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Anti-staphylococcal activity and antibiotic-modulating effect of *Olax subscorpioidea, Piper guineense, Scorodophloeus zenkeri, Fagara leprieurii,* and *Monodora myristica* against resistant phenotypes

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Abstract

Background: The development of new drugs from plants seems to be an interesting alternative approach to overcoming microbial resistance. This study aims to investigate the anti-staphylococcal and antibiotic-potentiating activity of methanolic extracts of five Cameroonian dietary plants namely *Piper guineense*, *Fagara leprieurii*, *Olax subscorpioïdea*, *Monodora myristica* and *Scorodophloeus zenkeri* against a panel of multidrug resistance (MDR) *Staphylococcus aureus* clinical isolates.

Methods: All antibacterial assays were done by broth microdilution method.

Results: Our results revealed that the studied extracts displayed antibacterial activities with minimal inhibitory concentration (MIC) values ranging from 256 to 2048 µg/mL. Seeds extract of *F. leprieurii* (FLS) showed the most extensive antibacterial activity, with an inhibitory spectrum of 96% of the tested bacterial strains. The lowest MIC value of 256 µg/mL was obtained with extracts of *Piper guineense* seed (PGS) and *Scorodophloeus zenkeri* seeds (SZS) against *S. aureus* MRSA4 and SA68, respectively. Antibiotic-modulating effects against more than 70% of the *S. aureus strains* tested were obtained when *Scorodophloeus zenkeri* bark (SZB) and FLS (mostly at MIC/2) were combined with chloramphenicol, tetracycline and ceftriaxone.

Conclusions: The present study demonstrated that PGS, FLS, and SZS had anti-staphylococcal effects, and that, some studied extracts and mostly FLS, and SZB could be used as antibiotic resistance modulators to fight against resistant strains of *staphylococcus aureus.*

Key words: Anti-staphylococcal activity; antibiotic-modulating effect; resistant phenotypes.

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Background

Bacterial infections are major public health problems nowadays. The World Health Organization estimated that these infections are responsible for more than 560,000 deaths each year worldwide [1]. Antibiotic-resistant infections place a substantial health and economic burden on the health care system and population all over the world [2]. The Centers for Disease Control and Prevention (CDCP) reported that at least 23,000 people die each year in the United States as a direct result of the infections due to multidrug resistance (MDR) bacteria [3]. Among the bacterial genera responsible for human infections, staphylococci cause a wide variety of community-based and nosocomial human infections such as septicemia, endocarditis and skin infections [4]. Among drug resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) have been designated as serious public threats [3]. Based on the above observations, reversal of antibiotic resistance is therefore a high priority for researchers over the world.

Plant sources have been used since ancient times for their remarkable pharmacological properties among which their antimicrobial potentials appear as an interesting alternative for the discovery of new antibacterial substances against MDR bacteria [5, 6]. Several studies have shown the antibacterial activities as well as the antibiotic-potentiating effects of plant derived substances [7-9]. Previous studies showed that Cameroonian edible plants or their derived compounds have direct or indirect activities against sensitive and MDR bacteria [10-13]. In the same light, this study was designed first; to evaluate the anti-staphylococcal and antibiotic-potentiating activity of the methanolic extracts of five Cameroonian dietary plants namely: *Piper guineense* (Schum and Thonn) (Piperaceae), *Fagara leprieurii* (Guill and Perr) Engl. (Rutaceae), *Olax subscorpioïdea* Oliv. (Olacaceae), *Monodora myristica* Dunal (Annonaceae) and *Scorodophloeus zenkeri* Harms (Caesalpiniaceae) against a panel of *staphylococcus aureus* strains mainly constituted of MDR clinical isolates.

Methods

Plant materials and extraction

The spices used in this work were purchased from Dschang local market (West Region, Cameroon) in October 2017. These plant samples included the seeds of *Piper guineense* (Schum and Thonn) (Piperaceae), *Fagara leprieurii* (Guill and Perr) Engl. (Rutaceae), *Olax subscorpioïdea* Oliv. (Olacaceae), *Monodora myristica* Dunal (Annonaceae), barks and seeds of *Scorodophloeus zenkeri* Harms (Caesalpiniaceae). Plants were identified at the National Herbarium (Yaoundé, Cameroon), where voucher specimens were deposited under a reference number (Table 1). The extracts were obtained by methanol (MeOH) maceration as previously described [32].

Chemicals for antimicrobial assay

Six reference antibiotics (RA) purchased from Sigma-Aldrich (St Quentin Fallavier, France) were tested: chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), kanamycin (KAN), streptomycin (STR), tetracycline (TET) and ceftriaxone (CEF); *p* Iodonitrotetrazolium chloride (INT; Sigma-Aldrich) was used as microbial growth indicator; and Dimethylsulfoxyde (DMSO; Sigma-Aldrich) was used to dissolve crude extracts and antibiotics.

Bacteria, Culture Media, and Growth Conditions

Panels of *Staphylococcus aureus* used included a reference strain obtained from American Type Culture Collection (ATCC; ATCC 25923), 8 methicillin-resistant *S. aureus* (MRSA) strains (MSSA1, MRSA3, MRSA4, MRSA6, MRSA8, MRSA9, MRSA11, and MRSA12) (obtained from the culture collection of the Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, the University of Tokyo, Japan, and provided by Dr Jean P. Dzoyem, University of Dschang), and 16 resistant clinical laboratory strains of *S. aureus* (SA01, SA07, SA18, SA23, SA36, SA39, SA56, SA68, SA88, SA114, SA116, SA124,SA126, SA127, SA135, and SA139) available in our laboratory collection and previously isolated from patients in Ad-Lucem Hospital in Banka-Bafang (West Region of Cameroon). Their bacterial features are summarized in Table S1 (Supplementary Materials). Bacteria strains were maintained on agar slant at 4°C and sub-cultured on a fresh *Mueller Hinton Agar* (MHA) 24 h prior to any antibacterial test. The *Mueller Hinton Broth* (MHB) was used as liquid culture medium for susceptibility tests.

