Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Antibacterial and antibiotic-potentiating activities of nine Cameroonian medicinal plants against multidrug-resistant bacteria expressing active efflux pumps

Olive Monique F. Demgne[1], Cedric F. Tchinda[2], Armelle T. Mbaveng[3], Veronique P. Beng[1], Victor Kuete[3]*

Abstract

Background: In the last ten years, the resistance of Gram-negative and Gram-positive bacteria continuously increased. The present study was designed to evaluate the antibacterial and antibiotic-potentiating activities of nine Cameroonian medicinal plants: *Cucumeropsis mannii, Lagenaria siceraria, Citrullus lanatus, Cucurbita moschata, Salix ledermannii, Gouania longispicata, Psychotria mapourioides, Conyza aethiopica* and *Conyza sumatrensis* against resistant phenotypes.
Methods: Liquid broth microdilution method was used for the determination of antibacterial activities, while standard methods were used for phytochemical screening to detect the major classes of secondary metabolites in the extracts.
Results: The result of phytochemical screening revealed that the secondary metabolite classes were selectively detected in the extracts. The studied extracts showed antibacterial activities with minimum inhibitory concentrations (MIC) ranging from 64 to 1024 µg/mL on the tested strains. The *Gouanea longispicata* extract showed the greatest spectrum of action notably against 86.4% of the bacterial strains tested. The synergistic effects of the extracts and antibiotics observed varied from 20 to 60%. *Salix ledermannii* leaf extract in combination with cloxacilin and ciprofloxacin, showed the highest synergistic effects (60%) towards the tested pathogens.
Conclusion: The present study provides information on the possible use of the tested Cameroonian medicinal plants in the control of bacterial infections, especially those caused by resistant phenotypes. It also indicates that extracts of *Gouania longispicata* and *Salix ledermannii* leaves can be used as natural modulators of antibiotic resistance to control MDR bacteria.

Keywords. Antibacterial; antibiotic modifying activity; Cameroon; medicinal plants; multi-drug resistance.

*Correspondence: Tel.: 237677355927; E-mail address: kuetevictor@yahoo.fr; ORCID: http://orcid.org/0000-0002-1070-1236 (Victor Kuete)

[1]Department of Biochemistry, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon; [2]Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, Ministry of Scientific Research and Innovation, Yaoundé, Cameroon; [3]Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon

Other authors:
E-mail: princessdemleur@yahoo.fr (Olive Monique F. Demgne); E-mail: tchindafossicedric@yahoo.fr (Cedric F. Tchinda); E-mail: armbatsa@yahoo.fr; ORCID: https://orcid.org/0000-0003-4178-4967 (Armelle T. Mbaveng) ; E-mail: v.penlap@yahoo.fr; (Veronique P. Beng)

Citation on this article: Demgne OMF, Tchinda CF, Mbaveng AT, Beng VP, Kuete V. Antibacterial and antibiotic-potentiating activities of nine Cameroonian medicinal plants against multidrug-resistant bacteria expressing active efflux pumps. Investigational Medicinal Chemistry and Pharmacology (2022) 5(1):58; Doi: https://dx.doi.org/10.31183/imcp.2022.00058

Background

Today, bacterial infections remain a serious health problem worldwide and particularly in developing countries. They are a leading cause of hospital and community-acquired infections, ranging from common infections such as skin and soft tissue infections to life-threatening infections. An estimated 17 million deaths from infectious diseases occur each year out of a total of 62 million observed cases [1]. Of this incidence, 70% of mortality is associated with pathogenic bacteria [2]. These figures affect all age groups, and the World Health Organization (WHO) estimated the global number of neonatal deaths at 2.8 million in 2015, 47.6% of which were due to bacterial infections [3]. This situation is more alarming as we are witnessing the emergence of infectious outbreaks that are difficult to manage due to the occurrence of multi-resistant phenotypes to the usual antibiotics. Among these infections, those caused by multi-resistant bacteria are involved in several therapeutic failures and are, for the most part, involved in nosocomial infections [4, 5]. As an illustration, nosocomial infections caused by Gram-negative bacteria such as Enterobacteriaceae (*Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii*) and *Pseudomonas aeruginosa*, but also *Staphylococcus aureus* (Gram-positive bacteria) have become difficult to treat due to their ability to resist several antibiotics [6, 7]. Several biochemical mechanisms of resistance have been developed by these bacteria including enzymatic inactivation of the antibiotic, modification of its target, and decrease of the intracellular concentration of the antibiotic by reduction of permeability and active efflux [8]. Bacterial resistance and/or multidrug resistance contribute to therapeutic failures and result in an economic burden, which, together with the adverse side effects of synthetic antibiotics, complicates the control of bacterial infections [9, 10]. The resistance of these bacteria to antimicrobial agents may be associated with the presence of membrane transport systems called efflux pumps that are thought to be responsible for the overexpression of the multidrug resistance phenomenon [11]. The increase of multidrug resistance (MDR) which is more and more frequent in the bacterial world, and the lack of new antibiotics encourage the search for new effective antibacterial agents and/or resistance modulators via medicinal plants. Since ancient times, plants have been considered a source of inspiration for new drug compounds, as plant-derived medicines have contributed greatly to human health and well-being [12]. Medicinal plants are a rich source of compounds with pharmacological activities, such as antimicrobial activities, because of the diversity of secondary metabolites [13]. The Cameroonian flora abounds with a diversity of plants that have demonstrated their potential in the control of various human ailments, including bacterial infections [14-16]. Therefore, the exploration of this flora appears to be an interesting strategy in the discovery of new antibacterial drugs. In addition, several studies carried out in Cameroon have shown antibacterial activities and modulating effects of antibiotic activity of extracts of many medicinal plants, food plants, and derived products against bacteria (Gram-positive and Gram-negative) sensitive or resistant/multidrug-resistant to common antibiotics [17-19]. In our continuous search for antibacterials from botanical sources, we designed the present work to study *in vitro*, the antibacterial activity of methanolic extracts of nine Cameroonian medicinal plants, namely *Cucumeropsis mannii* (Cucurbitaceae), *Lagenaria siceraria* (Cucurbitaceae), *Citrullus lanatus* (Cucurbitaceae), Cucurbita moschata (Cucurbitaceae), *Salix ledermannii* (Salicaceae), *Gouania longispicata* (Rhamnaceae), *Psychotria mapourioides* (Rubiaceae), *Conyza aethiopica* (Asteraceae) and *Conyza sumatrensis* (Asteraceae). This study was extended to evaluate the capacity of some extracts studied to potentiate the activity of antibiotics commonly used against resistant strains. The plants used in the present work are commonly used in traditional medicine in Cameroon in the treatment of several diseases which include, in addition to bacterial and fungal infectious diseases, cancers, and metabolic syndromes (Table 1). Some of them are also used in Cameroonian cuisine.

