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Cinnamomum zeylanicum, Dichrostachys glomerata and three other plants had anti-staphylococcal and antibiotic-modifying activity against drug-resistant phenotypes

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Abstract

Background: The increase resistance of *Staphylococcus aureus* clinically, propels the search of novel approaches to treat staphylococcal infections. This study aims to investigate the anti-staphylococcal activity and antibiotic-modulating effects of the methanol extracts of five Cameroonian dietary plants namely *Cinnamomum zeylanicum, Fagara xanthoxyloides, Imperata cylindrica, Dichrostachys glomerata, Pentadiplanara brazzeana* against a panel of *S. aureus* strains.

Methods: The plant extracts were prepared by maceration in methanol. The activity of extracts and the reference antibiotic (Ciprofloxacin) was evaluated against 25 strains of *S. aureus* including antibiotic-resistant phenotypes, using the broth microdilution method, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined.

Results: Ciprofloxacin displayed anti-staphylococcal activity against all tested strains whereas the five extracts had selective activities with recorded MIC values ranged between 256–2048 µg/mL. *Cinnamomum zeylanicum* leave's extract had the highest activity, with MIC values observed on 23/25 bacteria (92%). The lowest MIC value (256 µg/mL) was recorded against MRSA4 with extracts of *Fagara xanthoxyloides* seeds and *Dichrostachys glomerata* fruits. The most active extracts displayed bactericidal effects (MBC/MIC≤ 4). Antibiotic-modulating activity was observed on more than 70% of the tested *S. aureus* strains after the combination of CHL and TET with above extracts (at MIC/2 and MIC/4).

Conclusions: The overall data obtained highlight the suitability of the tested extracts, mainly those of *C. zeylanicum* and *D. glomerata* alone as well as in combination with chloramphenicol and tetracycline, as therapeutic agents for treatment of infections caused by resistant strains of *S. aureus*.

Keywords: *Cinnamomum zeylanicum, Dichrostachys glomerata,* anti-staphylococcal activity; antibiotic-modulating effect; drug-resistant phenotypes.

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Background

Bacterial infections account for more than one fifth of the 2.7 million neonatal deaths worldwide each year [1]. Among the most pandemic bacterial pathogens, *Staphylococcus aureus* is one which causes severe morbidity and fatal infections, ranging from minor skin and soft tissue infection to life-threatening pneumonia and toxinosis [2]. It is estimated that about 70% of staphylococci isolated from sepsis in intensive care are *Staphylococcus aureus* [3]. Rapid and indiscriminate use of antibiotics in the treatment of staphylococcal infections has caused the emergence and spread of resistant strains like methicillin-resistant S. aureus (MRSA) [4, 5]. In staphylococci, antibiotic resistance has been documented [6]. MRSA can use many mechanisms to evade the antibacterial action; this include (i) enzymatic drug modification or inactivation, (ii) modification of drug binding site, (iii) acquisition of novel drug resistant target and (iv) over-expression of endogenous efflux pumps [7]. Problems of antibiotic resistance in *Staphylococcus aureus* present propel the search of novel treatment's approaches.

Plants still serve as a rich source of many novel biologically active compounds because they are known to produce various antimicrobial molecules to protect themselves from other plants or environmental pathogens [8]. Plant-derived antimicrobials are potential sources of novel antibacterial drugs. Furthermore, drug combination strategies, in particular, phytochemical and antibiotic combination approaches have been recommended in several studies to combat multiple drug-resistant bacteria [8-13]. Previous studies have shown the antibacterial and/or potentiating (antibiotic) activity of many food plants in Cameroon against multi-resistant bacteria [14-17]. Hence, the aim of this study was to evaluate the *in vitro* antibacterial activity of methanol extracts of five Cameroonian dietary plants namely *Cinnamomum zeylanicum, Fagara xanthoxyloides, Imperata cylindrical, Dichrostachys glomerata* and *Pentadiplanara brazzeana* against a panel of *S. aureus* strains mainly constituted of multidrug resistant (MDR) clinical isolates. We have extended the study to the combination of studied extracts and some commonly used antibiotics against selected MDR bacteria.