Antibacterial assay

The anti-staphylococcal activity on the different plant extracts was evaluated by microdilution method as described by Eloff [33] with some modifications [34]. Briefly, the samples were dissolved in 10% dimethyl-sulfoxide (DMSO) /Mueller Hinton Broth (MHB) and serially diluted two fold (in a 96-well microplate). Next, 100 µL of inoculum (1×10^6 CFU/mL) in MHB were added in each well. Ciprofloxacin was used as reference antibiotic and the well containing the vehicle (DMSO 2.5%) as control. Each plate was then

covered with a sterile plate sealer and gently shaken, After 18 h of incubation at 37 °C, the MIC of each sample, defined as the lowest sample concentration that inhibited complete bacteria growth was detected following addition of 40 µL INT (0.2 mg/mL) and incubation at 37 °C for 30 min. Viable bacteria reduced the yellow dye to pink. The MBC value was determined by adding 50 µL aliquots of the preparations, which did not show any growth after incubation during MIC assays, to 150 µL of MHB. Then, these preparations were incubated at 37 ºC for 48 h. The MBC was regarded as the lowest concentration of samples, which did not produce a color change after addition of INT as mentioned above [34]. All assays were performed in triplicate and repeated thrice.

Antibiotic Modulation Assays

To determine the antibiotic potentiating activity of the plant extracts, *P. guineense* seeds (PGS); *F. leprieurii* seeds (FLS); *O. subscorpioïdea* seeds (OSS); *M. myristica* seeds (MMS) and *S. zenkeri* bark (SZB) and seeds (SZS) to modulate drug resistance of *S. aureus*. MICs of CIP, CHL, TET, KAN, ERY and CEF (ranging from 2 to 256 g/mL) were determined alone and in combination first with extracts at sub-MICs (MIC/2, MIC/4, MIC/8 and MIC/16) for preliminary study against *S. aureus* SA18, then with extracts at selected sub-MICs (MIC/2 and MIC/4) against seven (07) drug resistant *S. aureus*. Briefly, after serial dilutions of antibiotics, extract was added to each well at its sub-MICs, the bacterial inoculation was done, and the MICs were determined. Rows receiving antibiotic dilutions without extracts were used for the determination of the MIC of the antibiotics. Modulation factors (MF), calculated as MIC of antibiotic alone divided by the MIC of antibiotic + extract; was used to express the antibiotic-potentiating effects of the plant extracts [7, 35].

Results

Antibacterial Activity of extracts

Results of the antibacterial activities of the 6 tested extracts and ciprofloxacin are presented in Table 2. It indicates that studied extracts possess selective antibacterial activities within a MIC range of 256–1024 µg/mL against 24/25 (96%) tested bacteria for *Fagara leprieurii* seeds (FLS), 15/25 (60%) for *Scorodophloeus zenkeri* seeds (SZS), 14/25 (56%) for *Piper guineense* seeds (PGS), 13/25 (52%) for *Olax subscorpioïdea* seeds (OSS), 12/25 (48%) for *Scorodophloeus zenkeri* bark (SZB) and *Monodora myristica* seeds (MMS). The lowest MIC value of 256 µg/mL was obtained with MMS and PGS against MRSA4 strain; with extracts at and with SZS against SA68 strain. The MBC values presented by the tested extracts (512–1024 µg/mL) were globally equal or less than 4 times the corresponding MIC values.

Antibiotic Modulation Activity of extracts

Six plant extracts: *PGS*, FLS, OSS, MMS, SZB and *SBS* were first tested at their various sub-MICs (MIC/2, MIC/4, MIC/8, and MIC/16) in combination with 8 antibiotics: CIP, CHL, TET, KAN, ERY and CEF against *S. aureus* SA18 strain. The results summarized in Table S2 (Supplementary Materials) show that better antibiotics modulation activity was obtained with all extracts FLS, OSS, MMS, and SZB at MIC/2 and MIC/4. At these concentrations, two-fold or more increase of antibiotic activities was obtained with these extracts against at least 2 of the 6 tested antibiotics. Consequently, we have selected extracts FLS, OSS, MMS, and SZB and tested in combination with the above antibiotics against seven (07) drug resistant *S. aureus* (MRSA3, MRSA4,MRSA9, SA07, SA36, SA88, SA127) at MIC/2 and MIC/4.

The results in Table 3-6 show that selected extracts presented two-fold or more antibiotic-modulating effects against more than 70% of the drug resistant *S. aureus* used. Antibiotic-modulating effects were obtained when FLS was combined with CHL (100% and 88.89 % at MIC/2 and MIC/4, respectively), with TET and CEF at MIC/2 and MIC/4 (71.42%, Table 3); when SZB was combined with CHL (85.71% and 71.42% at MIC/2 and MIC/4, respectively), TET (71.42% at MIC/2 and MIC/4), and CEF (71.42% at MIC/2) (Table 4); when OSS was combined with CHL at MIC/2 and MIC/4 (71.42%), and TET at MIC/2 (71.42%) (Table 5), and when MMS was combined with CHL at MIC/2 (71.42%) (Table 6).