Methods

Plant material and extraction

The nine medicinal plants used in this work were collected from Moungo (Littoral region, Cameroon), Dschang (Western region, Cameroon), and Mberenka (Southwest region, Cameroon) during the period from March 2016 to June 2017. Plant samples collected were leaves, fruits, and seeds of *Cucumeropsis mannii, Lagenaria siceraria, Citrullus lanatus,* and *Cucurbita moschata*, the whole plant of *Psychotria mapourioides, Conyza aethiopica, Conyza sumatrensis* and *Gouania longispicata,* and bark and leaves of *Salix ledermannii*. Plants were identified at the National Herbarium (Yaoundé, Cameroon) where reference specimens were deposited under a reference number (see Table 1). Each plant sample was air-dried at laboratory temperature (22°C) and then powdered. The resulting powder was extracted with methanol (1:3 w/v) for 48 h at room temperature. The extract was then concentrated under reduced pressure at about 40ºC, to give a residue that constitutes the crude extract. All extracts were then stored at 4ºC until further use.

Chemicals for antimicrobial assay

In this study, the reference antibiotics used were ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), kanamycin (KAN), erythromycin (ERY), tetracycline (TET), streptomycin (STR), norfloxacin (NOR), cloxacillin (CLO) and doxycycline (DOX), which were all obtained from Sigma-Aldrich (St. Quentin Fallavier). Dimethylsulfoxide (DMSO, Sigma-Aldrich) was used to dissolve the tested samples. The microbial growth indicator used was p-iodonitrotetrazolium chloride ≥97% (INT, Sigma-Aldrich) [20].

Bacterial strains and culture media

A panel of 22 bacteria (Gram-negative and Gram-positive) was used in this work. They included resistant strains of *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The bacterial strains were obtained from the American Type Culture Collection (ATCC) or were clinical laboratory isolates. Their bacterial characteristics were given previously (Additional file 1; Table S1) [12, 21]. Bacterial strains were maintained on agar plates at 4°C and subcultured onto appropriate fresh agar plates 24 hours before any antibacterial test. Mueller Hinton agar (MHA; Sigma) was used for bacterial activation and Mueller Hinton broth (MHB; Sigma) was used for minimum concentration [22] and modulation factor determination [23].

Antibacterial testing

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations on the used bacterial strains were performed using a rapid colorimetric *p*-iodonitrotrazolium chloride (INT) test [20, 24]. The different plant extracts and the reference drug were dissolved in DMSO-MHB. The bacterial inoculum used was 1.5×10^6 CFU/mL and the incubation conditions were 37 °C for 18 h. DMSO with concentrations less than 2.5% was used as control solvent while CHL and CIP were used as positive controls. A preliminary test was performed by evaluating a combination of the plant extracts at different sub-inhibitory concentrations (MIC/2, MIC/4, MIC/8, and MIC/16) with 10 antibiotics (AMP, CLO, DOX, CIP, NOR, CHL, TET, KAN, ERY, and STR) on PA124 (see Additional file 1; Table S2), which allowed us to select the appropriate sub-inhibitory concentration to further potentiate the effect on other bacteria. Therefore, MIC/2 and MIC/4 values were subsequently used for the combination of antibiotics in the sample on a larger number of bacteria [25 - 28]. Fractional inhibitory concentrations were calculated as the ratio of the MIC of the antibiotic in the combination to that of the antibiotic alone ($MIC_{\text{of the antibiotic in the combination}}/MIC_{\text{of the antibiotic alone}}$) and interpretation was made as synergistic (≤ 0.5), indifferent (1 to 4), or antagonistic (> 4) [29, 30].