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 roots of *Pentadiplandra brazzeana* Baill. (Capparaceae) and *Imperata cylindrica* Beauv. var. koenigii Durand and Schinz (Gramineae), leaves of *Cinnamomum zeylanicum* (Linn) Cor. (Lauraceae), seeds of *Fagara xantoxyloides* Watern. (Rutaceae), and fruits of *Dichrostachys glomerata* (Forsk) Chuov (Mimosaceae). Plants were identified at the National Herbarium (Yaoundé, Cameroon), where voucher specimens were deposited under a reference number (Table 1). For extraction, each plant material was cleaned and air-dried, and the powder (200 g) was soaked in methanol (MeOH, 0.6 L) for 48 h at room temperature. The extract obtained was collected by filtration and concentrated under reduced pressure using a rotary evaporator to yield a residue which constituted the crude extract. All extracts were then kept at 4 ºC until further use.

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 strains and culture media

The panel of *Staphylococcus aureus* strains 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 antibiotic susceptibility features are summarized in Table S1 (Supplementary file). All bacteria strains were maintained on *Mueller Hinton Agar* (MHA) slant at 4°C and sub-cultured 24 h prior to any antibacterial test. *Mueller Hinton Broth* (MHB) was used for the determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) [13].

MIC and MBC determinations

The anti-staphylococcal activity on the different plant extracts was evaluated using rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay [32] with some modifications [25, 33]. Briefly, the samples were dissolved in 10% dimethyl-sulfoxide (DMSO) /Mueller Hinton Broth (MHB) and serially diluted two fold (in a 96-well microplate). Then, 100 µL of inoculum (1×10^6 CFU/mL) prepared in MHB were added in each well. Ciprofloxacin was used as reference drug and the well containing the vehicle (DMSO 2.5%) as control. Microplates were further covered with a sterile plate sealer and gently shacked. 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 [33]. The assays were performed trice in triplicate.

Antibiotic modulation assay

The evaluation of the extracts as antibiotic activity modifiers was performed according to Coutinho et al. [34] with some modifications [12]. Briefly, after serial dilutions (2 and 256 µg/mL) of antibiotics (CIP, CHL, TET, KAN, ERY and CEF), the extracts were added at their sub-inhibitory concentration (MIC/2, MIC/4, MIC/8 and MIC/16). Rows receiving antibiotic dilutions without extracts were used for the determination of the MIC of the antibiotics. Controls were prepared as described above. The plates were incubated at 37°C for 24 h and read through the addition of INT. Modulation factors (MF), calculated as MIC of antibiotic alone

divided by the MIC of antibiotic + extract; was used to express the antibiotic-modulating effects of the plant extracts [12, 35]. The assays were performed trice in triplicate.

Results

Tested extracts have potent anti-staphylococcal activity

The anti-staphylococcal activity of the tested extracts as well as that of ciprofloxacin (RA) was evaluated against 25 *S. aureus* strains including antibiotic-resistant phenotypes. Ciprofloxacin displayed anti-staphylococcal activity against all tested strains whereas the five extracts had selective activities with MIC values range between 256–2048 µg/mL (Table 2). Extract of *Cinnamomum zeylanicum* (CZL) had the highest activity, with MIC values observed on 23/25 bacteria (92%); followed by the extracts of *Dichrostachys glomerata* (DGF) (22/25; 88%), *Fagara xanthoxyloides* (FXS) (21/25; 84%), *Imperata cylindrical* (ICR) (17/25; 68%) and finally by *Pentadiplanara brazzeana* (PBR) (4/25; 16%). The lowest MIC value (256 µg/mL) was recorded against MRSA4 with the extracts FXS and DGF. The MBC values of the tested extracts (512–1024 µg/mL) were globally equal or less than 4 times the MIC values (Table 2).

