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Anti-staphylococcal and antibiotic-potentiating activities of seven Cameroonian edible plants against resistant phenotypes

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Abstract

Background: In this study, we evaluated the anti-staphylococcal and antibiotic-potentiating activities against resistant phenotypes of seven Cameroonian dietary plants: *Arachis hypogaea; Cola pachycarpa; Curcuma longa; Lycopersicon esculentum; Manihot esculenta; Passiflora edulis* and *Rubus fellatae*.

Methods: The liquid broth microdilution method was used for the determination of antibacterial activities, while standard methods were used for phytochemical screening to detect the main classes of secondary metabolites in the extracts.

Results: The phytochemical screening revealed that classes of secondary metabolites were selectively detected in extracts. The studied extracts displayed antibacterial activities with minimal inhibitory concentrations (MICs) values ranging from 32 and 2048 µg/mL on the majority of 20 tested staphylococcus strains. Extracts from rhizome of *Curcuma longa* and leaves of *Rubus fellatae* presented the broadest spectrum of activity by inhibiting the growth of 95 % and 85 % of tested bacteria strains respectively. The lowest MIC value 32 µg/mL (best activity) was displayed by *Curcuma longa*. The extracts of *Rubus fellatae, Passiflora edulis* and *Manihot esculenta* improved the activity of antibiotics (ceftriaxone, chloramphenicol, tetracycline and erythromycin) towards more than 80 % of tested pathogens.

Conclusion: The present study provides information on the possible use of the tested Cameroonian edible plants in the control of staphylococcal infections including resistant phenotypes. It also indicates that extracts of *Rubus fellatae*, *Passiflora edulis* and *Manihot esculenta* can be used as naturally occurring antibiotic-resistance modulators to tackle MDR (multi-drug resistance) bacteria.

Key words: Anti-staphylococcal activities; Cameroon; edible plants; phenotypes; resistance

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Background

Staphylococcus aureus is an ubiquitous and opportunistic pathogen that causes serious infections in humans worldwide. *S. aureus* is responsible for diseases of varying severity, sometimes benign, such as muco-cutaneous or severe infections such as endocarditis and sepsis. It is one of the most common causes of skin, soft tissue, community and nosocomial infections [1]. The emergence and spread of multidrug-resistant staphylococci in human populations has become a serious public health concern [2]. In 2007, the prevalence of methicillin-resistant *S. aureus* (MRSA) in Africa was estimated at 10 to 57% in general, a high prevalence in Black Africa and lower frequency (less than 10%) in countries of Maghreb [3]. The biochemical mechanisms of resistance developed by these bacteria include the enzymatic inactivation of the antibiotic, the modification of its target and the decrease of the intracellular concentration of the antibiotic by the reduction of the permeability and the active efflux [2]. Resistance and/or multidrug-résistance of bacteria contribute to therapeutic failures and limit the armory of available antibiotics. The discovery of new and effective antimicrobials and/or resistance modulators is necessary to combat the spread of resistance or to reverse the multi-drug resistance. The scarcity of the development of new antibiotics propels development of alternative medicine including phytotherapy. Since ancient times, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Medicinal plants are a rich source of compounds with pharmacological activities, such as antimicrobial activities, due to the diversity of secondary metabolites [4]. African flora in general, and Cameroonian flora in particular, are very rich and have shown a good potential to fight various human ailments including bacterial infections [5-7]. Therefore, exploring Cameroonian flora for antibacterial drug discovery appears as an attractive strategy. In Cameroon, several studies have shown antibacterial activities and modulatory effects of antibiotic activity extracts of many medicinal plants, food plants and derived products against bacteria (Gram-positive and Gram-negative) sensitive or resistant / multi-resistant to common antibiotics [8-10]. In addition, many classes of molecules with antibacterial activities have been isolated or highlighted in many plant extracts investigated. In our continuous quest for naturally occurring bioactive compounds to tackle bacterial multi-drug resistance, the present study was designed to evaluate the anti-staphylococcal activity of methanol extracts of seven Cameroonian dietary plants namely *Arachis hypogaea* L. *(*Fabaceae), *Cola pachycarpa* K Schum. (Sterculiaceae), *Curcuma longa* L. (Zingiberaceae), *Lycopersicon esculentum* L. (Solanaceae), *Manihot esculenta* Crantz. (Euphorbiaceae), *Passiflora edulis* Sims. (Passifloraceae) and *Rubus fellatae* A. Chev. (Rosaseae). The study was extended to the evaluation of the ability of some selected studied extracts to potentiate the activity of commonly used antibiotics towards resistant strains of *S. aureus*. The plants used in the present work are commonly used in traditional medicine in Cameroon in the treatment of many illnesses that include in addition to bacterial infectious diseases, cancers and metabolic syndromes (see more details in Table 1). They also have the particularity of being commonly used in Cameroonian cuisine. Many molecules belonging to different classes of secondary metabolites (terpenes, phenolic compounds and alkaloids) have been isolated or detected in these plants, whose metabolites are responsible for the biological activities mentioned above.