Phytochemical screening

The presence of the major classes of secondary metabolites, namely alkaloids, polyphenols, flavonoids, triterpenes, sterols, and saponins (Table 2) was determined using the standard phytochemical methods described by [23].

Results

Phytochemistry of the studied plants

The results of Table 2 reporting the qualitative phytochemical analysis indicated that the classes of secondary metabolites are randomly distributed in the extracts of the plants studied. Only the *Salix ledermannii* leaf extract tested contained all the secondary metabolites highlighted in this work. Except for the extract from the leaves of *Lagenaria siceraria*, all the other crude extracts contained polyphenols (Table 2).

Antibacterial activity

The MIC results as compiled in Table 3 indicate that the extracts studied possess antibacterial activity with values ranging from 64 to 1024 µg/mL. *Gouanea longispicata* extract presented the highest spectrum of action notably against 86.4% of the tested bacterial strains. The other extracts presented an inhibition spectrum between 4.5% - 31.8% except those of *Cucumeropsis mannii* seeds, *Lagenaria siceraria* seeds, *Citrullus lanatus* seeds and leaves, *Salix ledermannii* bark, *Psychotria mapourioides* and *Conyza aethiopica* which did not show antibacterial activity. The lowest MIC value of 64µg/mL was recorded with *Cucurbita moschata* against *Providencia stuartii* NEA16. The other extracts showed low activities against a limited number of strains studied. According to the MBC/MIC ratio values, the effects of the studied extracts were mostly bacteriostatic (MBC/MIC > 4). The most active extract, *Gouanea longispicata*, showed low bactericidal activity with only one MBC against 2/22 (9.1%). CHL and CIP were used as reference antibiotics on Gram-negative and Gram-positive bacteria, respectively. CHL was active on 94.4% of Gram-negative bacteria tested and CIP on all Gram-positive bacteria (100%) with MICs ranging from 8 µg/mL to 256 µg/mL and <0.5 µg/mL to 2 µg/mL respectively.

Antibiotic-potentiating effect of the crude extracts

Based on the results obtained in a preliminary study performed on *Pseudomonas aeruginosa* PA124, the three selected extracts (*Gouania longispicata, Salix ledermannii* leaves, and *Conyza sumatrensis*) were combined with ten antibiotics (AMP, CHL, CIP, CLO, DOX, ERY, KAN, NOR, STR, and TET) commonly used in bacterial chemotherapy in order to check their potentiating capacities. Tables 4-6 show that the selected extracts potentiated the effect of the antibiotics in varying proportions depending on the antibiotic and the bacterial strain at sub-inhibitory concentrations of MIC/2 and MIC/4. These synergistic effects vary from 20 to 60% on the different microorganisms with all extracts. Potentiation of the antibiotic effect on more than 70% of the strains tested at the different concentrations (MIC/2 and MIC/4) was not obtained with the selected extracts (Table 4-6). Nevertheless, *Salix ledermannii* leaf extract in combination with COL and CIP, showed the highest synergistic effects (60%). On the other hand, *Gouania longispicata* extract, the most active extract according to MIC determinations, showed a low percentage of synergy (Table 5), the maximum of which was obtained against 40% tested bacteria when combined with NOR and TET. Several cases of indifference and antagonism were also observed.

Discussion

Phytochemistry

The biological activity of plant extracts depends on the active ingredients (secondary metabolites) it contains [32, 33]. This activity depends not only on the presence of secondary metabolites, but also on the types, and the quantity of the metabolites, as well as on the possible interactions between the constituents. These parameters can vary from one plant to another but also from one part to another in the same plant and depend on the harvesting period. The phytochemical screening carried out in the framework of this study allowed us to highlight the presence of polyphenols, flavonoids, alkaloids, triterpenes, sterols, and saponins (Table 2).

Antibacterial effects

The search for new and more efficient ways to fight MDR bacteria remains a real public health issue. Thus, the presence of secondary metabolites in the different plant extracts used in this study could justify their observed antibacterial activities. According to Kuete's classification [14], A plant extract was considered significantly active when MIC<100 µg/mL, moderately active for 100≤ MIC≤624 µg/mL, and weakly active when MIC>2048 µg/mL. However, the spectrum of activity and the level of resistance of the strains studied should be taken into consideration. Thus, in this work, the antibacterial activity observed varies from one extract to another and from one bacterial strain to another. The extract of *Gouania longispicata* presented the highest spectrum of activity with an inhibitory power on 19 of the 22 strains tested (86.4%). The extract of *Cucurbita moschata* seeds had a strong activity on 1