Tested extracts potentiate the activity of antibiotics when used in combination

Table S2 (Supplementary file) displays the results of the preliminary study performed by combining 6 antibiotics (CIP, CHL, TET, KAN, ERY and CEF) with the tested extracts at their various sub-MICs (MIC/2, MIC/4, MIC/8, and MIC/16) against *S. aureus* SA18. It allowed selecting MIC/2 and MIC/4 as the sub-MIC values of the plant extracts which were to show a considerable antibiotic-modulation activity. At these concentrations, two-fold increase of antibiotic activities and more were obtained. Selected extracts were further tested in combination with the above antibiotics against seven drug resistant *S. aureus* (MRSA3, MRSA4, MRSA9, SA07, SA36, SA88, SA127) at MIC/2 and MIC/4. The results summarized in Table 3-7 show that all the extracts had more than two-fold antibiotic-modulating effects against more than 70% of the selected drug resistant *S. aureus*. It was the case after combinations CZL and CHL (100% and 85.71%, at CMI/2 and CMI/4 respectively), CZL and TET (71.42%, at CMI/4) (Table 3); combinations between ICR and CHL (100% and 71.42% at CMI/2 and CMI/4, respectively), ICR and TET (85.71% and 71.42%, at CMI/2 and CMI/4, respectively) (Table 4); combinations between DGF and CHL (85.71% and 71.42%, at CMI/2 and CMI/4, respectively), DGF and TET (71.42%, at CMI/2 and CMI/4), DGF and CEF (71.42% at CMI/2) (Table 5); combinations between PBR and CHL (100% and 85.71% at CMI/2 and CMI/4 respectively), PBR and CEF (71.42% at CMI/2) (Table 6); after combinations of FXS and CHL (100% and 71.42%, at CMI/2 and CMI/4, respectively), FXS and TET (71.42% at CMI/2 and CMI/4), FXS and CEF (85.71% and 71.42% at CMI/2 and CMI/4, respectively) (Table 7).

Discussion

The spread of multi-drug resistant clinical isolates of *S. aureus* represents a serious heath concern, due to lack of selective therapeutic options [36]. Therefore, the development of novel antibacterial substances or those which may delay the emergence of resistance is required to combat such MDR infections. Plant derivatives, in recent year have shown their potential as antibacterial as well as drug resistance reversal agents by usually use deferent mechanisms than conventional antibiotics [37, 38]. Herein, we explored the anti-staphyloccocal activity of selected Cameroonian dietary plants. Data reported in Table 2 indicated that extracts from seeds of *Fagara xanthoxyloides*, fruits of *Dichrostachys glomerata* (DGF) and seeds of *Cinnamomum zeylanicum* had MIC values between 256 and 512 µg/ mL against 6, 3 and 2 bacterial strains, respectively. According to Tamokou *et al.* [39], these extracts, mainly that of *Fagara xanthoxyloides* presented significant anti-staphyloccocal activity (100 ≤ MIC ≤ 512 µg/ mL) against some *S. aureus strains* including SA23, SA116, MRSA4 and MRSA9; whereas *Cinnamomum zeylanicum* and *Imperata cylindrical* had moderate activity (512 <MIC ≤ 2058 µg/mL). This clearly indicates that seeds extracts of *Fagara xanthoxyloides* possesses good anti-staphylococcal potential. It should be mentioned that the obtained data are in accordance with previous works. Voukeng et al. [21] demonstrated that the methanol extracts of *Cinnamomum zeylanicum* and *Imperata cylindrica* were active against Gram-negative bacteria including MDR phenotypes of *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Parkavi *et al*. [27] showed that Ethanol leaf extract of *Imperata cylindrica* is active on *E. coli*. It was also reported that the methanol fruits extract of *Dichrostachys glomerata* and seed extract of *Fagara xanthoxyloides* were active against selected MDR Gram-negative bacteria [25]. This study was focused on Gram-positive resistant phenotypes, particularly on pathogenic *S. aureus* strains and therefore provides additional information on the antibacterial activity of the tested plants.