Discussion

Antibacterial Potential of Extracts

Nowadays, scientists are in search of novel antimicrobial compounds which are efficient against a wide range of MDR bacteria and having fewer or no side effects. As one of the main sources, they are exploring the variety of plants among which medicinal and edible plants. This is mainly due to the diversity of plant secondary metabolites [6, 36]. Based on established criteria, MIC values ranging from 100

to1000 µg/mL are indication that the plant extract have antimicrobial activities [37]. Also, the antibacterial activity of extract from edible plant is considered as very active, if values are below 100 µg/mL; significant, if 100 ≤ MIC ≤ 512 µg/ mL and ; moderate if 512 <MIC ≤ 2058 µg/mL [38]. Herein, we explored the anti-staphyloccocal activity of selected Cameroonian dietary plants. Results depicted in Table 2 showed that seeds extracts of *Piper guineense* (PGS) and *Scorodophloeus zenkeri* (SZS) had MIC values between 100 and 512 µg/ mL against six (6) bacterial strains. The seeds extract of *Monodora myristica* (MMS) also presented significant activity (MIC=256 µg/mL) against *S. aureus* MRSA4 strains (Table 2). These results clearly indicate that seeds extracts from *Piper guineense* and *Scorodophloeus zenkeri* have good anti-staphylococcal potentials. The data obtained are in accordance with previous antibacterial investigations of the active plants. For instance, Voukeng *et al*. [22] showed that methanolic extract of *Fagara leprieurii* is active against Gram-negative bacteria. The antifungal activity of its ethanolic extract has been demonstrated [25]. The antimicrobial activities of the essential oil [18] and the aqueous and ethanolic extracts [19] of *Piper guineense* against some bacteria including *S. aureus* strain are known. *Scorodophloeus zenkeri* stem barks' essential oil as well as the methanol extract have previously presented antimicrobial activity [22, 23]. This study focused on resistant phenotypes and therefore provides additional information on the good antibacterial activity of these plants and the ability of the methanol seeds extracts of *Piper guineense, Scorodophloeus zenkeri*, and *Fagara leprieurii* to combat resistant phenotypes.

Antibiotic Modulation Activity

Staphylococcus appears as one of the major pathogens of hospital acquired infections due to their resistance to regularly prescribed antibiotics. The main biochemical resistance mechanisms developed by these bacteria include the productions of β-lactamase and the expression of efflux pumps (TetK, msrA transporters) systems [39]. The clinical isolated species of *Staphylococcus aureus* used in this study showed resistance against some tested antibiotics (Table S1). Several plant extracts and plant derived compounds are known for their ability to modulate the antibiotic resistance [8, 40]. Molecules which are able to potentiate the activity of antibiotics on more than 70% of bacteria have been suggested as potential effux pumps inhibitors [41]. In this study, antibiotic-modulating activity of extracts at MIC/2 and MIC/4 on more than 70% of the tested MDR *S. aureus* strains was obtained with the association of OSS, FLS and SZB extracts with CHL and TET (Table 3-5). This was also the case with the combination of FLS and SZB with CEF. Thus, these extracts and mostly the bark extract of *Scorodophloeus zenkeri* as well as the seeds extract of *Fagara leprieurii* can be exploited as potential efflux pump inhibitors. Nevertheless, the modulation effect observed with the combination of the same extracts with CEF could also suggest the inhibition of the enzymatic degradation of the antibiotics, which is one of the main resistance mechanisms among staphylococci species. These data show that this plant could be used in combination with some antibiotics to combat bacterial resistance to antibiotics. This is in accordance with previous studies. Voukeng *et al*. [22] showed that the above plants were able to reverse the antibiotic activity in some MDR gram negative bacteria. Many Cameroonian plants have also showed antibiotic-modulating activity against some MDR bacteria [13, 42, 43].

Table 1. Information on the studied plants

Species (family); voucher number	Traditional uses	Bioactive or potentially bioactive components	Known antimicrobial activities of plants
Piper guineense (Piperaceae) 6018/SRFC	Respiratory infections, female infertility, aphrodisiac [14], spice [15], cough, bronchitis, rheumatism, anaemia, stomach ache and cancer [16].	β-pinene, D-limonene, caryophyllene, car-z-ene and dodecetrien-z-ol,3,7,11-trimethyl [17].	Activity of: the essential oil on Pa [18]; the aqueous and ethanol extract on Sa [19]; on Ec, Sa, Bs, Pa, Ca, An [20, 21].
Scorodophloeus zenkeri (Caesalpiniaceae) 44803/SRF-Cam	Headache, cough, rheumatism, constipation, spice [15].	Alkaloids, flavonoids, phenols and tannins [22].	Activity of: the essential oil of stem bark on Ec, Sa, Bs, Cu [23]; the methanol extract from fruit on Ec, Ea, Ecl, Kp, Ps, Pa [22].
Fagara leprieurii (Rutaceae) 37632/HNC	Abdominal pain, asthma, appendicitis, toothache [24], spice [15].	Alkaloids, anthraquinones, flavonoids, phenols, tannins and triterpenes [22].	Activity of: the ethanol extract of the seeds on Ca, Cn, Mg, Tm, Tr, Bci, Af, Afl, Sb [25]; the methanol extract from seeds on Ec, Ea, Ecl, Kp, Ps, Pa [22].
Monodora myristica (Annonaceae) 2949/SRFC	Constipation, uterine hemorrhage, diuretic, fever, [26], spice [15].	α-phellanarene, p-cymene, α-pinene, cis-sabinol and limonen [27].	Activity of: the fruit essential on Mt and Sa [27], the aqueous seed extract on Sa and three other bacteria [28].
Olax subscorpioidea (Olacaceae) 3528/SRFK	Constipation, yellow fever, jaundice, venereal diseases, Guinea worm [29], arthritis and rheumatism [30], spice [15].	Alkaloids, glycosides, saponins, and steroids [31].	Methanol extract from seeds on Ec, Ea, Ecl, Kp, Ps, Pa [10].

