13 bacterial strains.

Bacterial strains	MCPL		MCPS		MCPR		MKL		MKS		MKR		MCL		MCS		MCR		Ciprofloxacin	
	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R	MIC (+PAβN)	R
E. coli																				
ATTC10536	16 (16)	1	16 (4)	4	4 (4)	1	32 (16)	2	32 (8)	4	16 (2)	8	128 (64)	2	8 (2)	4	16 (2)	8	1 (0.125)	8
AG102	32 (16)	2	32 (8)	4	4 (2)	2	128 (64)	2	16 (4)	4	16 (1)	16	16 (16)	1	8 (1)	8	64 (8)	8	1 (0.125)	8
E. aerogenes																				
ATCC13048	32 (16)	2	8 (2)	4	16 (16)	1	32 (32)	1	32 (4)	8	8 (1)	8	- (32)	nd	32 (4)	8	64 (16)	4	1 (0.25)	4
EA27	128 (32)	4	8 (1)	8	4 (4)	1	64 (32)	2	32 (16)	2	16 (4)	4	128 (16)	8	8 (2)	4	64 (8)	8	1 (0.125)	8
K. pneumoniae																				
ATCC11296	32 (4)	8	32 (8)	4	8 (2)	4	16 (8)	2	8 (8)	1	8 (4)	2	32 (16)	2	8 (8)	1	128 (16)	8	2 (0.5)	4
KP55	32 (4)	8	16 (4)	4	8 (8)	1	64 (32)	2	32 (8)	4	8 (2)	4	16 (4)	4	16 (16)	1	- (16)	nd	1 (0.125)	8
P. stuartii																				
PS2636	16 (4)	4	8 (4)	2	32 (16)	2	64 (32)	2	32 (32)	1	8 (8)	1	32 (8)	4	32 (8)	4	32 (32)	1	2 (0.25)	8
NEA16	16 (2)	8	16 (4)	4	8 (8)	1	- (64)	nd	32 (16)	2	32 (4)	8	32 (16)	2	16 (8)	2	64 (32)	2	1 (0.125)	8
P. aeruginosa																				
PA01	32 (32)	1	32 (16)	2	32 (16)	2	128 (32)	4	16 (16)	1	16 (4)	4	128 (64)	2	32 (16)	2	64 (8)	8	4 (1)	4
PA124	64 (32)	2	32 (16)	2	32 (4)	8	128 (16)	8	32 (32)	1	16 (4)	4	32 (64)	<1	16 (16)	1	64 (16)	4	2 (0.5)	4
S. aureus																				
ATCC25923	8 (2)	4	4 (4)	1	8 (2)	4	16 (4)	4	8 (8)	1	4 (1)	4	8 (4)	2	8 (8)	1	8 (4)	2	1 (0.5)	2
MRSA3	4 (4)	1	8 (2)	4	8 (2)	4	8 (2)	4	8 (2)	4	16 (8)	2	16 (2)	8	16 (16)	1	16 (4)	4	1 (0.25)	4
MRSA6	4 (4)	1	4 (4)	1	4 (2)	2	16 (2)	8	8 (4)	2	8 (2)	4	16 (4)	4	8 (8)	1	16 (8)	2	2 (0.5)	4

MCPL: *Macaranga capensis* leaves; MCPS: *Macaranga capensis* stem; MCPR: *Macaranga capensis* root; MKL: *Macaranga kilimandscharica* leaves; MKS: *Macaranga kilimandscharica* stem; MKR: *Macaranga kilimandscharica* root; MCL: *Macaranga conglomerata* leaves; MCS: *Macaranga conglomerata* stem; MCR: *Macaranga conglomerata* root; MIC: minimal inhibitory concentration; PAβN: phenylalanine beta naphthylamide; R=Ameliorating Factor: $MIC_{sample\ alone}/MIC_{sample+PA\beta N}$ ratio (this means the factor which determines the improvement of the activity of samples by PAβN; the activity of a sample was considered to be improved when its AIF was > 2); (-): MIC >512 µg/mL for crude extract; nd: not determined (as no MIC and MBC values were not observed till 512 µg/mL)

compounds, which results in increased affinity to cell membranes and an improved interaction with target proteins.[45]