Drug synergism between antibiotics and bioactive botanicals or phytochemicals is one of the novel ways to overcome multidrug resistance in pathogenic bacteria [10, 38, 39]. From the present study, it was demonstrated that the tested extracts possess high synergistic activity with two-fold increase of antibiotic-modulating effects (Table 3-7). This is very promising since the observed effect could lead to new options for the treatment of drug resistance *S. aureus* infectious. Since MRSA can use many mechanisms to evade the antibiotic actions; such as enzymatic drug modification or inactivation, modification of drug binding site, acquisition of novel drug resistant target and over-expression of endogenous efflux pumps [7]; We hypothesized a role of the tested extract in the inhibition of drug resistance proteins in MRSA. Furthermore, bacterial efflux pumps are an important mechanism of antibiotic resistance and are required for many pathogens to cause infection [40]. In this study, antibiotic-modulating activity were observed on more than 70% of the tested MDR *S. aureus* strains after the combination of the extracts CZL, ICR, and DGF extracts with CHL and TET (Table 3-5). This suggests that these extracts could be considered as potential sources of efflux pumps inhibitors [41]. This is in accordance with previous studies, such as those of Voukeng et al. [21] and Fankam et al. [25] which showed that some of the above plant extracts were able to reverse the antibiotic activity in Gram negative MDR bacteria. Many others Cameroonian plants previously showed antibiotic-modulating activity against MDR bacteria [12,13, 16, 42]. Finally, the tested plant extracts and mainly those of *Cinnamomum zeylanicum, Imperata cylindrica* and *Dichrostachys glomerata* could be used in combination with antibiotics to combat MDR bacteria infections.

Table 1. Information on the studied plants

Species (family); voucher number	Traditional uses	Bioactive or potentially bioactive components	Known antimicrobial activities of plants
Cinnamomum zeylanicum (Lauraceae) 22309/SRFC	Intestinal worms and skin parasites [18].	Cinnamaldéhyde, eugénol, benzaldéhyde [19], acétate3-phénylpropyle, benzoate de benzyle, α-caryophyllène, α-cadinène, r-humulène, r-copaène, ç-cadinène, r-muurolène, germacrene-B, germacrene-D [20].	Essential oil of the bark on 21 bacteria including Sa [19]; methanol extract from fruit on Ec, Ea, Ecl, Kp, Ps, Pa [21].
Dichrostachys glomerata (Fabaceae-Mimosoideae) 15220/SRF-Cam	Antidotes, analgesic, arthritis, swelling, edema, venereal diseases [22].	Emodin, 3-geranyloxyemodin, 2-geranylemodin [23].	Methanol leaf extract on Sa [24]; methanol seed extract on Ec, Ea, Ecl, Kp, Ps, Pa [25].
Imperata cylindrica (Ramineae) 30139/SRFC	Diuretic, anti-inflammatory, dysentery, urinary tract infections, cancer [26].	Phytochemical screening highlights alkaloids, anthocyanins, anthraquinones, flavonoids, phenols and triterpenes [21].	Ethanolic leaf extract on Ec and Sa [27]; methanol extract from fruit on Ec, Ea, Ecl, Kp, Ps, Pa [21].
Pentadiplanara brazzeana (Capparaceae) 42918/HNC	Gastric ulcer and cancer [28].	Benzylisothiocyanate and benzylcyanide have been isolated from essential oil roots [29].	Hydroethanolic leaf extract on Pv[30]; Essential oil on Ec, Sa, Pa and six fungal species[29].
Fagara xanthoxyloides (Rutaceae) 21793/HNC/SRF	Elephantiasis, toothache, sexual impotence, gonorrhea, malaria, dysmenorrheal, abdominal pain [31].	Alkaloids, anthraquinones, flavonoids, phenols and tannins [25].	Methanol seed extract on Ec, Ea, Ecl, Kp, Ps, Pa [25].

HNC: Cameroon National Herbarium; SRF: Société des reserves forestières; Cam: Cameroon.
Sa : *Staphylococcus aureus* ; *Ec* : *Escherichia coli* ; *Ea* : *Enterobacter aerogenes* ; *Ecl* : *Enterobacter cloacae* ; *Kp* : *Klebsiella pneumoniae* ; *Ps* : *Providencia stuartii* ; *Pa* : *Pseudomonas aeruginosa* ; *Pv* : *Proteus vulgaris.*