of the strains that were sensitive to it with a bactericidal effect on it. On the other hand, this extract had a moderate activity on 2 strains with bacteriostatic effects and a weak activity on 4 strains with 1 bactericidal effect and 3 bacteriostatic effects. The extracts of leaves and fruit of *Cucumeropsis mannii, Lagenaria siceraria, Cucurbita moschata*, the fruit of *Citrullus lanatus*, leaves of *Salix ledermannii,* and the extract of *Conyza sumatrensis* showed activities with spectra ranging from 4.5% (1 strain out of 22) to 31.8% (7 strains out of 22). However, extracts from *Cucumeropsis mannii* seeds, *Lagenaria siceraria* seeds, *Citrullus lanatus* seeds and leaves, *Salix ledermannii* bark, *Psychotria mapourioides,* and *Conyza aethiopica* showed no activity. The different distribution of secondary metabolites within the same plant would justify the activity observed with our cucurbitaceae extracts. The methanolic extract of *Salix ledermannii* leaves was only active on 1 bacterial strain, whereas the phytochemical screening revealed the presence of all classes of secondary metabolites. This activity could therefore be related to the amount (concentration) of secondary metabolites present. The methanolic extracts of *Lagenaria siceraria* fruit and leaf were weakly active while the seed extract showed no activity (Table 3). On the other hand, many studies have shown antioxidant, anticancer, antibacterial properties of the seeds of this plant [34 - 37]. Also, the seeds and pulps of this plant are used in traditional medicine in the treatment of many diseases and against pain. Thus, the presence of some metabolites and not others could be justified by the composition of the soil, the temperature, and the light intensity. *Cucurbita moschata* has received considerable attention in recent years because of the nutritional and protective values of its fruits and seeds. This plant has several therapeutic properties and is traditionally used to prevent chicken pox, skin diseases, jaundice, insomnia, colic, eye disorder, reduces cell damage, cancer and improves immune function. In this study, the methanolic extracts of the different parts of this plant showed variable activities with the best activity found in the seed extract and the lowest in the fruit extract. This activity corroborates those demonstrated by [38-39] on sensitive strains and adds to the other activities listed, namely its antidiabetic, anticancer, antihypertensive, antioxidant, antitumor, immunomodulatory, anti-inflammation, antifungal, and antihyperlipidemic activity [40-43]. The antibacterial activity obtained with the extract of *Gouania longispicata* is significant. Indeed, not only was this extract active on 19 of the 22 strains tested, but we also noted MICs=128 µg/mL on 4 of the strains, including one MRSA strain (*E. coli* AG102, *K. pneumoniae* KP55, ATCC11296, and *S. aureus* MRSA9). The differences in susceptibility found for the same extract with different strains could be explained by intrinsic differences in the chemical composition of the bacterial wall and/or in genetic elements of resistance that may or may not be transferable between strains such as plasmids or transposons [44]. The presence of efflux pumps in bacteria is responsible for therapeutic failures because they are involved in resistance to several antibiotics such as tetracycline, chloramphenicol, rifampin, quinolones, and certain β-lactams, thus leading to an increase in MICs above therapeutic thresholds [45-47]. This activity observed on both Gram-negative and Gram-positive bacteria is due to the presence of phytochemicals with antibacterial potential. It should be noted that no study has yet been done regarding the antibacterial potential of *Gouania longispicata*. Olapeju *et al.* [48] demonstrated some sensitivity of some bacteria and fungi towards methanol, ethyl acetate and n-hexane extracts of *Conyza sumatrensis* with clear zones of inhibition at high concentrations. However, in the present study, low activity (MIC = 512 µg/mL) was noted on only one of the strains tested (NEA16). Moreover, we noted as much as Olapeju *et al.* [48], during the phytochemical screening, the presence of secondary metabolites with biological activity namely flavonoids, terpenes, polyphenols, and tannins. Thus, the low activity noted in the present work could be justified by the concentration of metabolites, associated with the quality of the soil and the harvest period. Indeed, the composition of the soil and the harvesting period are factors that condition the concentration of metabolites present in the plants.

Antibiotic-modulation activity

Synergistic or modulating effects following the combination of selected plant extracts (*Gouania longispicata, Salix ledermannii* leaves and *Conyza sumatrensis*) with antibiotics on the tested bacteria were noted. The work of Braga *et al*. [28] demonstrated synergistic effects of the combination of plant extracts with the usual antibiotics on clinical isolates of *S. aureus*, this work also showed that when a percentage of synergy is greater than or equal to 70%, a possible existence of EPI in the extracts studied could be noticed. Synergistic effects were obtained in this work, only the extract of *Salix ledermannii* leaves in combination with CLO and CIP, showed the most important synergistic effects (60%). 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 1 strain of multidrug-resistant bacteria. Cases of indifference of some extracts towards certain antibiotics have also been reported; this can 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 antibiotics could be explained by a possible neutralization of the active function of the antibiotic by some plant components [12].