HNC: Cameroon National Herbarium; SRF: Société des reserves forestières; Cam: Cameroon.
Pa : *Pseudomonas aeruginosa* ; *Sa* : *Staphylococcus aureus* ; *Ec* : *Escherichia coli* ; *Bs : Bacillis subtilis; Ca : Candida albicans; An: Aspergillus niger; Cu : Candida utilis; Cn : Cryptococcus neoformans ; Mg : Microsporum gypseum ; Tm: Trichophyton mentagrophytes ; Tr: Trichophyton rubrum ; Bci : Botrytis cinerea ; Af : Aspergillus fumigatus ; Afl : Aspergillus flavus; Sb: Scopulariopsis brevicaulis ; Ea* : *Enterobacter aerogenes* ; *Ecl* : *Enterobacter cloacae ; Kp* : *Klebsiella pneumoniae* ; *Ps* : *Providencia stuartii* ; *Mt* : *Micobacterium tuberculosum*.

Table 2. Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the plant extracts and ciprofoxacin against *Staphylococcus aureus* strains

***Staphylococcus aureus* strains**	**Tested sample, MIC, and MBC in µg/mL**													
	PGS		**SZS**		**SZB**		**FLF**		**OSS**		**MMS**		**Ciprofloxacine**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
SA 01	2048	-	2048	-	-	-	2048	-	-	-	-	-	1	-
SA 07	-	-	-	-	-	-	-	-	-	-	-	-	1	-
SA 18	1024	-	**512**	-	2048	-	1024	-	1024	-	2048	-	16	32
SA 23	**512**	1024	**512**	2048	2048	-	1024	2048	1024	1024	-	-	1	1
SA 36	**512**	1024	1024	-	2048	-	2048	2048	2048	-	1024	2048	2	2
SA 39	-	-	-	-	-	-	2048	-	-	-	-	-	<0.5	-
SA 56	-	-	-	-	-	-	2048	-	-	-	2048	-	16	-
SA 68	1024	-	**256**	2048	-	-	1024	-	1024	2048	-	-	<0.5	<0.5
SA 88	-	-	-	-	-	-	2048	-	-	-	-	-	1	-
SA 114	-	-	2048	-	2048	-	1024	-	1024	-	1024	-	1	16
SA 116	2048	-	**512**	-	2048	-	**512**	-	1024	-	2048	-	<0.5	-
SA 124	-	-	-	-	-	-	2048	-	-	-	-	-	1	-
SA 126	-	-	-	-	-	-	1024	-	-	-	-	-	2	2
SA 127	-	-	-	-	2048	-	2048	-	-	-	-	-	2	16
SA 135	2048	-	-	-	-	-	2048	-	-	-	-	-	1	-
SA 139	-	-	-	-	-	-	1024	-	-	-	-	-	<0.5	-
MRSA 1	**512**	1024	**512**	512	1024	2048	2048	2048	1024	-	-	-	2	2
MRSA 3	-	-	-	-	-	-	1024	2048	-	-	-	-	8	-
MRSA 4	**256**	-	1024	-	2048	-	1024	-	1024	-	**256**	-	1	16
MRSA 6	**512**	2048	1024	-	2048	-	2048	-	1024	-	-	-	<0.5	1
MRSA 8	2048	-	1024	-	-	-	2048	-	-	-	-	-	1	-
MRSA 9	1024	1024	1024	-	2048	-	1024	-	1024	-	-	-	<0.5	-
MRSA 11	**512**	1024	**512**	512	2048	-	1024	2048	1024	1024	-	-	8	16
MRSA 12	1024	2048	**512**	1024	2048	-	**512**	-	1024	1024	-	-	<0.5	8
*ATCC*25923	-	-	2048	-	-	-	2048	-	2048	-	-	-	1	-

PGS : Piper guineenses seeds *; SZS: Scorodophloeus zenkeri* seeds ; *SZB: Scorodophloeus zenkeri* bark; *FLS: Fagara leprieurii seeds ; OSS: Olax subscorpioidea* seeds ; *MMS:Monodora myristica* seeds. - : >2048 µg/mL; MIC value in bold: signifcant activity [44-46].

Table 3. Resistance-modulating effects of the methanol extract from *Scorodophloeus zenkeri* bark (SZB) at MIC/2 and MIC/4.