Methods

Plant materials and extraction

The seven edible plants used in this work were harvested at Melong (Littoral Region, Cameroon), Bapa and Dschang (West Region, Cameroon) during the period of December 2016 to February 2017. The collected plant samples were the leaves of *Cola pachycarpa, Lycopersicon esculentum* and *Rubus fellatae,* leaves and stem of *Arachis hypogaea*, the stem and leaves of *Passiflora edulis*, the tubers of *Manihot esculenta* and rhizomes of *Curcuma longa*. The plants were further identified at the National Herbarium (Yaoundé, Cameroon) where voucher specimens were deposited under a reference number (See Table 1). Each plant part was air dried and then powdered. The powdered air-dried sample from each plant was extracted with methanol (1:3 w/v) for 48 h at room temperature. The extract was then concentrated under reduced pressure to give a residue that constituted the crude extract. They were then kept under 4°C until further use.

Chemicals for antimicrobial assay

Six antibiotics ceftriaxone (CEF), kanamycine (KAN), erythromycine (ERY), tetracycline (TET), chloramphenicol (CHL) and ciprofloxacin (CIP) (Sigma-Aldrich, St Quentin Fallavier, France) were used as reference antibiotics (RA). *p*-Iodonitrotetrazolium chloride 0.2 % (INT; Sigma-Aldrich) was used as microbial growth indicator [11] while Dimethylsulfoxyde 2.5 % (DMSO; Sigma-Aldrich) was used to dissolve crude extract.

Table 1. Information on the studied plants

Species (family); voucher number	Traditional uses	Bioactive or potentially bioactive components	Known antimicrobial activities of plants
Arachis hypogaea L. (Fabaceae); (42592/HNC)	Aphrodisiac, cholecystosis, decoagulant, anti-inflammatory, nephritis, cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders [29, 30]	Quercetin, rutin, resveratrol [30]	Antimicrobial, antiviral, anti-inflammatory, antiproliferative antioxidant activities [30]
Cola pachycarpa K Schum. (Sterculiaceae); (48643/HNC)	Coughs internal heat, fever [31], bacterial infections [32]	glycosides, saponins, steroids, caffeine, theobromine [31, 33]	Not reported
Curcuma longa L. (Zingiberaceae) (42173/HNC)	Anticancer, anti-inflammatory, microbial infections, diabetes, arthritic, muscular disorders, biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders and sinusitis [34]	Germacrone, turmerone, turmerones, *β*-bisabolene, *α*-curcumene, zingiberene, *β*-sesquiphellandene, bisacurone, curcumenone, curcumenol, turmeronols, *β*-sitosterol, 2-hydroxymethyl anthraquinone, sabinene , cineol, borneol [34]	Antioxidant, antineoplastic, antiviral, anti-inflammatory, antibacterial, antifungal, anti-diabetic, anticoagulant, anti-fertility, cardiovascular protective, hepatoprotective and immuno-stimulant activities (crude extract and cucurmin)[34]
Lycopersicon esculentum L. (Solanaceae); (43088/HNC)	Anti-inflammatory, antimicrobial, heart diseases and age-related diseases[35]	Lycopene, β-carotene, lutein, zeaxanthin, flavonoids, hydroxycinnamic acid, glycosides [36]	Anti-inflammatory and antibacterial activities (crude extract) [37]
Manihot esculenta Crantz. (Euphorbiaceae) (18619/SRFCam)	Rheumatism, fever, headache, diarrhea, loss of appetite, ringworm, conjunctivitis, abscess and sores [38]	Coumarins [39]	Antimicrobial activity of seed oil against *Sa, Pac* , Ec, Po and *Ca* [38]
Passiflora edulis Sims. (Passifloraceae) (651054/HNC)	Treatment of cancer, fungal infections, inflammation, insomnia and anxiety, antihypertensive [40], gastric trouble [41], antioxidant [42]	Ionone-I, ionone-II, megastigma-5,8-dien-4-1, megastigma-5,8(*Z*)-diene-4-1, 4,4*a*-Epoxy-4, 4*a*-dihydroedulan, 3-hydroxyedulan, edulan-I, edulan-II, passifloric acid methyl ester [42]	Antimicrobial activities of methanol extract against *Ec, Kp, Ea, Pa, Ps, Sa, Ef, Bs, , Pv* and *St* [42]
Rubus fellatae A. Chev. (Rosaseae) (44163/HNC)	Hypogycaemic, angina, diarrhea, anti-inflammatory [43, 44]	Flavonoids anthocyanins (Wu et al., 2004) Hydroxycinnamic and hydroxybenzoic acids [45]	Antibacterial activity (crude extract) [46]

/ : not reported *;* Po : *Pityrosporum ovale;Sa : Staphylococcus aureus ; Ca: Candida albicans ; Ec : Escherichia coli ; Pa : Pseudomonas aeruginosa ; ,* Pac : *Propionibacterium acnes ; Pv : Proteus vulgaris ; St : Salmonella typhi ;; Ef : Enterococcus faecium ; Bs : Bacillus subtilis ;Kp : Klebsiella pneumoniae; Ea : Enterobacter aerogenes;Ps : Providencia stuarti ; Bacillus subtilus*