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 samples, MIC, and MBC in µg/mL**											
	Cinnamomum zeylanicum		***Fagara xanthoxyloides***		***Imperata cylinaricum***		***Dichrostachys glomerata***		***Pentadiplanara brazzeana***		**Ciprofloxacin**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
SA01	2048	-	-	-	2048	-	2048	-	-	-	1	-
SA07	1024	-	-	-	-	-	2048	-	-	-	1	-
SA18	1024	2048	2048	-	1024	2048	2048	-	-	-	16	32
SA23	1024	2048	**512**	-	2048	2048	2048	-	2048	-	1	1
SA36	1024	2048	2048	2048	1024	-	**512**	-	1024	2048	2	2
SA39	2048	-	2048	-	-	-	1024	-	-	-	<0.5	-
SA56	2048	-	2048	-	2048	-	2048	-	-	-	16	-
SA 68	2048	2048	2048	-	1024	2048	1024	2048	1024	-	<0.5	<0.5
SA88	2048	-	1024	-	-	-	-	-	-	-	1	-
SA114	2048	-	1024	-	2048	-	2048	-	-	-	1	16
SA116	2048	-	**512**	-	**512**	-	1024	-	2048	-	<0.5	-
SA124	1024	-	2048	-	-	-	2048	-	-	-	1	-
SA126	-	-	1024	-	2048	-	2048	-	-	-	2	2
SA127	-	-	2048	-	-	-	2048	-	-	-	2	16
SA135	**512**	-	2048	-	-	-	-	-	-	-	1	-
SA139	2048	-	**512**	-	-	-	**512**	-	-	-	<0.5	-
MSSA1	2048	-	2048	-	2048	2048	1024	2048	-	-	2	2
MRSA3	**512**	-	-	-	2048	-	-	-	-	-	8	-
MRSA4	2048	-	**256**	-	1024	-	**256**	-	-	-	1	16
MRSA6	2048	2048	1024	-	2048	-	2048	-	-	-	<0.5	1
MRSA8	2048	-	1024	-	-	-	2048	-	-	-	1	-
MRSA9	1024	-	**512**	1024	2048	-	1024	-	-	-	<0.5	-
MRSA11	2048	-	2048	-	2048	2048	2048	2048	-	-	8	16
MRSA12	2048	-	**512**	1024	2048	-	1024	-	-	-	<0.5	8
ATCC25923	1024	-	-	-	2048	-	2048	-	-	-	1	-

MIC: Minimal Inhibitory Concentration ; MBC : Minimal bactericidal concentrations ; *MRSA :* Methicillin-resistant *Staphylococcus aureus; SA : Staphycoccus aureus.*
- : >2048 µg/mL

Table 3. Resistance-modulating effects of the leaves methanol extract from *Cinnamomum zeylanicum* (CZL) at its MIC/2 and MIC/4.

Antibiotics	**Extract concentrations**	**Bacterial strains, MIC (µg/mL) of antibiotics in the absence (0) and presence of the extract**							**Antibiotic-modulating effect (%)**
		MRSA3	**MRSA4**	**MRSA9**	**SA07**	**SA36**	**SA88**	**SA127**	
	0	32	4	4	32	128	32	32	
CHL	MIC/2	≤2(**≥16**)	≤2(**≥2**)	≤2(**≥2**)	≤2(**≥16**)	16(**8**)	16(**2**)	4(**8**)	**100**
	MIC/4	16(**2**)	≤2(**≥2**)	4(1)	≤2(**≥16**)	16(**8**)	16(**2**)	4(**8**)	**85.71**
	0	16	32	2	≤0.5	1	8	64	
TET	MIC/2	≤0.5(**≥32**)	2(**16**)	1(**2**)	≤0.5(na)	≤0.5(**≥2**)	8(1)	64(1)	57.14
	MIC/4	≤0.5(**≥32**)	2(**16**)	1(**2**)	≤0.5(na)	≤0.5(**≥2**)	8(1)	32(**2**)	**71.42**
	0	≤2	8	≤2	≤0.5	≤2	>64	8	
ERY	MIC/2	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	16(>**4**)	8(1)	28.57
	MIC/4	8(≤0.25)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	16(>**4**)	8(1)	28.57
	0	32	16	256	256	32	32	4	
CEF	MIC/2	≤2(**≥16**)	16(1)	4(**64**)	16(**16**)	16(**2**)	32(1)	4(1)	57.14
	MIC/4	8(**4**)	16(1)	256(1)	16(**16**)	16(**2**)	32(1)	8(0.5)	42.85
	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
CIP	MIC/2	≤0.5(**≥8**)	≤0.5(**≥16**)	≤0.5 na)	1(≤0.5)	2(1)	≤0.5(na)	≤0.5(na)	28.57
	MIC/4	≤0.5(**≥8**)	≤0.5(**≥16**)	≤0.5(na)	1(≤0.5)	4(0.5)	≤0.5(na)	≤0.5(na)	28.57
	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
KAN	MIC/2	8(≤0.25)	16(≤0.125)	≤2(na)	≤2(**≥32**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57
	MIC/4	≤2(1)	16(≤0.125)	≤2(na)	≤2(**≥32**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : Ceftriaxon; (): Modulating factor; na: not applicable ; MIC Minimal Inhibitory Concentration ; 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 roots methanol extract from *Imperata cylindrica*(ICR) at its MIC/2 and MIC/4.