To the best of our knowledge, the antibacterial activity of *M. capensis*, *M. kilimandscharica*, and *M. conglomerata,* which are among the 4 species found in Kenya, has to date been reported so far against a panel MDR phenotypes. However, a literature survey showed that phytochemical and pharmcological studies carried out in *Macaranga*-type propolis have been documented. For instance, chemical investigation of Indonesian plant, *M. trichocarpa* for antibacterial principles was undertaken by Fareza and co-authors. Chromatographic separation of acetone fraction led them to the isolation of one active prenylated flavonoids, macatrichocarpin A which showed antibacterial effect towards *Bacillus subtilis*.[46] Lee *et al*. showed that, propolin D obtained from *M. tanarius* at 5 to10 µg/ml significantly inhibited biofilm formation by three *S. aureus* strains, and *S. epidermidis* strain, with an MIC of 10 µg/ml.[47]

Furthermore, the low antibacterial activity observed in some extracts would be due to the presence within the tested bacteria of efflux systems which are responsible, when they are over-expressed, for the phenomenon of multi-resistance.[48] Efflux pumps have been shown to decrease the intracellular concentration of antibiotics and consequently their activity.[49] The pathogens used in this work are known for their ability to express efflux pumps to a high degree.[31] These efflux pumps can be competitively or non-competitively blocked by an efflux inhibitor, thereby restoring not only the intracellular concentration, but also the activity of the antibiotics,[50] as well as plant extracts. The increase in the antibacterial activity of the different extracts with respect to the strains tested in the presence of PAβN, would reflect the fact that the active ingredients contained in the extracts tested would have been discharged by the AcrAB-TolC type efflux pumps of the Enterobacteriaceae or MexAB-OprM type of *P. aeruginosa*.[31] This inhibitor would therefore have blocked the efflux pumps and led to an increase in the intracellular concentration of the active ingredient(s) contained in the extracts. This would have favoured the strong action of the compounds contained in the extracts through various antibacterial mechanisms, leading to the progressive death of the bacterial cells.[40,43]

CONCLUSION

The overall results showed that of the 9 plant extracts investigated, the majority of them displayed strong to moderate antibacterial activity against the selected pathogenic microbes. This suggests that the plant extracts under investigation can be a promising starting point in the search for new antibacterial drugs towards alleviating human suffering.

ACKNOWLEDGEMENT

IH is grateful to the Tertiary Education Trust Fund (TETFund) for a PhD scholarship to study at University of Nairobi, Kenya. Authors are also thankful to Mr. Patrick Mutiso of the Herbarium, School of Biological Science, University of Nairobi for authentication of the plant material. Mr Justus Mukavi for his assistance during plant collection. We acknowledged the financial support of the International Science Programme (ISP) through the KEN-02 project.

Authors' Contribution

Data collection: IH; carried out the study: IH, V-A. N-N, M-GFG and ATM; wrote the manuscript IH, V-A. N-N and LKO; designed the experiments and supervised the work: LKO, JMO, SMM and VK; provided the bacterial strains and facilities for antibacterial assays: VK; all authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

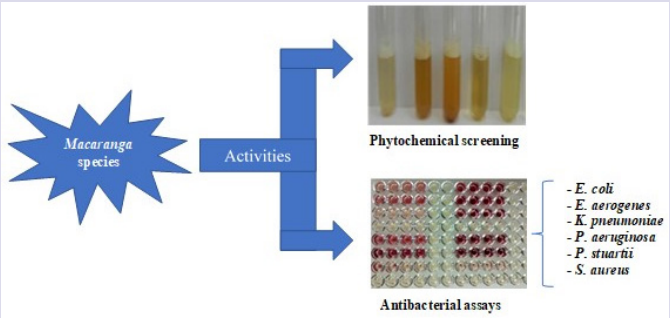
MCPL: *Macaranga capensis* leaves; **MCPS:** *Macaranga capensis* stem; **MCPR:** *Macaranga capensis* root; **MKL:** *Macaranga kilimandscharica* leaves; **MKS:** *Macaranga kilimandscharica* stem; **MKR:** *Macaranga kilimandscharica* root; **MCL:** *Macaranga conglomerata* leaves; **MCS:** *Macaranga conglomerata* stem; **MCR:** *Macaranga conglomerata* root; ***E. coli:*** *Escherichia coli*; ***E. aerogenes:*** *Enterobacter aerogenes*; ***K. pneumoniae:*** *Klebsiella pneumoniae;* ***P. stuartii:*** *Providencia stuartii;* ***P. aeruginosa:*** *Pseudomonas aeruginosa;* ***S. aureus:*** *Staphylococcus aureus*.