Table 2. Extraction yields and phytochemical composition of the plant extract

Plant extract and part used		Yields (%)	Alk	Pol	Flav	Anthr	Anth	Tan	Tri	Ster	Sap
Arachis hypogaea	Leaves	9.3	+	+	-	-	-	-	+	+	-
	Stem	7.87	+	+	-	-	-	+	+	+	+
Cola pachycarpa	Leaves	4.15	+	-	-	-	-	-	+	+	-
Curcuma longa	Rhizome	10.31	+	+	+	+	+	-	-	-	-
Lycopersicon esculentum	Leaves	9.17	+	-	-	-	-	-	+	+	-
Manihot esculenta	Tubers	0.73	+	+	-	-	-	+	+	+	+
Passiflora edulis	Stem	7.38	-	+	+	+	-	-	+	+	-
	Leaves	13.21	+	+	+	+	-	-	+	+	-
Rubus fellatae	Leaves	16.32	+	+	+	+	-	+	+	+	-

(-): Absent; (+): Present; yield calculated as the ratio of the mass of the obtained methanol extract/mass of the plant powder; Alk : Alcaloids ; Anth : Anthocyanins ; Anthr : anthraquinones ; Flav : flavonoids ; Pol : polyphenols ; Sap : saponins ; Ster : steroids ; Tan : tannins ; Tri : Triterpenes

Bacteria strains and culture media

The studied bacteria included twenty resistant strains of *S. aureus* obtained from the American Type Culture Collection (ATCC) or clinically (Laboratory collection) (See additional file Table: S1). Bacteria strains were maintained on agar slant at 4°C and sub-cultured on a fresh appropriate agar plates 24 h prior to any antibacterial test. Mueller Hinton Agar (MHA) was used for the activation of bacteria and the Mueller Hinton Broth (MHB) was used for the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and Modulating factor determinations [12].

INT colorimetric assay

The MIC and MBC determinations on the tested bacteria strains were conducted using rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay as described by Eloff [11] with some modifications [13].

To evaluate the antibiotic-resistance modulating activity of extracts, a preliminary assay was performed to determine the MICs of antibiotics in the absence and presence of these extracts using broth micro-dilution method as previously described [8, 9, 11, 14]. *S. aureus SA18* was used for preliminary assays and samples were tested at various sub-inhibitory concentrations (MIC/2, MIC/4, MIC/8 and MIC/16) with 6 antibiotics (CIP, CHL, TET, KAN, ERY and CEF) and most active studied dietary plants (roots of *Manihot esculenta*, leaves of *Passiflora edulis* and *Rubus fellatae*, rhizomes of *Curcuma longa*). Results allowed selecting MIC/2 and MIC/4 as sub-inhibitory concentrations for further experiments on selected *S. aureus* strains. Briefly, after serial dilution of antibiotic, extract was added to each well at its sub-inhibitory concentration and the bacterial inoculation was done; the MIC was further determined. Rows receiving antibiotic dilutions without extracts were used for the determination of the MICs of the antibiotics. The modulation factor was defined as the ratio of the MIC of antibiotic alone versus that of antibiotic in the presence of extract. Modulation factor ≥ 2 was set as the cut-off for biologically significance of antibiotic resistance modulating effects [15].

Phytochemical screening.

The presence of major secondary metabolite classes, namely, alkaloids, polyphenols, flavonoids, anthraquinones, anthocyanins, tannins, triterpenes, sterol and saponins (Table2) was determined using standard phytochemical methods as described by [16].

Results

The results of qualitative analysis showed that classes of secondary metabolites are selectively distributed in studied plants extracts. Alkaloids, polyphenols, triterpenes and sterols are most detected classes on crude extracts samples (Table 2).

MIC results as compiled in Table 3 indicate that studied extracts possess an antibacterial activity with values ranging from 32-2048 µg/mL. Extracts from *Curcuma longa* and leaves of *Rubus fellatae* presented the largest spectrum of action respectively against 95% and 85% of tested *S. aureus* strains. Other extracts presented a spectrum of inhibition between 20-50% except the extract of *Cola pachycarpa* which did not present any antibacterial activity. The lowest MIC value of 32µg/mL was recorded with *Curcuma longa* against *S. aureus ST68* and *ST114*. Other extracts exhibited weak activities against a limited number of studied strains. According to MBC/MIC ratio values, the effects of studied extracts are in majority bacteriostatic (MBC/MIC > 4). The most active extract *Curcuma longa* show low bactericidal activities displaying MBC only against 1/20 (5%) while *Rubus fellatae* show the best bactericidal spectrum with MBC values against 8/20 (40%) of tested strains.

Six antibiotics (CHL, CIP, TET, CEF, KAN, ERY) commonly used in bacterial chemotherapy were combined with four selected extracts (tubers of *Manihot esculenta*, leaves of *Passiflora edulis* and *Rubus fellatae*, rhizomes of *Curcuma longa*) and tested in a preliminary assay against *S. aureus SA18*. The results (Table 4) show that at sub-inhibitory concentrations of MIC/2 and MIC/4, activities of antibiotics are improved compared to MIC/8 and MIC/16 concentrations. Thus, these give the explanation of why we selected the sub-inhibitory concentrations of MIC/2 and MIC/4 for further experiments (Tables 5-8).