Antibiotics	Extract concentration	Bacterial strains, MIC (µg/mL) of antibiotics in the absence (0) and presence of the extract (MIC/2 and MIC/4)							Antibiotic-modulating effect (%)
		MRSA3	MRSA4	MRSA9	SA07	SA36	SA88	SA127	
CHL	0	32	4	4	32	128	32	32	
	MIC/2	≤2(**≥16**)	≤2(**≥2**)	≤2(**≥2**)	≤2(**≥16**)	≤2(**≥64**)	32(1)	8(**4**)	**85.71**
	MIC/4	≤2(**≥16**)	≤2(**≥2**)	16(0.25)	≤2(**≥16**)	16(**8**)	32(1)	8(**4**)	**71.42**
TET	0	16	32	2	≤0.5	1	8	64	
	MIC/2	≤0.5(**≥32**)	1(**32**)	≤0.5(**≥4**)	≤0.5(na)	≤0.5(**≥2**)	4(**2**)	64(1)	**71.42**
	MIC/4	≤0.5(**≥32**)	1(**32**)	≤0.5(**≥4**)	≤0.5(na)	≤0.5(**≥2**)	4(**2**)	64(1)	**71.42**
ERY	0	≤2	8	≤2	≤0.5	≤2	8	8	
	MIC/2	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	8(1)	4(**2**)	28.57
	MIC/4	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	8(1)	4(**2**)	28.57
CEF	0	32	16	256	256	32	32	4	
	MIC/2	≤2(**≥16**)	8(**2**)	128(**2**)	16(**16**)	≤2(**≥16**)	32(1)	4(1)	**71.42**
	MIC/4	≤2(**≥16**)	8(**2**)	256(1)	4(**4**)	32(1)	32(1)	4(1)	42.85
CIP	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
	MIC/2	≤0.5(≥**8**)	16(0.5)	≤0.5(na)	≤0.5(na)	2(1)	≤0.5(na)	1(≥0.5)	14.28
	MIC/4	≤0.5(≥**8**)	16(0.5)	≤0.5(na)	≤0.5(na)	2(1)	≤0.5(na)	1(≥0.5)	14.28
KAN	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
	MIC/2	≤2(na)	8(≤0.25)	≤2(na)	8(**8**)	≤2(na)	≤0.5(na)	4(1)	14.28
	MIC/4	≤2(na)	8(≤0.25)	≤2(na)	8(**8**)	≤2(na)	≤0.5(na)	4(1)	14.28

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : Ceftriaxone; (): Modulating factor; na: not applicable ; MIC Minimal Inhibitory Concentration ; Percentage of antibiotic's modulating effect by the plant extracts; Values in bold represent modulating factor ≥ 2 and modulating effect observed on more than 70% of the tested MDR bacteria.

Table 4. Resistance-modulating effects of the methanol extract from *Fagara leprieurii* seeds (FLS) at its MIC/2 and MIC/4.

Antibiotics	Extract concentration	Bacterial strains, MIC (µg/mL) of antibiotics in the absence (0) and presence of the extract (MIC/2 and MIC/4)							Antibiotic-modulating effect (%)
		MRSA3	MRSA4	MRSA9	SA07	SA36	SA88	SA127	
CHL	0	32	4	4	32	128	32	32	
	MIC/2	≤2(**≥16**)	≤2(≥**2**)	≤2(≥**2**)	≤2(≥**16**)	16(**8**)	16(**2**)	8(**4**)	**100**
	MIC/4	32(1)	≤2(≥**2**)	≤2(≥**2**)	≤2(≥**16**)	16(**8**)	16(**2**)	8(**4**)	**85.71**
TET	0	16	32	2	≤0.5	1	8	64	
	MIC/2	≤0.5(≥**32**)	2(**16**)	≤0.5(≥**4**)	≤0.5(≥na)	≤0.5(≥**2**)	≤0.5(≥**16**)	64(1)	**71.42**
	MIC/4	≤0.5(≥**32**)	2(**16**)	≤0.5(≥**4**)	≤0.5(na)	≤0.5(≥**2**)	1(**8**)	64(1)	**71.42**
ERY	0	≤2	8	≤2	≤0.5	≤2	>64	8	
	MIC/2	≤2(na)	≤2(≥**4**)	≤2(na)	≤0.5(na)	≤2(na)	16(>**4**)	≤2(≥**4**)	42.85
	MIC/4	32(≤0.5)	≤2(≥**4**)	≤2(na)	≤0.5(na)	≤2(na)	16(>**4**)	≤2(≥**4**)	42.85
CEF	0	32	16	256	256	32	32	4	
	MIC/2	4(**8**)	16(1)	32(**8**)	16(**16**)	8(**4**)	≤2(≥**16**)	4(1)	**71.42**
	MIC/4	16(**2**)	16(1)	64(**4**)	16(**16**)	8(**4**)	4(**8**)	4(1)	**71.42**
CIP	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
	MIC/2	1(**4**)	4(**2**)	≤0.5(na)	≤0.5(na)	2(1)	≤0.5(na)	≤0.5(na)	28.57
	MIC/4	1(**4**)	16(0.5)	≤0.5(na)	≤0.5(na)	2(1)	≤0.5(na)	≤0.5(na)	14.28
KAN	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
	MIC/2	≤2(na)	16(≤0.1)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	≤2(≥**2**)	28.57
	MIC/4	4(≤0.5)	16(≤0.1)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	≤2(≥**2**)	28.57

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : Ceftriaxone; (): Modulating factor; na: not applicable ; MIC Minimal Inhibitory Concentration ; Percentage of antibiotic's modulating effect by the plant extracts; Values in bold represent modulating factor ≥ 2 and modulating effect observed on more than 70% of the tested MDR bacteria.

Table 5. Resistance-modulating effects of the methanol extract from *Olax subscorpioidea* seeds (OSS) at its MIC/2 and MIC/4.