REFERENCES

1. Syed MA, Bana NF. Developing Countries Need Action Plans to Combat the Challenge of Antimicrobial Resistance. Arch Clin Microbiol. 2016;7:12.
2. Founou RC, Founou LL, Essack SY. Clinical and Economic Impact of Antibiotic Resistance in Developing Countries: A Systematic Review and Meta-Analysis. PLoS One. 2017;12(12):1-18.
3. Ayukekbong JA, Ntemgwa M, Atabe AN. The Threat of Antimicrobial Resistance in Developing Countries: Causes and Control Strategies. Antimicrob Resist Infect Control. 2017;6(1):1-8.
4. Rice LB. The Clinical Consequences of Antimicrobial Resistance. Curr Opin Microbiol. 2009;12(5):476-81.
5. Al-judaibi E. Infection and Antibiotic Resistant Bacteria in Developing Countries: A Genetic Review. J Microbiol Res. 2014;4(6A):10-7.
6. Fankam AG, Kuiate JR, Kuete V. Antibacterial and Antibiotic Resistance Modulatory Activities of Leaves and Bark Extracts of *Recinodindron heudelotii* (Euphorbiaceae) against Multidrug-Resistant Gram-Negative Bacteria. BMC Complement Altern Med. 2017;17(1):1-6.
7. Khan MF, Tang H, Lyles JT, Pineau R, Mashwani ZR, Quave CL. Antibacterial Properties of Medicinal Plants from Pakistan against Multidrug-Resistant ESKAPE Pathogens. Front Pharmacol. 2018;9:1-17.
8. Wamba BEN, Nayim P, Mbaveng AT, Voukeng IK, Dzotam JK, Ngalani OJT, *et al. Syzygium jambos* Displayed Antibacterial and Antibiotic-Modulating Activities against Resistant Phenotypes. Evidence-based Complement Altern Med. 2018;5124735.
9. Tchana MES, Fankam AG, Mbaveng AT, Nkwengoua ET, Seukep JA, Tchouani FK, *et al.* Activities of Selected Medicinal Plants against Multi-Drug Resistant Gram-Negative Bacteria in Cameroon. Afr Health Sci. 2014;14(1):167-72.
10. Anand U, Jacobo HN, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites. 2019;9(11):1-13.
11. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-Derived Secondary Metabolites as the Main Source of Efflux Pump Inhibitors and Methods for Identification. J Pharm Anal. 2020;10(4):277-90.
12. Siregar M, Konservasi P, Kebun T. Floristic Composition of Peat Swamp Forest in Mensemat-Sambas, West Kalimantan. In Proceedings of the International Symposium on Tropical Peatlands; Hokkaido University and Indonesian Institute of Sciences: Bogor, Indonesia. 2000;153-64.
13. Zakaria R, Fadzly N, Rosely N, Mansor M, Zakaria MY. The Distribution of *Macaranga*, Genus (Family *Euphorbiaceae*) in Penang Island, Peninsular Malaysia. J Biosci. 2008;19(2):91-9.
14. Magadula JMJ. Phytochemistry and Pharmacology of the Genus *Macaranga*: A Review. J Med Plants Res. 2014;8(12):489-503.
15. Kokwaro JO. Medicinal plants of East Africa. 2nd ed. University of Nairobi Press. 1993;15-230.
16. Lim TY, Lim YY, Yule CM. Evaluation of Antioxidant, Antibacterial and Anti-Tyrosinase Activities of Four *Macaranga* Species. Food Chem. 2009;114(2):594-9.
17. Phommart S, Sutthivaiyakit P, Chimnoi N, Ruchirawat S, Sutthivaiyakit S. Constituents of the Leaves of *Macaranga tanarius*. J Nat Prod. 2005;68(6):927-30.
18. Mahidol C, Prawat H, Prachyawarakorn V, Ruchirawat S. Investigation of Some Bioactive Thai Medicinal Plants. Phytochem Rev. 2002;1(3):287-97.
19. Sutthivaiyakit S, Unganont S, Sutthivaiyakit P, Suksamrarn A. Diterpenylated and Prenylated Flavonoids from *Macaranga denticulata*. Tetrahedron. 2002;58(18):3619-22.
20. Amina K, Rahman M, Akter A, Islam S, Akter M, Kabir S. Bioactivity of the Bark of *Macaranga indica* Wight Ic (*Euphorbiaceae*). World J Pharm Res. 2014;13(10):15.
21. Qi WY, Shen Y, Wu Y, Leng Y, Gao K, Yue JM. Deheiculatins A-L, 20-Oxygenated Cembranoids from *Macaranga deheiculata*. Phytochemistry. 2017;136:101-7.