Tables 5-8 show that selected extracts have potentiated the effect of antibiotics to varying proportions depending on the antibiotic and the bacterial strain at the sub-inhibitory concentrations of MIC/2 and MIC/4. The highest percentage of synergy reached (2-fold or more increases of activity) was 83.33% of the tested strains. The potentiation of the effect of antibiotics on more than 70% of the tested strains (all at MIC/2) was obtained with the extract from tubers of *Manihot esculenta* in combination with ceftriaxone (83.33%) (Table 5); leaves extract of *Passiflora edulis* (Table 6) in combination with tetracycline and erythromycin (83.33%); the leaves extract of *Rubus fellatae* (Table 7, 8) combined with chloramphenicol and tetracycline (83.33%). On the other hand, the extract from rhizomes of *Curcuma longa*, the most active extract following MIC determinations, showed a low percentage of synergy (Table 7), the maximum of which was obtained on 66.67% when combined with ceftriaxone.

Discussion

The plant extracts used in this work displayed antibacterial activities that varied from one extract to another. Many works have shown that the antimicrobial properties of plants are mainly attributed to the secondary metabolites they contain [4, 17]. This variability of antibacterial action obtained can be due to the difference in composition of each plant extract as demonstrated by phytochemical screening (Table2). According to the classification scale established by Tamokou et al. [18], an extract of a food plant or extract of a part of a food plant is considered as very active when its MIC <100 µg/mL, significantly active if 100 ≤ MIC ≤ 512 µg/ mL, moderately active if 512 <MIC ≤ 2058 µg/mL, weakly active if MIC> 2048 µg/mL and considered not active if MIC> 10 mg/ml. On the basis of this scale, only the extract from the rhizomes of *Curcuma longa* was very active vis-à-vis 85% of tested strains/isolates of *S. aureus* (SA68, SA114, SA18, SA23, SA36, SA88, SA126, SA127, MRSA1, MRSA3, MRSA4, MRSA6, MRSA8, MRSA9, MRSA11 and ATCC25923).This same extract exhibited significant activity against MRSA12, SA07 and SA01; otherwise, the extracts from leaves of *Rubus fellatae*, *Pasiflora edulis* as well as extracts of *Lycopersicon esculentum* presented significant activities with regard to a few staphylococcal strains. However, it is important to note that the activity of a plant extract does not depend solely on the presence alone of secondary metabolites, but also on the types present, their quantity and the possible interactions with other components of the plant [19]. Ankur et al. [20] showed that the rhizomes of *Curcuma longa* contain alkaloids, flavonoids, glycosides and hydrates of carbons; which corroborate the results obtained in this work. These same works demonstrated that all fractions of *Curcuma longa* rhizomes such as the ether, chloroform, methanol and water fractions are highly active on strains of *Staphylococcus aureus* ATCC 6571 and clinical isolates. These results are very similar to those we obtained in this work. According to investigation done by Cordell et al. [21], antibacterial activities presented by this plant extract would be attributed to the presence in the latter of bioactive components such as alkaloids and flavonoids. Investigations by Asirvathamdoss et al. [22] have demonstrated the important antimicrobial activity of the leaves extract of *Passiflora edulis*

against a large number of strains including *Staphylococcus aureus*, which has also been demonstrated in this study. The antibacterial activity of this plant is attributed to the presence of alkaloids, polyphenols and triterpenes, many of which have already had to demonstrate their antibacterial activities [4]. The investigations conducted by Afif et al. [23] on a plant of the genus *Rubus* (*Rubus fruticosus*) showed a remarkable inhibitory activity against resistant bacteria of the respiratory tract such as *S. aureus*; activity that is attributed to polyphenols, especially flavonoids contained in this plant. The present study enhances the antibacterial effect of plants of the genus *Rubus*, which in this case showed a spectrum of inhibition on 85% of resistant *S. aureus* tested. Other plant extracts showed inhibition spectra of less than or equal to 50% of the bacterial strains tested with predominantly low MICs. This can be explained by the low proportion of secondary metabolites in these extracts or the multidrug-resistant phenotype expressed by tested bacterial strains. Thus, the results of this study could be considered very important given the clinical significance of the tested pathogens.