Antibiotics	Extract concentration	Bacterial strains, MIC (µg/mL) of antibiotics in the absence and presence of the extract							Antibiotic-modulating effect (%)
		MRSA3	MRSA4	MRSA9	SA07	SA36	SA88	SA127	
	0	32	4	4	32	128	32	32	
CHL	MIC/2	≤2(**≥16**)	≤2(**≥2**)	≤2(**≥2**)	≤2(**≥16**)	16(**8**)	16(**2**)	16(**2**)	**100**
	MIC/4	32(1)	≤2(**≥2**)	≤2(**≥2**)	≤2(**≥16**)	32(**4**)	32(1)	16(**2**)	**71.42**
	0	16	32	2	≤0.5	1	8	64	
TET	MIC/2	1(**16**)	4(**8**)	≤0.5(**≥4**)	≤0.5(na)	≤0.5(**≥2**)	≤0.5(**≥16**)	32(**2**)	**85.71**
	MIC/4	≤0.5(**≥32**)	4(**8**)	1(**2**)	≤0.5(na)	≤0.5(**≥2**)	2(**4**)	64(1)	**71.42**
	0	≤2	8	≤2	≤0.5	≤2	>64	8	
ERY	MIC/2	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	16(>**4**)	4(**2**)	42.85
	MIC/4	16(**≤8**)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	16(>**4**)	4(**2**)	57.14
	0	32	16	256	256	32	32	4	
CEF	MIC/2	16(**2**)	16(1)	256(1)	32(**8**)	≤2(**≥16**)	≤2(**≥16**)	4(1)	57.14
	MIC/4	16(**2**)	16(1)	256(1)	32(**8**)	16(**2**)	≤2(**≥16**)	4(1)	57.14
	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
CIP	MIC/2	1(**4**)	4(**2**)	≤0.5(na)	≤0.5(na)	≤0.5(≥**4**)	≤0.5(na)	≤0.5(na)	42.85
	MIC/4	≤0.5(**≥8**)	16(0.5)	1(na)	1(≤0.5)	4(0.5)	≤0.5(na)	≤0.5(na)	14.28
	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
KAN	MIC/2	≤2(na)	16(≤0.125)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57
	MIC/4	4(≤0.5)	16(≤0.125)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : Ceftriaxon; (): Modulating factor; na: not applicable ; MIC Minimal Inhibitory Concentration ; 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 fruits methanol extract from *Dichrostachys glomerata* (DGF) at its MIC/2 and MIC/4.