22. Grosvenor PW, Supriono A, Gray DO. Medicinal Plants from Riau Province, Sumatra, Indonesia. Part 2: Antibacterial and Antifungal Activity. J Ethnopharmacol. 1995;45(2):97-111.
23. Yoder BJ, Cao S, Norris A, Miller JS, Ratovoson F, Razafitsalama J. *et al*. Antiproliferative Prenylated Stilbenes and Flavonoids from *Macaranga alnifolia* from the *Madagascar* Rainforest. J Nat Prod. 2007;70(3):342-6.
24. Zakaria I, Ahmat N, Jaafar FM, Widyawaruyanti A. Flavonoids with Antiplasmodial and Cytotoxic Activities of *Macaranga triloba*. Fitoterapia. 2012;83(5):968-72.
25. Salah MA, Bedir E, Toyang NJ, Khan IA, Harries MD, Wedge DE. Antifungal Clerodane Diterpenes from *Macaranga monandra* (L) Muell. et Arg. (Euphorbiaceae). J Agric Food Chem. 2003;51(26):7607-10.
26. Ngoumfo RM, Ngounou GE, Tchamadeu CV, Qadir MI, Mbazoa CD, Begum A, *et al*. Inhibitory Effect of Macabarterin, a Polyoxygenated Ellagitannin from *Macaranga barteri*, on Human Neutrophil Respiratory Burst Activity. J Nat Prod. 2008;71(11):1906-10.
27. Vu LTN, Ngan TB, Phuong L, Huong DTM, Litaudon M, Van HN, *et al*. Chemical Constitutents from Fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2) . Vietnam J Chem. 2018;56(4):516-20.
28. Poumale HMP, Hamm R, Zang Y, Shiono Y, Kuete V. Coumarins and Related Compounds from the Medicinal Plants of Africa. Med Plant Res Afri. 2013;261-300.
29. Raaman N. Qualitative Phytochemical Screening. In Phytochemical techniques; New India Publishing Agency. 2006;19-22.
30. Harborne JB. Phytochemcial Methods. Chapman and Hall, Ltd: London. 1973.
31. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Albert FS, Ngadjui BT, *et al*. Efflux Pumps are Involved in the Defense of Gram-Negative Bacteria against the Natural Products *Isobavachalcone* and *Diospyrone*. Antimicrob Agents Chemother. 2010;54(5):1749-52.
32. Chevalier J, Pagès JM, Eyraud A, Malléa M. Membrane permeability modifications are involved in antibiotic resistance in *Klebsiella pneumonia*. Biochem Biophys Res Commun. 2000;274(2):496-9.
33. Mallèa M, Chevalier J, Eyraud A, Pagès JM. Inhibitors of antibiotic efflux pumps in resistant *Enterobacter aerogenes* strains. Biochem Biophys Res Commun. 2002;293(5):1370-3.
34. Ghisalberti D, Masi M, Pagès JM, Chevalier J. Chloramphenicol and expression of multidrug efflux pump in *Enterobacter aerogenes*. Biochem Biophys Res Commun. 2005;328(4):1113-8.
35. Lorenzi V, Muselli A, Bernardini AF, Berti L, Pagès JM, Amaral L, *et al*. Geraniol restores antibiotic activities against multidrug resistant isolate from gram-negative species. Antimicrob Agents Chemother. 2009;53(5):2209-11.
36. Paudel A, Hamamoto H, Kobayashi Y, Yokoshima S, Fukuyama T, Sekimizu K. Identification of Novel Deoxyribofuranosyl Indole Antimicrobial Agents. J Antibiot. 2012;65(2):53-7.
37. Dzoyem JP, Hamamoto H, Ngameni B, Ngadjui BT, Sekimizu K. Antimicrobial Action Mechanism of Flavonoids from *Dorstenia* Species. Drug Discov Ther. 2013;7(2):66-72.
38. Eloff JN. A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. Planta Med. 1998;64(8):711-3.
39. Mativandlela SPN, Lall N, Meyer JJM. Antibacterial, Antifungal and Antitubercular Activity of (the Roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) Root Extracts. South African J Bot. 2006;72(2):232-7.
40. Kuete V, Ngameni B, Simo CCF, Tankeu RK, Ngadjui BT, Meyer JJM, *et al*. Antimicrobial Activity of the Crude Extracts and Compounds from *Ficus chlamydocarpa* and *Ficus cordata* (Moraceae). J Ethnopharmacol. 2008;120(1):17-24.
41. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Med. 2010;76(14):1479-91.
42. Kuete V, Efferth T. Cameroonian medicinal plants: Pharmacology and derived natural products. Front Pharmacol. 2010;1:123.
43. Fankam AG, Kuiate JR, Kuete V. Antibacterial Activities of *Beilschmiedia obscura* and Six Other Cameroonian Medicinal Plants against Multi-Drug Resistant Gram-Negative Phenotypes. BMC Complement Altern Med. 2014;14(1):1-9.
44. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. Pharmacol Clin Pharm Res. 2019;4(1):1-4.
45. Chen X, Mukwaya E, Wong MS, Zhang Y. A Systematic Review on Biological Activities of Prenylated Flavonoids. Pharm Biol. 2014;52(5):655-60.
46. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial Flavanones and Dihydrochalcones from *Macaranga trichocarpa*. Zeitschrift fur Naturforsch C. 2014;69(9-10):375-80.
47. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and Antibiofilm Activities of Prenylated Flavanones from *Macaranga tanarius*. Phytomedicine. 2019;63:153033.
48. Cattoir V. Efflux-Mediated Antibiotics Resistance in Bacteria. Pathol Biol. 2004;52(10):607-16.
49. Van BF, Pages JM, Lee VJ. Inhibitors of Bacterial Efflux Pumps as Adjuvants in Antibacterial Therapy and Diagnostic Tools for Detection of Resistance by Efflux. Front Anti-Infective Drug Discov. 2012;3:138-75.
50. Pagès JM, Amaral L. Mechanisms of Drug Efflux and Strategies to Combat Them: Challenging the Efflux Pump of Gram-Negative Bacteria. Biochim Biophys Acta. 2009;1794(5):826-33.