Table 3. MIC and MBC of the plant extracts and ciprofloxacin against *Staphylococcus aureus* strains

Bacterial strains	Samples, MIC and MBC in µg/mL (in Bracket)									
	Plant Extracts									Antibiotic
	AHS	AHL	CPL	CLR	LEL	MET	PEL	PES	RFL	CIP
ATCC25923	2048 (-)	2048 (-)	-	64 (1024)	-	2048(-)	1024	2048 (-)	1024 (-)	<0.5 (16)
SA01	-	-	-	512 (-)	-	-	-	-	-	<0.5 (4)
SA07	-	-	-	256 (1024)	-	-	-	-	-	<0.5 (1)
SA18	-	2048 (-)	-	64 (512)	-	-	2048 (-)	-	1024 (1024)	<0.5 (8)
SA23	2048 (-)	-	-	64 (512)	-	-	1024 (2048)	2048 (-)	1024 (2048)	<0.5 (<0.5)
SA36	2048 (-)	-	-	64 (512)	-	1024 (-)	-	2048 (-)	256 (1024)	1 (8)
SA56	-	2048 (-)	-	-	512 (2048)	-	-	-	-	<0.5 (4)
SA68	-	-	-	32 (512)	-	1024 (-)	2048 (-)	-	1024	<0.5 (<0.5)
SA88	2048 (-)	-	-	64 (1024)	-	-	-	-	1024 (2048)	<0.5 (2)
SA114	-	-	-	32 (1024)	-	2048 (-)	2048 (-)	-	1024	<0.5 (<0.5)
SA126	-	-	-	64 (512)	-	1024 (-)	2048 (-)	-	1024	<0.5 (<0.5)
SA127	-	-	-	64 (1024)	-	-	-	-	512 (512)	<0.5 (<0.5)
MSSA1	2048 (-)	-	-	64 (1024)	2048 (-)	2048 (-)	-	-	1024 (-)	2 (16)
MRSA3	-	-	-	64 (512)	-	2048 (-)	2048 (-)	-	1024 (2048)	2 (16)
MRSA4	2048 (-)	2048 (-)	-	64 (2048)	2048 (-)	1024 (-)	2048 (-)	2048 (-)	512 (2048)	1 (16)
MRSA6	2048 (-)	2048 (-)	-	64 (2048)	2048 (-)	-	-	-	512 (2048)	2 (8)
MRSA8	2048 (-)	2048 (-)	-	64 (1924)	-	2048 (-)	512 (-)	2048 (-)	1024 (-)	2 (8)
MRSA9	-	-	-	64 (1024)	-	2048 (-)	2048 (-)	-	1024 (-)	2 (16)
MRSA11	2048 (-)	-	-	64 (1024)	-	-	-	-	2048 (-)	2 (16)
MRSA12	-	-	-	128 (2048)	-	-	-	-	2048 (-)	2 (4)

AHS: Arachis hypogaea stem; AHL: Arachis hypogaea leaves; CPL: Cola pachycarpa leaves; CLR: Curcuma longa rhizome; LEL: Lycopersicon esculentum leaves; MET: Manihot esculenta tubers; PEL:Passiflora edulis leaves; PES: Passiflora edulis Stem; EFL: Rubus fellatae leaves; CIP: ciprofloxacin; -: > 2048 (MIC); MIC : Minimal Inhibitory Concentration ; MBC : Minimal bactericidal Concentration.

The extracts mostly presented a bacteriostatic effect based on low MBC/ MIC> 4 [24]. Only the extract from the leaves of *Rubus fellatae* had a bactericidal effect (CMB/CMI≤4) against 40% strains; extract from rhizomes of *Curcuma longa which* has shown the highest inhibition spectrum wasbactericidal vis-à-vis a single strain (ST07) as leaves extracts of *Passiflora edulis* (ST23) and *Lycopersicon esculentum* (ST36). The low bactericidal effects could be explained by the multidrug-resistant phenotypes of tested pathogens. Tests which consist of maintaining the effectiveness of conventional antibiotics or modulation of the resistance can be found in combinations with other products which are a treatment option face to infections by resistant *Staphylococcus aureus*. Tegos et al. [25] concluded at the end of their work that plants can constitute a possible source of efflux pumps inhibitors (EPI) that could restore the activity of antibiotics. Indeed, previous studies have shown synergistic effects of the combination of plant extracts and/or derived products with antibiotics against bacteria over-expressing

active efflux [12, 26, 27]. Similarly, the work of Braga et al. [28] demonstrated synergistic effects of the combination of plant extracts with the usual antibiotics on clinical *S. aureus* isolates, also this work showed that when a percentage of synergy is greater or equal 70%, a possible existence of EPI in the studied extracts could be noticed. Synergistic effects were obtained in this work, extracts of *Manihot escculenta*, *Passiflora edulis* and *Rubus fellatae* improved the activities of ceftriaxone, chloramphenicol, tetracycline and erythromycin by more than 80% of the tested strains (at MIC/2). This can be explained by the fact that the extracts act as preferential substrates for efflux pumps, or by inhibiting the synthesis of transmembrane proteins involved in the efflux phenomenon. The other plant extracts showed synergistic effects with at least one antibiotic against at least one strain of multidrug-resistant staphylococcus. Cases of indifference of certain extracts with regard to some antibiotics have also been reported; thiscan be explained by the fact that the extracts do not act on the resistance mechanisms developed by the bacteria. The antagonisms observed in combination with the antibiotics could be explained by a possible neutralization of the active function of the antibiotic by certain components of the plant.