Table 1. Information on the studied plants

Species (family); voucher number	Traditional uses	Bioactive or potentially bioactive components*	Bioactivity of crude extract
Cucumeropsis mannii (Cucurbitaceae); 1874/SFRK	Healing ointment	/	/
Lagenaria siceraria (Cucurbitaceae); 34058/HNC	Eczema, malaria, respiratory disorders, jaundice, diabetes, ulcer, hypertension and infectious diseases, deworming, insomnia, epilepsy [49]	Cucurbitacin, polyphenols, sterols (campester and sitosterol), flavonoids, vitamins, saponins [49]	Methanolic extract: anti-hepatoprotective, antioxidant, hypolipidemic, immunoprotective, antiproliferative, antidepressant, cardioprotective, and antitumor [49]
Cucurbita moschata (Cucurbitaceae); 42958/HNC	Anthelmintic, skin conditions, inflammation [50]	Polysaccharides, para aminobenzoic acid, sterols, protein and peptides, carotenoids, aminobutyric acid [50]	Methanolic extract: Antifungal, antidiabetic, antioxidant, antitumor, antimicrobial, anti-inflammatory [50]
Citrullus lanatus (Cucurbitaceae); 55594/HNC	Epilepsy, asthma, bronchitis, eye pain, smallpox, rheumatism, diarrhea, dysentery, vermifuge, diuretic, tonic, inflammation, hypotensive, kidney stones [51]	Lycopene, glucose, vitamin C, β-carotene, cucurbitacin, triterpenes, sterols, and alkaloids [51]	Methanolic and aqueous extract: antioxidant, cytoprotective [51]
Salix ledermannii (Salicaceae) ; 42587 / HNC	/	/	/
Gouania longispicata (Rhamnaceae) ; 4447/SRFK	Dysentery, diarrhea, irritation of the stomach, intestines. Catarrhal infections of the kidneys, dysuria and gonorrhoea, syphilis, dental caries [52]	Flavonoids, tannins, anthracene, sterols and triterpenes, glycosides, saponins, steroids [52]	Methanol hexane extract, chloroform water: antibacterial, antifungal [52]
Psychotria mapourioides (Rubiaceae); 25860 / SRF Cam	/	/	/
Conyza aethiopica (Asteraceae); 5604/SRF/Cam	Epilepsy, asthma, bronchitis, eye pain, smallpox, rheumatism, diarrhea, dysentery [53]	/	/
Conyza sumatrensis (Asteraceae); 52936 / HNC	Asthma, burns tumors, diarrhea, fever, nausea splenosis, gastric distress, deworming, fungicide, bactericide, whitlow, leprosy, dermatoses, scabies, mycoses, snake bite, microfilaria [54]	/	/

/ : not reported ; HNC: Cameroon National Herbarium; SRF/Cam/ K : *Section des Réserves Forestières du Cameroun / Kamerun.*; * Several classes of secondary metabolites detected are potential antibacterial, anticancer, anti-inflammatory agents, etc [55-61].

Table 2. Qualitative phytochemical composition of the plant extracts

Classes	Studied plants and composition																	
	Lagenaria siceraria			***Cucumeropsis mannii***			***Citrullus lanatus***			***Cucurbita moschata***			***Salix ledermannii***		***Gouania longispicata***	***Psychotria mapourioides***	***Conyza aethiopica***	***Conyza sumatrensis***
	S	F	L	S	F	L	S	F	L	S	F	L	B	L	W	W	W	W
Polyphenols	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+
Alkaloids	-	+	+	-	+	+	-	+	-	-	+	-	+	+	-	+	-	+
Triterpenes	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+
Sterols	-	-	+	-	-	-	-	+	+	-	-	+	-	+	+	+	+	-
Saponins	+	+	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+

(-): Absent; (+): Present; the tested extracts were obtained from (L: Leaves; B: bark; F: Fruit; S: Seed; W: whole plant).

Table 3. MICs and MBCs in µg/mL of methanol extracts from the studied plants, chloramphenicol, and ciprofloxacin

Bacterial strains	Tested samples, MIC and MBC (in bracket) values (µg/mL)																		
	LS			CM			CL			CMo				SL	GLo	Pm	Ca	Cs	ATB
	S	F	L	S	F	L	S	F	L	S	F	L	B	L	W	W	W	W	CHL
Escherichia coli																			
ATCC8739	-	-	256 (1024)	-	-	512 (-)	-	128 (-)	-	128 (1024)	512 (512)	512 (-)	-	-	256(-)	-	-	-	8(256)
ATCC10536	-	-	1024 (-)	-	-	-	-	-	-	512 (-)	-	1024 (-)	-	-	256 (512)	-	-	-	16(32)
AG100A$_{TET}$	-	-	512(-)	-	-	-	-	512 (1024)	-	-	-	1024 (1024)	-	-	512(-)	-	-	-	64 (128)
AG102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	128 (1024)	-	-	-	64(64)
MC4100	-	-	1024 (-)	-	-	-	-	1024 (-)	-	1024 (-)	-	-	-	-	512(-)	-	-	-	128(128)
W3110	-	-	-(-)	-	-	-	-	1024 (-)	-	-	-	-	-	-	256(-)	-	-	-	64(128)
Enterobacter aerogenes																			
ATCC13048	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	256(-)	-	-	-	**8**(32)
CM64	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	1024(-)	-	-	-	128(-)
EA27	-	1014 (-)	-	-	512 (-)	-	-	-(-)	-	-	-	-	-	-	256(-)	-	-	-	-(-)
EA289	-		-	-	-	-	-	-(-)	-	-	-	-	-	-	512(-)	-	-	-	256(-)
Klebsiella pneumoniae																			
KP55	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	128(-)	-	-	-	32(128)
KP63	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	256 (1024)	-	-	-	128(-)
ATCC11296	-	-	-	-	-	-	-	-(-)	-	1024 (-)	-	-	-	-	128(-)	-	-	-	8(256)
Providencia stuartii																			
NEA16	-	-	-	-	-	-	-	-(-)	-	**64 (64)**	-	512 (512)	-	512 (512)	512(-)	-	-	512 (512)	256(-)
ATCC29916	-	-	-	-	512 (-)	-	-	-(-)	-	-	-	-	-	-	512 (-)	-	-	-	16(32)
PS2636	-	1024 (-)	-	-	101 **4 (-)**	-	-	-(-)	-	-	-	-	-	-	512 (-)	-	-	-	32(32)
Pseudomonas aeruginosa																			
PA01	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	512 (-)	-	-	-	128(-)
PA124	-	-	-	-	-	-	-	-(-)	-	1024 (1024)	-	-	-	-	1024 (-)	-	-	-	256(-)
Staphlococcus aureus																			
ATCC25923	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	-(-)	-	-	-	**<0,5**(16)
MRSA3	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	-(-)	-	-	-	**2**(16)
MRSA8	-	-	-	-	-	-	-	-(-)	-	-	-	-	-	-	-(-)	-	-	-	**2(8)**
MRSA9	-	-	-	-	-	-	-	-(-)	-	1024 (-)	-	-	-	-	128(-)	-	-	-	**2**(16)