PICTORIAL ABSTRACT



SUMMARY

- Nine crude extracts from three *Macaranga* species were screened for antibacterial activities.
- Screened extracts displayed MIC values ranging from 4 – 128 µg/mL against the tested micro-organisms.
- Most of the extracts exhibited a bactericidal effect against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus* with MBC/MIC ratio ≤ 4.
- Flavonoids, terpenoids, saponins and coumarins were the major secondary metabolites found in all the plant extracts.

ABOUT AUTHORS

Mr Ibrahim Hashim is a Ph.D student at the Department of Chemistry, University of Nairobi, Kenya where he is working on the phytochemical investigation of medicinal plants for bioactive principles. He has published (as author/co-author) more than 10 articles in different peer-reviewed journals. Hashim is a member of Chemical Society of Nigeria (CSN) and American Chemical Society (ACS), as well as an associate member of Royal Society of Chemistry (RSC).

Prof. Leonidah Kerubo Omosa is an Associate Professor in Organic Chemistry, Department of Chemistry, University of Nairobi, Kenya. Her research interest includes: Drug discovery from Kenyan ethnomedicinal flora with anti-plasmodial, anti-microbial anti-oxidant and anti-cancer activities. She has published > 40 research articles in different peer reviewed journals. She is currently the Dean, School of Physical Sciences (SPS), University of Nairobi (UoN).

Dr. Vaderament-Alexe Nchiozem-Ngnitedem is a Researcher at the Department of Chemistry at University of Nairobi, Kenya. His main research interest is on isolation, structure elucidation and hemisynthesis of bioactive natural products from medicinal plants with antimicrobial, anti-inflammatory, and anti-cancer properties.