Table 4. Preliminary evaluation of antibiotic-resistance modulatory activity of selected extracts at sub-inhibitory concentrations against *S. aureus SA18*

Plant extracts[a]	Extract concentrations	MIC of antibiotic (µg/mL) alone and in combination with extracts and fold increase of activity (in bracket)					
		CIP	CHL	TET	KAN	ERY	CEF
	0	32	4	64	128	128	16
Manihot esculenta tubers	CMI/2	2 **(16)**	-	8 **(8)**	≤2 **(≥64)**	- (≤0.5)	≤2 **(≥8)**
	CMI/4	8 **(4)**	-	32**(2)**	8 **(16)**	- (≤0.5)	4 **(4)**
	CMI/8	8 **(4)**	-	32 (1)	16 **(8)**	- (≤0.5)	32 (0.5)
	CMI/16	32 (1)	-	32 (1)	32 **(4)**	- (≤0.5)	32 (0.5)
*Passiflora bedulis l*eaves	CMI/2	≤ 0,5 **(≥64)**	≤1 **(≥4)**	≤0.5 **(≥128)**	≤2 **(≥64)**	≤2 **(≥64)**	≤2 **(≥8)**
	CMI/4	2 **(16)**	≤1 **(≥4)**	≤0.5 **(≥128)**	≤2 **(≥64)**	- (≤0.5)	≤2 **(≥8)**
	CMI/8	8 **(4)**	≤1 **(≥4)**	4 **(16)**	32 **(4)**	- (≤0.5)	8 **(2)**
	CMI/16	16 **(2)**	2 **(2)**	32 **(2)**	64 **(2)**	- (≤0.5)	16 (1)
Curcuma longa Rhizome	CMI/2	16 **(2)**	≤1 **(≥4)**	32 **(2)**	64 **(2)**	256 (0.5)	16 (1)
	CMI/4	16 **(2)**	≤1 **(≥4)**	32 **(2)**	128 (1)	256 (0.5)	32 (0.5)
	CMI/8	16 **(2)**	≤1 **(≥4)**	32 **(2)**	128 (1)	- (≤0.5)	32 (0.5)
	CMI/16	32 (1)	2 **(2)**	32 **(2)**	128 (1)	- (≤0.5)	32 (0.5)
Rubus fellatae leaves	CMI/2	2 **(16)**	≤1 **(≥4)**	≤0.5 **(≥128)**	≤2 **(≥64)**	≤2 **(≥64)**	4 **(4)**
	CMI/4	32 (1)	≤1 **(≥4)**	32 **(2)**	64 **(2)**	- (≤0.5)	4 **(4)**
	CMI/8	32 (1)	2 **(2)**	32 **(2)**	64 **(2)**	- (≤0.5)	16 (1)
	CMI/16	32 (1)	2 **(2)**	64 (1)	128 (1)	- (≤0.5)	64 (0.25)

CIP: ciprofloxacin; TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF: Ceftriaxone; −: MIC not detected at up to 256 µg/mL; (in bracket): Modulating factor; MIC Minimal Inhibitory Concentration; Values in bold represent modulating factor ≥ 2.

Table 5. Resistance-modulating effects of the methanol extract from tubers *Manihot esculenta* at its MIC/2 and MIC/4 on selected strains of *Staphyloccus aureus*

Antibiotics	**Extract concentration**	**Bacterial strains, MIC (µg/mL) of antibiotics in the absence and presence (in bracket) of the extract (in bracket)**						**Antibiotic-modulating effect (%)**
		MRSA3	**SA 07**	**SA 23**	**SA 36**	**SA 68**	**ATCC 25923**	
CIP	0	0.125	1	≤ 0.062	4	0.125	0.125	
	MIC/2	0.5 (0.25)	2 (0.5)	≤0.062(na)	1 **(4)**	0.25 (0.5)	0.25 (0.5)	16.67
	MIC/4	0.5 (0.25)	≤0.06**(≥16)**	≤0.062(na)	4(1)	0.25 (0.5)	0.25 (0.5)	16.67
CHL	0	2	256	4	256	64	2	
	MIC/2	2 (1)	32 **(8)**	≤2**(≥2)**	128 **(2)**	64 (1)	32 (0.062)	50
	MIC/4	2 (1)	32 **(8)**	≤2 **(≥2)**	256 (1)	256 (0.25)	-(na)	33.33
TET	0	0.5	-	2	4	16	0.5	
	MIC/2	≤0.25 **(≥2)**	64 **(≥2)**	≤0.5 **(≥4)**	8 (0.5)	16 (1)	0.5 (1)	50
	MIC/4	≤0.25 **(≥2)**	64 **(≥2)**	1 **(2)**	32 (0.125)	-(na)	0.5 (1)	50
ERY	0	64	256	4	32	32	4	
	MIC/2	≤ 2 **(≥32)**	256 (1)	≤ 2 **(≥2)**	64 (0.5)	64 (0.5)	≤ 2 **(≥2)**	50
	MIC/4	≤ 2 **(≥32)**	256 (1)	≤ 2 **(≥2)**	64 (0.5)	128 (0.25)	≤ 2 **(≥2)**	50
KAN	0	4	32	4	4	16	8	
	MIC/2	≤ 2 **(≥2)**	≤ 2 **(≥16)**	≤ 2 **(≥2)**	32 (0.125)	64 (0.25)	≤ 2 **(≥4)**	66.67
	MIC/4	4 (1)	4 **(64)**	≤ 2 **(≥2)**	16 (0.25)	32 (0.5)	4 **(2)**	50
CEF	0	64	16	16	32	128	32	
	MIC/2	≤2 **(≥32)**	≤ 2 **(≥8)**	≤ 2 **(≥8)**	64 (0.5)	16 **(8)**	16 **(2)**	**83.33**
	MIC/4	16 **(4)**	8 **(2)**	4 **(4)**	128 (0.25)	16 **(8)**	32 (1)	66.67