– : >1024 (MIC) or not determined; the tested extracts were obtained from (L: Leaves; B: bark; F: Fruit; S: Seed; W: whole plant); CHL: chloramphenicol; CIP: ciprofloxacin; *Ca: Conyza aethiopica; CM: Cucumeropsis mannii ; CL: Citrullus lanatus ; CMo: Cucurbita moschata ; Cs: Conyza sumatrensis; GLo : Gouania longispicata; LS: Lagenaria siceraria ; Pm: Psychotria mapourioides: SL : Salix ledermannii*; Values in bold: Significant activity [61-64]; MICs and MBC: were determined by the broth microdilution method [65, 66].

Table 4. MIC of antibiotics after the association with *Salix ledermannii* (leaves) at MIC/2 and MIC/4 against five MDR bacteria strains

Antibiotics[a]	Bacterial strains[b], MIC (µg/mL) of antibiotics in the absence and presence of *Salix ledermannii* (leaves)						PSBS (%)
	Extract concentration	MRSA3	ATCC29916	ATCC13048	AG102	ATCC25923	
STR	0	0.25	0.125	1	2	/	
	MIC/2	0.25 (1)I	0.0625 (0.5)S	8 (8)A	/ (nc)A	/	1/5 (20%)
	MIC/4	0.25 (1)I	0.0625 (0.5)S	2 (2)I	/ (nc)A	/	1/5 (20%)
CLO	0	32	16	32	32	64	
	MIC/2	4 (0.125)S	2 (0.125)S	32 (1)I	32 (1)I	32 (0.5)S	3/5 (60%)
	MIC/4	4 (0.125)S	2 (0.125)S	32 (1)I	32 (1)I	32 (0.5)S	3/5 (60%)
ERY	0	4	4	/	/	/	
	MIC/2	4 (1)I	2 (0.5)S	/	/	/	1/5 (20%)
	MIC/4	4 (1)I	2 (0.5)S	/	/	/	1/5 (20%)
NOR	0	2	≤0.5	2	1	16	
	MIC/2	1 (0.5)S	16 (≥32)A	16 (8)A	64 (64)A	64 (4)A	1/5 (20%)
	MIC/4	1 (0.5)S	16 (≥32)A	2 (1)I	64 (64)A	64 (4)A	1/5 (20%)
CHL	0	8	2	2	2	8	
	MIC/2	2 (0.25)S	2 (1)I	4 (2)I	4 (2)I	4 (0.5)S	2/5 (40%)
	MIC/4	2 (0.25)S	2 (1)I	4 (2)I	2 (1)I	2 (0.25)S	2/5 (40%)
DOX	0	≤0.5	≤0.5	8	8	8	
	MIC/2	≤0.5 (1)I	≤0.5 (1)I	16 (2)I	16 (2)I	16 (2)I	0/5 (0%)
	MIC/4	≤0.5 (1)I	≤0.5(1)I	8 (1)I	8 (1)I	8 (1)I	0/5 (0%)
AMP	0	/	/	/	/	/	
	MIC/2	/	/	/	/	/	0/5 (0%)
	MIC/4	/	/	/	/	/	0/5 (0%)
CIP	0	0.25	0.125	/	2	/	
	MIC/2	0.25 (1)I	0.25 (2)I	0.5 (nc)S	0.5 (0.25)S	0.5 (nc)S	3/5 (60%)
	MIC/4	0.25 (1)I	0.25 (2)I	/	1 (0.5)S	1 (nc)S	2/5 (40%)
TET	0	8	8	32	16	16	
	MIC/2	≤0.5 (≤0.06)S	≤0.5 (≤0.06)S	32 (1)I	32 (2)I	32 (2)I	2/5 (40%)
	MIC/4	2 (0.25)S	≤0.5 (≤0.06)S	32 (1)I	32 (2)I	32 (2)I	2/5 (40%)
KAN	0	0.5	0.125	2	2	2	
	MIC/2	0.125 (0.25)S	0.25 (2)I	2 (1)I	2 (1)I	2 (1)I	1/5 (20%)
	MIC/4	0.5 (1)I	1 (8)A	2 (1)I	2 (1)I	2 (1)I	0/5 (0%)

[a]Antibiotics [CIP Ciprofloxacin, CHL Chloramphenicol, ERY Erythromycin, CLO Cloxacilin, DOX Doxycicline, AMP Ampicillin, KAN Kanamycin, NOR Norfloxacin, STR Streptomycin, TET Tetracyclin]. [b]Bacteria: *Escherichia coli* [AG102], *Enterobacter aerogenes* [ATCC13048], *Providencia stuartii* [ATCC29916], *Staphlococcus aureus* [ATCC25923, MRSA3]. PBSS: Percentage of bacteria strain on which synergism has been observed; S: Synergy; I: Indifference; A: antagonism; (): FIC (Fractional Inhibitory Concentration) of the antibiotics after association with extracts; 0: MIC of the antibiotic alone; /: MIC >1024 µg/mL; nc: not calculated.