Antibiotics	Extract concentration	Bacterial strains, MIC (µg/mL) of antibiotics in the absence (0) and presence of the extract (MIC/2 and MIC/4)							Antibiotic-modulating effect (%)
		MRSA3	MRSA4	MRSA9	SA07	SA36	SA88	SA127	
CHL	0	32	4	4	32	128	32	32	
	MIC/2	≤2(≥**16**)	≤2(≥**2**)	8(0.5)	≤2(≥**16**)	≤2(≥**64**)	32(1)	8(**4**)	**71.42**
	MIC/4	≤2(≥**16**)	≤2(≥**2**)	16(0.25)	≤2(≥**16**)	8(**16**)	32(1)	8(**4**)	**71.42**
TET	0	16	32	2	≤0.5	1	8	64	
	MIC/2	≤0.5(≥**32**)	2(**16**)	1(**2**)	≤0.5(na)	≤0.5(≥**2**)	≤0.5(≥**16**)	64(1)	**71.42**
	MIC/4	≤0.5(≥**32**)	2(**16**)	2(1)	≤0.5(na)	≤0.5(≥**2**)	1(**8**)	64(1)	57.14
ERY	0	≤2	8	≤2	≤0.5	≤2	8	8	
	MIC/2	≤2(na)	≤2(≥**4**)	≤2(na)	≤0.5(na)	≤2(na)	8(1)	4(**2**)	28.57
	MIC/4	≤2(na)	≤2(≥**4**)	≤2(na)	≤0.5(na)	≤2(na)	8(1)	4(**2**)	28.57
CEF	0	32	16	256	256	32	32	4	
	MIC/2	≤2(≥**16**)	8(**2**)	256(1)	16(**16**)	≤2(≥**16**)	64(0.5)	4(1)	57.14
	MIC/4	≤2(≥**16**)	8(**2**)	>256(<1)	16(**16**)	16(**2**)	64(0.5)	8(0.5)	57.14
CIP	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
	MIC/2	≤0.5(≥**8**)	16(0.5)	2(≤0.25)	≤0.5(na)	2(1)	≤0.5(na)	1(≤0.5)	14.28
	MIC/4	≤0.5(≥**8**)	16(0.5)	2(≤0.25)	≤0.5(na)	4(0.5)	≤0.5(na)	1(≤0.5)	14.28
KAN	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
	MIC/2	≤2(na)	8(≤0.25)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	4(1)	14.28
	MIC/4	≤2(na)	8(≤0.25)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	4(1)	14.28

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : Ceftriaxone; (): Modulating factor; na: not applicable ; MIC Minimal Inhibitory Concentration ; Percentage of antibiotic's modulating effect by the plant extracts; Values in bold represent modulating factor ≥ 2 and modulating effect observed on more than 70% of the tested MDR bacteria.

Table 6. Resistance-modulating effects of the seeds methanol extract from ***Monodora myristica*** at its MIC/2 and MIC/4.

Antibiotics	Extract concentration	Bacterial strains, MIC (µg/mL) of antibiotics in the absence (0) and presence of the extract (MIC/2 and MIC/4)							Antibiotic-modulating effect (%)
		MRSA 3	*MRSA* 4	*MRSA* 9	*SA* 07	*SA* 36	*SA* 88	*SA* 127	
CHL	0	32	4	4	32	128	32	32	
	MIC/2	≤2(**≥16**)	≤2(**≥2**)	4(1)	≤2(**≥16**)	32(**4**)	32(1)	16(**2**)	**71.42**
	MIC/4	32(1)	4(1)	8(0.5)	≤2(**≥16**)	≤2(**≥64**)	32(1)	16(**2**)	42.85
TET	0	16	32	2	≤0.5	1	8	64	
	MIC/2	1(**16**)	4(**8**)	16(0.125)	≤0.5(na)	32(0.031)	8(1)	>64(<1)	28.57
	MIC/4	≤0.5(**≥32**)	2(**16**)	16(0.125)	≤0.5(na)	≤0.5(**≥2**)	8(1)	>64(<1)	42.85
ERY	0	≤2	8	≤2	≤0.5	≤2	>64	8	
	MIC/2	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	16(> **4**)	4(**2**)	42.85
	MIC/4	32(≤0.062)	≤2(**≥4**)	8(≤0.25)	≤0.5(na)	64(≤0.031)	64(>1)	4(**2**)	28.57
CEF	0	32	16	256	256	32	32	4	
	MIC/2	≤2(**≥16**)	16(1)	256(1)	32(**8**)	64(0.5)	32(1)	32(0.125)	28.57
	MIC/4	16(**2**)	16(1)	256(1)	32(**8**)	32(1)	32(1)	32(0.125)	28.57
CIP	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
	MIC/2	≤0.5(**≥8**)	2(**4**)	1(≤0.5)	1(≤0.5)	2(1)	≤0.5(na)	≤0.5(na)	28.57
	MIC/4	≤0.5(**≥8**)	4(**2**)	1(≤0.5)	≤0.5(na)	2(1)	≤0.5(na)	≤0.5(na)	28.57
KAN	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
	MIC/2	4(≤0.5)	32(≤0.062)	≤2(na)	32(**2**)	16(≤0.125)	≤0.5(na)	≤2(**≥2**)	28.57
	MIC/4	≤2(na)	32(≤0.062)	4(≤0.5)	32(**2**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : Ceftriaxone; (): Modulating factor; na: not applicable ; MIC Minimal Inhibitory Concentration ; Percentage of antibiotic's modulating effect by the plant extracts; Values in bold represent modulating factor ≥ 2 and modulating effect observed on more than 70% of the tested MDR bacteria.

Conclusions

In this study, the ability of the methanol seeds extracts of *Piper guineense, Scorodophloeus zenkeri* and *Fagara leprieurii* to fight against resistant strains of *Staphylococcus aureus* was demonstrated. This work also indicates that *Scorodophloeus zenkeri* and *Fagara leprieurii* bark and seeds extracts, respectively could be used as antibiotics resistance modulators, providing a new alternative to fight against bacterial infections involving resistant phenotypes.

Additional file

Supplementary file.docx. Table S1. Further details on the antibiotic-resistance profiles of tested bacteria.
Supplementary file.docx. Table S2. Preliminary antibiotic-resistance modulation activity.