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : ceftriazone; −: MIC not detected at up to 256 µg/mL; (): 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 Leaves methanol extract from *Passiflora edulis* at its MIC/2 and MIC/4 on selected strains of *Staphyloccus aureus*

Antibiotics	**Extract concentration**	**Bacterial strains, MIC (µg/mL) of antibiotics in the absence and presence (in bracket) of the extract (in bracket)**						**Antibiotic-modulating effect (%)**
		MRSA3	**SA 07**	**SA 23**	**SA 36**	**SA 68**	**ATCC 25923**	
CIP	0	0.125	1	≤ 0.062	4	0.125	0.125	
	MIC/2	≤0.062 **(≥2)**	1 (1)	≤ 0.062 (1)	8 (0.5)	≤0.062 **(≥2)**	≤0.062 **(≥2)**	50
	MIC/4	≤0.062 **(≥2)**	1 (1)	≤ 0.062 (1)	4 (1)	≤0.062 **(≥2)**	≤0.062 **(≥2)**	50
CHL	0	2	256	4	256	64	2	
	MIC/2	≤2 (≥1)	32 **(8)**	≤2 **(≥2)**	128 **(2)**	128 (0.5)	≤2 (≥1)	50
	MIC/4	≤2 (≥1)	128 **(2)**	4 (1)	256 (1)	128 (0.5)	≤2 (≥1)	16.67
TET	0	0.5	-	2	4	16	0.5	
	MIC/2	≤0.25 **(2)**	32 (**≥4**)	1 **(2)**	4 (1)	8 **(2)**	≤0.25 **(≥2)**	**83.33**
	MIC/4	2 (0.25)	64 (**≥2**)	2 (1)	32 (0.125)	64 (0.25)	≤0.25 **(≥2)**	33.33
ERY	0	64	256	4	32	32	4	
	MIC/2	≤ 2 (**≥32**)	64 **(4)**	≤ 2 (**≥2**)	32 (1)	16 **(2)**	≤ 2 (**≥2**)	**83.33**
	MIC/4	≤ 2 (**≥32**)	64 **(4)**	≤ 2 (**≥2**)	128 (0.25)	128 (0.25)	≤ 2 (**≥2**)	66.67
KAN	0	4	32	4	4	16	8	
	MIC/2	≤ 2 (**≥2**)	≤ 2 (**≥16**)	≤ 2 (**≥2**)	32 (0.125)	32 (0.5)	≤ 2 (**≥4**)	66.67
	MIC/4	≤ 2 (**≥2**)	16 **(2)**	≤ 2 (**≥2**)	32 (0.125)	32 (0.5)	≤ 2 (**≥4**)	66.67
CEF	0	64	16	16	32	128	32	
	MIC/2	16 (4)	16 (1)	8 **(2)**	64 (0.5)	32 **(4)**	64 (0.5)	33.33
	MIC/4	64 (1)	8 **(2)**	16 (1)	16 **(2)**	64 **(2)**	64 (0.5)	50

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : ceftriazone; −: MIC not detected at up to 256 µg/mL; (): 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 7. Resistance-modulating effects of the Rhizomes methanol extract from *Curcuma longa* at its MIC/2 and MIC/4 on selected strains of *Staphyloccus aureus*