Table 5. MIC of antibiotics after the association with *Gouania longispicata* at MIC/2 and MIC/4 against five MDR bacteria strains

Antibiotics[a]	Bacterial strains[b], MIC (µg/mL) of antibiotics in the absence and presence of *Gouania longispicata*						PSBS (%)
	Extract concentration	MRSA3	ATCC29916	ATCC13048	AG102	ATCC25923	
STR	0	0.25	0.125	1	2	/	
	MIC/2	2 (8)A	0.5 (4)A	/ (nc)A	/ (nc)A	/	0/5 (0%)
	MIC/4	0.5 (2)I	0.5 (4)A	/ (nc)A	/ (nc)A	/	0/5 (0%)
CLO	0	32	16	32	32	64	
	MIC/2	64 (2)I	/ nc)A	32 (1)I	64 (2)I	/ (nc)A	0/5 (0%)
	MIC/4	64 (2)I	32 (2)I	32 (1)I	64 (2)I	64 (1)I	0/5 (0%)
ERY	0	4	4	/	/	/	
	MIC/2	4 (1)I	4 (1)I	/	/	/	0/5 (0%)
	MIC/4	4 (1)I	4 (1)I	/	/	/	0/5 (0%)
NOR	0	2	≤0.5	2	1	16	
	MIC/2	1 (0.5)S	≤0.5 (1)I	2 (1)I	64 (64)A	64 (4)A	1/5 (20%)
	MIC/4	1 (0.5)S	≤0.5 (1)I	2 (1)I	1 (1)I	8 (0.5)S	2/5 (40%)
CHL	0	8	2	2	2	8	
	MIC/2	2 (0.25)S	16 (8)A	8 (4)A	4 (2)I	16 (2)I	1/5 (20%)
	MIC/4	8 (1)I	16 (8)A	8 (4)A	4 (2)I	4 (0.5)S	1/5 (20%)
DOX	0	≤0.5	≤0.5	8	8	8	
	MIC/2	≤0.5 (1)I	≤0.5 (1)I	16 (2)I	8 (1)I	8 (1)I	0/5 (0%)
	MIC/4	≤0.5 (1)I	≤0.5 (1)I	8 (1)I	8 (1)I	8 (1)I	0/5 (0%)
AMP	0	/	/	/	/	/	
	MIC/2	/	/	/	/	/	0/5 (0%)
	MIC/4	/	/	/	/	/	0/5 (0%)
CIP	0	0.25	0.125	/	2	/	
	MIC/2	0.5 (2)I	1 (8)A	1 (nc)S	/ (nc)A	/	1/5 (20%)
	MIC/4	0.5 (2)I	0.25 (2)I	1 (nc)S	/ (nc)A	/	1/5 (20%)
TET	0	8	8	32	16	16	
	MIC/2	1 (0.125)S	≤0.5 (≤0.06)S	32 (1)I	32 (2)I	16 (1)I	2/5 (40%)
	MIC/4	1 (0.125)S	≤0.5 (≤0.06)S	32 (1)I	32 (2)I	32 (2)I	2/5 (40%)
KAN	0	0.5	0.125	2	2	2	
	MIC/2	1 (2)I	0.25 (2)I	2 (1)I	2 (1)I	1 (0.5)S	1/5 (20%)
	MIC/4	0.5 (1)I	0.5 (4)A	2 (1)I	2 (1)I	2 (1)I	0/5 (0%)

[a]Antibiotics [CIP Ciprofloxacin, CHL Chloramphenicol, ERY Erythromycin, CLO Cloxacilin, DOX Doxycicline, AMP Ampicillin, KAN Kanamycin, NOR Norfloxacin, STR Streptomycin, TET Tetracyclin]. [b]Bacteria: *Escherichia coli* [AG102], *Enterobacter aerogenes* [ATCC13048], *Providencia stuartii* [ATCC29916], *Staphlococcus aureus* [ATCC25923, MRSA3]. PBSS: Percentage of bacteria strain on which synergism has been observed; S: Synergy; I: Indifference; A: antagonism; (): FIC (Fractional Inhibitory Concentration) of the antibiotics after association with extracts; 0: MIC of the antibiotic alone; /: MIC >1024 µg/mL; nc: not calculated.