Abbreviations

CEF: ceftriaxone; CHL: chloramphenicol; CIP: ciprofloxacin; DMSO: dimethylsulfoxide; ERY: erythromycin; FLS: *Fagara leprieurii* seeds ; *HNC : Herbier National du Cameroun ;* INT: *p*-iodonitrotetrazolium chloride; KAN: kanamycin; MBC: Minimal bactericidal concentration; MDR: Multi-drug resistant; MHA: *Mueller Hinton Agar;* MHB: *Mueller Hinton Broth;* MIC: Minimal inhibitory concentration; MMS: *Monodora myristica;* OSS: *Olax subscorpioïdea;* PGS: *Piper guineense* seeds; RA: Reference antibiotics; *SRF-Cam: Société des Réserves Forestières du Cameroun ;* SZB: *Scorodophloeus zenkeri* bark ; SZB: *Scorodophloeus zenkeri* seeds; TET: tetracycline.

Authors' Contribution

GB, PN and BRNW carried out the study; AGF and VK wrote the manuscript; VK designed the experiments, supervised the work, and provided the bacteria strains; all authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

1. World Health Organization. Global Health Observatory (http://www.who.int/gho/child_health/en/index.html, accessed on 28 April 2017.
2. Golkar Z, Bagazra O, Pace DG. 2014. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *J Infect Dev Ctries.* 8(2):129–136.
3. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. Available at: http://www.cdc.gov/drugresistance/threat-report-2013. Accessed on January 07, 2018.
4. Pantosti A. 2012. Methicillin-resistant *Staphylococcus aureus* associated with animal and its relevance to human. *Front Microbiol.* 3(127): 12.
5. Cowan MM. 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev,* 12(4):564-582.
6. Compean KL, Ynalvez RA. 2014. Antimicrobial activity of plant secondary metabolites: A review. *Res J Med Plant.* 8(5): 204–213.
7. Stavri M, Piddock LJV, Gibbons S. 2007. Bacterial efflux pumps 1inhibitors from natural sources. *J Antimicrob Chemother*. 59(6):1247–1260.
8. Aiyegoro OA, Okoh AI. 2009. Use of Bioactive plant products in combination with Standard Antibiotics: Implications in antimicrobial chemotherapy. *J Med Plants Res*. 3(13):1147–52.
9. [9] Saleem M, Nazir M, Ali MS, Hussain H, Lee YS, Riaz N, Jabbar A. 2010. Antimicrobial natural products: an update on future antibiotic drug candidates. *Nat Prod Rep*, 27(2):238–254.
10. Fankam AG, Kuiate JR, Kuete V. 2011. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug resistant phenotypes. *BMC Complement Altern Med*. 11:104.
11. Dzotam JK, Simo IK, Bitchagno GTM, Çelik I, Ekti's SF, Wackam VK, Tane P, Kuete V. 2018. Further antibacterial compounds from *Myristica fragrans. Invest Med Chem Pharm*. 1(2): 5.
12. Nguenang SG, Mbaveng TA, Fankam A G, Manekeng TH, Nayim P, Wamba BEN, Kuete V. 2018. *Tristemma hirtum* and five other cameroonian edible plants with weak or no antibacterial effects modulate the activities of antibiotics against Gram-Negative Multidrug-Resistant Phenotypes. *Sci World J.* Article ID 7651482, 12 pages.
13. Wamba BEN, Mbaveng AT, Nayim P, Dzotam JK, Ngalani OJT, Kuete V. 2018. Antistaphylococcal and Antibiotic Resistance Modulatory Activities of Thirteen Cameroonian Edible Plants against Resistant Phenotypes. *Int J Food Microbiol.* Article ID 1920198, 12 pages.
14. Noumi E, Amvam, ZPH, Lontsi D. 1998. Aphrodisiac plants used in Cameroon. *Fitoterapia.* 69(2):125–134**.**
15. Kuete V, Krusche B, Youns M, Voukeng I, Fankam AG., Tankeo S, Lacmata S, Efferth T. 2011. Cytotoxicity of some Cameroonian spices and selected medicinal plant extracts. *J Ethnopharmacol*, 134(3): 803–812.
16. Oparaeke AM, Bunmi JO. 2006. Bioactivity of two powdered spices *Piper guineense* thonn & schum and *Xylopia aethiopica* (dunal) A. Richard] as home-made insecticides against *Callosobruchus subinnotatus* (pic.) on stored bambarra groundnut. *Agricultura Tropica et Subtropica.* 39(2):132–134.
17. Olonisakin A, Oladimeji MO, Lajide L. 2006. Chemical composition and antibacterial activity of steam distilled oil of Ashanti Pepper (*Piper Guineense*) Fruits (Berries). *Journal of Applied Sciences.* 6(11): 2520-2522.
18. Oyedeji OA, Adeniyi BA, Ajayi O, König WA. 2005. Essential oil composition of *Piper guineense* and its antimicrobial activity. Another Chemotype from Nigeria. *Phytother Res.* 19(4): 362–364.
19. Olusimbo OA, Abimbola RE, Chinonye L A. 2011. Antimicrobial activities of some Nigerian spices on some pathogens. *ABJNA*, 2(8): 1187-1193.
20. Konning GH, Agyare C, Ennison B. 2004. Antimicrobial activity of some medicinal plants from Ghana. *Fitoterapia*, 75(1):65–67.
21. O Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO, Ogunniran KO. 2009. Antibacterial effects of extracts of *Ocimum*