Antibiotics	Extract concentration	Bacterial strains, MIC (µg/mL) of antibiotics in the absence and presence (in bracket) of the extract (in bracket)						Antibiotic-modulating effect (%)
		MRSA3	**SA 07**	**SA 23**	**SA 36**	**SA 68**	**ATCC 25923**	
CIP	0	0.125	1	≤ 0.062	4	0.125	0.125	
	MIC/2	≤0.062 **(2)**	2 (0.5)	≤0.062 (na)	2 **(2)**	0.125 (1)	≤0.062 **(2)**	50
	MIC/4	≤0.062 **(2)**	2 (0.5)	≤0.062 (na)	4(1)	0.125 (1)	≤0.062 **(2)**	33.33
CHL	0	2	256	4	256	64	2	
	MIC/2	≤ 1 **(≥2)**	32 **(8)**	128 (0.031)	256 (1)	256 (0.25)	2 (1)	33.33
	MIC/4	≤ 1 **(≥2)**	128 **(2)**	128 (0.031)	-(≤0.5)	256 (0.25)	2 (1)	33.33
TET	0	0.5	-	2	4	16	0.5	
	MIC/2	≤0.25 **(≥2)**	16**(≥4)**	-(na)	4 (1)	16 (1)	≤0.25 **(≥2)**	50
	MIC/4	≤0.25 **(≥2)**	16**(≥4)**	-(na)	8 (0.5)	4**(4)**	≤0.5 (1)	50
ERY	0	64	256	4	32	32	4	
	MIC/2	≤ 2 **(≥32)**	64 **(4)**	32 (0.125)	64 (0.5)	128 (0.25)	≤ 2 **(≥2)**	50
	MIC/4	≤ 2 **(≥32)**	64 **(4)**	128 (0.031)	128 (0.25)	256 (0.125)	≤ 2 **(≥2)**	50
KAN	0	4	32	4	4	16	8	
	MIC/2	≤ 2 **(≥2)**	≤ 2 **(≥16)**	4 (1)	64 (0.062)	64 (0.25)	≤ 2 **(≥4)**	50
	MIC/4	≤ 2 **(≥2)**	≤ 2 **(≥16)**	16 (0.25)	32 (0.125)	32 (0.5)	4 **(2)**	50
CEF	0	64	16	16	32	128	32	
	MIC/2	32 **(2)**	8 **(2)**	8 **(2)**	32 (1)	128 (1)	16 **(2)**	66.67
	MIC/4	16 **(4)**	8 **(2)**	8 **(2)**	64 (0.5)	128 (1)	64 (0.5)	50

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF :ceftriazone; −: MIC not detected at up to 256 µg/mL; (): 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.

Table 8. Resistance-modulating effects of the methanol extract from leaves of *Rubus fellatae* at its MIC/2 and MIC/4 on selected strains of *Staphyloccus aureus*

Antibiotics	Extract concentration	Bacterial strains, MIC (µg/mL) of antibiotics in the absence and presence (in bracket) of the extract (in bracket)						Antibiotic-modulating effect (%)
		MRSA3	SA 07	SA 23	SA 36	SA 68	ATCC 25923	
CIP	0	0.125	1	≤ 0.062	4	0.125	0.125	
	MIC/2	≤0.062 **(2)**	≤0.062 **(≥16)**	≤0.062 (1)	4 (1)	0.125 (1)	≤0.062 **(2)**	50
	MIC/4	≤0.062 **(2)**	≤0.062 **(≥16)**	≤0.062 (1)	8 (0.5)	0.125 (1)	≤0.062 **(2)**	50
CHL	0	2	256	4	256	64	2	
	MIC/2	≤1 **(2)**	≤ 2 **(≥128)**	≤ 2 **(≥2)**	256 (1)	8 **(8)**	≤1 **(2)**	**83.33**
	MIC/4	≤1 **(2)**	32 **(8)**	4 (1)	256 (1)	128 (0.5)	≤1 **(2)**	50
TET	0	0.5	-	2	4	16	0.5	
	MIC/2	2 (0.25)	≤0,5 **(≥128)**	≤0,5 **(4)**	2 **(2)**	8 **(2)**	0.125 **(4)**	**83.33**
	MIC/4	1 (0.5)	-(na)	2 (1)	2 **(2)**	8 **(2)**	0.5 (1)	33.33
ERY	0	64	256	4	32	32	4	
	MIC/2	≤ 2 **(≥32)**	256 (1)	≤ 2 **(≥2)**	32 (1)	16 **(4)**	≤ 2 **(≥2)**	66.67
	MIC/4	≤ 2 **(≥32)**	256 (1)	4 (1)	32 (1)	64 (0.5)	≤ 2 **(≥2)**	33.33
KAN	0	4	32	4	4	16	8	
	MIC/2	≤ 2 (**≥2**)	64 (0.5)	≤ 2 (**≥2**)	32 (0.125)	32 (0.5)	≤ 2 (**≥4**)	50
	MIC/4	≤ 2 (**≥2**)	64 (0.5)	≤ 2 (**≥2**)	8 (0.5)	16 (1)	4 **(2)**	50
CEF	0	64	16	16	32	128	32	
	MIC/2	32 **(2)**	4 (4)	≤ 2 **(≥8)**	32 (1)	64 **(2)**	32 (1)	50
	MIC/4	64 (1)	32 (0.5)	16 (1)	16 **(2)**	64 **(2)**	32 (1)	33.33

TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, CEF : ceftriazone; −: MIC not detected at up to 256 µg/mL; (): 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

The present study provides information on the possible use of the tested Cameroonian edible plants in the control of staphylococcal infections including resistant phenotypes. It also indicates that studied extracts, especially extracts of *Rubus fellatae*, *Passiflora edulis* and *Manihot esculenta* can be used as naturally occurring antibiotic-resistance modulators to tackle MDR bacteria.

Additional file

Supplementary file.docx. Table S1. Further details on the antibiotic-resistance profiles of tested Gram-negative bacteria. (DOCX, 127 Kb)

Authors' Contribution

HTM, GSN, BENW, PN and AGF carried out the study; ATM and VK designed the experiments. JAS, ATM and VK wrote the manuscript; NRY read and corrected the language; ATM and VK supervised the work and provided the facilities for antibacterial assays; 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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