Antibiotics	Extract concentration	Bacterial strains. MIC (µg/mL) of antibiotics in the absence and presence of the extract							Antibiotic-modulating effect (%)
		MRSA3	MRSA4	MRSA9	SA07	SA36	SA88	SA127	
	0	32	4	4	32	128	32	32	
CHL	MIC/2	≤2(**≥16**)	4(1)	≤2(**≥2**)	≤2(**≥16**)	16(**8**)	≤2(**≥16**)	8(**4**)	**85.71**
	MIC/4	≤2(**≥16**)	4(1)	16(0.25)	≤2(**≥16**)	32(**4**)	≤2(**≥16**)	8(**4**)	**71.42**
	0	16	32	2	≤0.5	1	8	64	
TET	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**)	≤0.5(**≥16**)	64(1)	**71.42**
	0	≤2	8	≤2	≤0.5	≤2	8	8	
ERY	MIC/2	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	≤0.5(**≥16**)	4(**2**)	42.85
	MIC/4	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	8(1)	4(**2**)	28.57
	0	32	16	256	256	32	32	4	
CEF	MIC/2	≤2(**≥16**)	8(**2**)	256(1)	64(**4**)	32(1)	≤2(**≥16**)	≤2(**≥2**)	**71.42**
	MIC/4	8(**4**)	8(**2**)	>256(<1)	128(**2**)	32(1)	≤2(**≥16**)	8(0.5)	57.14
	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
CIP	MIC/2	≤0.5(**≥8**)	8(1)	≤0.5(na)	≤0.5(na)	4(0.5)	≤0.5(na)	1(≤0.5)	14.28
	MIC/4	≤0.5(**≥8**)	16(0.5)	≤0.5(na)	≤0.5(na)	4(0.5)	≤0.5(na)	1(≤0.5)	14.28
	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
KAN	MIC/2	4(≤0.5)	32(≤0.06)	≤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 : Ceftriaxon; (): Modulating factor; na: not applicable ; MIC Minimal Inhibitory Concentration ; 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 roots methanol extract from *Pentadiplanara brazzeana* (PBR) 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							Antibiotic-modulating effect (%)
		MRSA3	MRSA4	MRSA9	SA07	SA36	SA88	SA127	
	0	32	4	4	32	128	32	32	
CHL	MIC/2	≤2(**≥16**)	≤2(**≥2**)	≤2(**≥2**)	≤2(**≥16**)	16(**8**)	16(**2**)	8(**4**)	**100**
	MIC/4	≤2(**≥16**)	≤2(**≥2**)	4(1)	≤2(**≥16**)	32(**4**)	16(**2**)	8(**4**)	**85.71**
	0	16	32	2	≤0.5	1	8	64	
TET	MIC/2	≤0.5(**≥32**)	8(**4**)	2(1)	≤0.5(na)	≤0.5(**≥2**)	≤0.5(**≥16**)	64(1)	57.14
	MIC/4	≤0.5(**≥32**)	8(**4**)	2(1)	≤0.5(na)	≤0.5(**≥2**)	8(1)	64(1)	42.85
	0	≤2	8	≤2	≤0.5	≤2	>64	8	
ERY	MIC/2	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	8(> **8**)	≤2(**≥4**)	42.85
	MIC/4	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	8(> **8**)	≤2(**≥4**)	42.85
	0	32	16	256	256	32	32	4	
CEF	MIC/2	16(**2**)	16(1)	16(**16**)	≤2(**≥128**)	16(**2**)	32(1)	≤2(**≥2**)	**71.42**
	MIC/4	16(**2**)	16(1)	16(**16**)	64(**4**)	32(1)	32(1)	≤2(**≥2**)	57.14
	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
CIP	MIC/2	≤0.5(**≥8**)	1(**8**)	1(≤0.5)	≤0.5(na)	2(1)	≤0.5(na)	≤0.5(na)	28.57
	MIC/4	≤0.5(**≥8**)	4(**2**)	2(≤0.25)	1(≤0.5)	2(1)	≤0.5(na)	≤0.5(na)	28.57
	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
KAN	MIC/2	≤2(na)	16(≤0.125)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57
	MIC/4	≤2(na)	32(≤0.062)	≤2(na)	16(**4**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57

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

Table 7. Resistance-modulating effects of the beans methanol extract from *Fagara xanthoxyloides* (FXB) at its MIC/2 and MIC/4.