gratissimum and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *Afr J Food Sci*, 3(3):77–81.
22. Voukeng IK, Kuete V, Dzoyem JP, Fankam AG, Noumedem JA, Kuiate JR, Pages JM. 2012. Antibacterial and antibiotic-potentiation activities of the methanol extract of some Cameroonian spices against Gram-negative multi-drug resistant phenotypes. *BMC Res Notes*. 5:299.
23. Kouokam JC, Jahns T, Becker H. 2002. Antimicrobial Activity of the Essential Oil and Some isolated Sulfur-Rich Compounds from *Scorodophloeus zenkeri*. *Planta Med.* 68(12):1082–1087.
24. Diniz MA, Martins ES, Gomes E, Silva O. 2007. Contribution to the knowledge of medicinal plants from Guinea-Bissau. *Port Acta Biol.* 19:417–427.
25. Ngono AN, Biyiti L, Amvam Zollo PH, Bouchet P. 2000. Evaluation of antifungal activity of extracts of two Cameroonian Rutaceae: *Zanthoxylum leprieurii* Guill. et Perr., and *Zanthoxylum xanthoxyloides* Waterm. *J Ethnopharmacol*. 70(3):335–342.
26. Okafor JC. 1987. Development of forest tree crops for food supplies in Nigeria. *For Ecol Manage*. 1: 235–247.
27. Tatsadjieu LN, Essia Ngang JJ, Ngassoum MB, Etoa FX. 2003. Antibacterial and antifungal activity of *Xylopia aethiopica, Monodora myristica, Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* from Cameroon. *Fitoterapia,* 74(5):469–472.
28. Ukaegbu-Obi K. M, Meribe M. O. et Odo C. E. 2015. Assessment of antimicrobial activity of aqueous and ethanolic extracts of Monodora myristica (ehuru) seeds. *MJPMS*, 4(3): 1-3.
29. Okoli RI, Aigbe JO, Ohaju-Obodo JO, Mensah JK. 2007. Medicinal herbs used for managing some common. *Pakistan J Nutr.* 6(5):490–496.
30. Oni SO. 2010. *In vitro* study of the effects of aqueous extracts of *Olax subscorpioidea* and *Lonchocarpus cyanescens* on monoamide oxidase activity from rat brain. Unpublished B.Sc. dissertation, *Federal University of Agriculture*, *Abeokuta*, Nigeria.
31. Otori AA, Mann A. 2014. Determination of chemical composition, minerals and antinutritional factors of two wild seeds from Nupeland, North Central Nigeria. *AJAC*, 1(1): 20-26.
32. Fankam AG, Kuiate JR, Kuete V. 2017. 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.* 17:168.
33. Eloff JN. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med*. 64(8):711–713.
34. Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. 2009. Antimicrobial activity of the crude extract, fractions and compounds from stem bark of *Ficus ovate* (Moraceae). *J Ethnopharmacol.* 124(3): 556–561.
35. Kovač J, Gavari N, Bucar F, Smole MS. 2014. Antimicrobial and resistance modulatory activity of *Alpinia katsumadai* seed extract, essential oil and post-distillation extract. *Food Technol Biotech*. 52(2):248–254.
36. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. 2017. Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. *Plants*. 6:16, doi: 10.3390/plants6020016.
37. Simões M, Bennett RN, Rosa EA. 2009. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. *Nat Prod Rep*, 26(6):746–757.
38. Tamokou JDD, Mbaveng AT, Kuete V. 2017. Chapter 8 - Antimicrobial Activities of African Medicinal Spices and Vegetables. *In: Medicinal Spices and Vegetables from Africa.* Academic Press, Cambridge, MA, USA. pp. 207-237.
39. Zmantar T, Slama RB, Fdhila K, Kouidhi B, Bakhrouf A, Chaieb K. 2017. Modulation of drug resistance and biofilm formation of Staphylococcus aureus isolated from the oral cavity of Tunisian children. *Braz J Infect Dis.* 2 1(1):27–34.
40. Sibanda T, Okoh AI. 2007. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. *Afr J Biotechnol*. 6(25):2886–96.
41. Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, ChartoneSouza E, Nascimento AMA. 2005. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Can J Microbiol*. 51(7):541–547.
42. Dzotam J.K, Kuete V. 2017. Antibacterial and antibioticmodifying activity of methanol extracts from six Cameroonian food plants against multidrug-resistant enteric bacteria. *Biomed Res Int.* Article ID 1583510, 19 pages.
43. Manekeng HT, Mbaveng AT, Nguenang GS, Seukep JA, Wamba BEN, Nayim N, Yinkfu NR, Fankam AG, Kuete V. 2018. Anti-staphylococcal and antibiotic-potentiating activities of seven Cameroonian edible plants against resistant phenotypes. *Invest Med Chem Pharm.*1(1): 7.
44. Fankam AG, Jules R Kuiate JR, Kuete V. 2014. Antibacterial activities of *Beilschmiedia obscura* and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes . *BMC Complement AlternMed.* 14:241.
45. Noumedem JAK, Mihasan M, Kuiate JR, Stefan M, Dzoyem JP, Cojocaru D, Kuete V. 2013. *In Vitro* Antibacterial and Antibiotic-potentiation Activities of four Edible Plants against Multidrug-resistant Gram-negative Species. *BMC Complement AlternMed.* 13:190.
46. Kuete, V., Wiench, B., Hegazy, M.E.F., Mohamed, T.A, Fankam, A.G., Shahat, A.A., Efferth, T. (2012): Antibacterial activity and cytotoxicity of selected Egyptian medicinal plants. *Planta Med.* 78: 193-199.