Antibiotics	Extract concentration	Bacterial strains. MIC (µg/mL) of antibiotics in the absence and presence of the extract MRSA3	MRSA4	MRSA9	SA07	SA36	SA88	SA127	Antibiotic-modulating effect (%)
	0	32	4	4	32	128	32	32	
CHL	MIC/2	≤2(**≥16**)	≤2(**≥2**)	≤2(**≥2**)	≤2(**≥16**)	≤2(**≥64**)	16(**2**)	16(**2**)	**100**
	MIC/4	32(1)	4(1)	≤2(**≥2**)	≤2(**≥16**)	≤2(**≥64**)	16(**2**)	16(**2**)	**71.42**
	0	16	32	2	≤0.5	1	8	64	
TET	MIC/2	≤0.5(**≥32**)	8(**4**)	≤0.5(**≥4**)	≤0.5(na)	≤0.5(**≥2**)	4(**2**)	64(1)	**71.42**
	MIC/4	≤0.5(**≥32**)	8(**4**)	1(**2**)	≤0.5(na)	≤0.5(**≥2**)	4(**2**)	64(1)	**71.42**
	0	≤2	8	≤2	≤0.5	≤2	>64	8	
ERY	MIC/2	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	≤2(na)	8(> **8**)	4(**2**)	42.85
	MIC/4	≤2(na)	≤2(**≥4**)	≤2(na)	≤0.5(na)	4(≤0.5)	16(> **4**)	4(**2**)	42.85
	0	32	16	256	256	32	32	4	
CEF	MIC/2	≤2(**≥16**)	16(1)	64(**4**)	128(**2**)	≤2(**≥16**)	16(**2**)	≤2(**≥2**)	**85.71**
	MIC/4	≤2(**≥16**)	16(1)	64(**4**)	128(**2**)	8(**4**)	64(0.5)	≤2(**≥2**)	**71.42**
	0	4	8	≤0.5	≤0.5	2	≤0.5	≤0.5	
CIP	MIC/2	≤0.5(**≥8**)	8(1)	≤0.5(na)	≤0.5(na)	≤0.5(**≥4**)	≤0.5(na)	≤0.5(na)	28.57
	MIC/4	≤0.5(**≥8**)	8(1)	≤0.5(na)	≤0.5(na)	≤0.5(**≥4**)	≤0.5(na)	≤0.5(na)	28.57
	0	≤2	≤2	≤2	64	≤2	≤0.5	4	
KAN	MIC/2	4(≤0.5)	32(≤0.062)	≤2(na)	≤0.5(**≥128**)	≤2(na)	≤0.5(na)	≤2(**≥2**)	28.57
	MIC/4	4(≤0.5)	32(≤0.062)	≤2(na)	≤0.5(**≥128**)	≤2(na)	≤0.5(na)	8(0.5)	14.28

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

Conclusions

This study investigated the potential of methanol extracts of *Cinnamomum zeylanicum, Fagara xanthoxyloides, Imperata cylindrica, Dichrostachys glomerata and Pentadiplanara brazzeana* against a panel of *S. aureus* strains. It was also focused on the evaluation of the antibiotic-modulating effects of the studied extracts against selected MDR bacteria. Overall, our results highlight the suitability of the tested extracts, mainly extracts of *Cinnamomum zeylanicum* and *Dichrostachys glomerata* alone as well as in combination with chloramphenicol and tetracycline as potential therapeutic agents for treatment of infections caused by MRSA. The above extracts can also potentiate the activity of chloramphenicol and tetracycline by inhibiting efflux pumps in *S. aureus*.

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
CZL: *Cinnamomum zeylanicum* leaves
DGF: *Dichrostachys glomerata* Fruits
DMSO: dimethylsulfoxide
ERY: erythromycin
FXS: *Fagara xanthoxyloides* seeds
HNC : Herbier National du Cameroun
ICR: *Imperata cylindrica*roots
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
PBR: *Pentadiplanara brazzeana* roots
RA: Reference antibiotics
SRF-Cam: Société des Réserves Forestières du Cameroun
TET: tetracycline

Authors' Contribution

GB, WBEN and PN carried out the study; GB, WBEN and ATM wrote the manuscript; VK and ATM designed the experiments; ATM supervised the work,; 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.

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