Table 6. MIC of antibiotics after the association with *Conyza sumatrensis* at MIC/2 and MIC/4 against five MDR bacteria strains

Antibiotics[a]	Bacterial strains[b], MIC (µg/mL) of antibiotics in the absence and presence of *Gouania longispicata*						PSBS (%)
	Extract concentration	MRSA3	ATCC29916	ATCC13048	AG102	ATCC25923	
STR	0	0.25	0.125	1	2	/	
	MIC/2	0.5 (2)I	1 (8)A	1 (1)I	/ (nc)A	/	0/5 (0%)
	MIC/4	1 (4)A	0.25 (2)I	4 (4)A	/ (nc)A	/	0/5 (0%)
CLO	0	32	16	32	32	64	
	MIC/2	32 (1)I	2 (0.125)S	32 (1)I	32 (1)I	64 (1)I	1/5 (20%)
	MIC/4	32 (1)I	2 (0.125)S	32 (1)I	32 (1)I	/ (nc)A	1/5 (20%)
ERY	0	4	4	/	/	/	
	MIC/2	2 (0.5)S	/ (nc)A	/	/	/	1/5 (20%)
	MIC/4	4 (1)I	/ (nc)A	/	/	/	0/5 (0%)
NOR	0	2	≤0.5	2	1	16	
	MIC/2	1 (0.5)S	1 (≥2)A	2 (1)I	64 (64)A	4 (0.25)S	2/5 (40%)
	MIC/4	2 (1)I	1 (≥2)A	2 (1)I	64 (64)A	2 (0.125)S	1/5 (20%)
CHL	0	8	2	2	2	8	
	MIC/2	2 (0.25)S	16 (8)A	2 (1)I	4 (2)I	16 (2)I	1/5 (20%)
	MIC/4	2 (0.25)S	4 (2)I	2 (1)I	2 (1)I	32 (4)A	1/5 (20%)
DOX	0	≤0.5	≤0.5	8	8	8	
	MIC/2	≤0.5 (1)I	≤0.5 (1)I	16 (2)I	8 (1)I	8 (1)I	0/5 (0%)
	MIC/4	≤0.5 (1)I	≤0.5 (1)I	16 (2)I	4 (0.5)S	8 (1)I	1/5 (20%)
AMP	0	/	/	/	/	/	
	MIC/2	/	/	/	/	/	0/5 (0%)
	MIC/4	/	/	/	/	/	0/5 (0%)
CIP	0	0.25	0.125	/	2	/	
	MIC/2	0.5 (2)I	1 (8)A	0.5 (nc)S	0.5 (0.25)S	/	2/5 (40%)
	MIC/4	0.25 (1)I	0.25 (2)I	1 (nc)S	4 (2)I	/	1/5 (20%)
TET	0	8	8	32	16	16	
	MIC/2	≤0.5 (≤0.06)S	≤0.5 (≤0.06)S	32 (1)I	32 (2)I	16 (1)I	2/5 (40%)
	MIC/4	4 (0.5)S	≤0.5 (≤0.06)S	32 (1)I	32 (2)I	32 (2)I	2/5 (40%)
KAN	0	0.5	0.125	2	2	2	
	MIC/2	0.5 (1)I	0.5 (4)A	2 (1)I	2 (1)I	2 (1)I	0/5 (0%)
	MIC/4	0.25 (0.5)S	0.5 (4)A	2 (1)I	2 (1)I	2 (1)I	1/5 (20%)

[a]Antibiotics [CIP Ciprofloxacin, CHL Chloramphenicol, ERY Erythromycin, CLO Cloxacilin, DOX Doxycicline, AMP Ampicillin, KAN Kanamycin, NOR Norfloxacin, STR Streptomycin, TET Tetracyclin]. [b]Bacteria: *Escherichia coli* [AG102], *Enterobacter aerogenes* [ATCC13048], *Providencia stuartii* [ATCC29916], *Staphlococcus aureus* [ATCC25923, MRSA3]. PBSS: Percentage of bacteria strain on which synergism has been observed; S: Synergy; I: Indifference; A: antagonism; (): FIC (Fractional Inhibitory Concentration) of the antibiotics after association with extracts; 0: MIC of the antibiotic alone; /: MIC >1024 µg/mL; nc: not calculated.

Conclusion

The present study provides information on the possible use of the Cameroonian medicinal plants tested in the control of bacterial infections, including resistant phenotypes. The results obtained suggest that the extracts of the plants studied, the extracts of *Gouania longispicata*, and the leaves of *Salix ledermannii* can be used as natural modulators of antibiotic resistance to control MDR bacteria.

Additional file

S1. Bacterial strains and features; S2. Preliminary evaluation of antibiotic-resistance modulatory activity of selected samples at sub-inhibitory concentrations against Pseudomonas aeruginosa PA124. Available at: https://www.investchempharma.com/icmp58-supplementary-file/

Abbreviations

AMP: Ampicillin; ATB: Antibiotic; ATCC: American Type Culture Collection; CFU: Colony Forming Unit; CHL: Chloramphenicol; CIP: Ciprofloxacin; CLO: Cloxacillin; DMSO: Dimethyl sulfoxide; DOX: Doxycycline; *E. aerogenes*: *Enterobacter aerogenes*; *E. coli*: *Escherichia coli*; EPI: Efflux Pumps Inhibitors; ERY: Erythromycin; INT: p-iodonitrotetrazolium chloride ≥97% (INT, Sigma-Aldrich); *K. pneumoniae*: *Klebsiella pneumoniae*; KAN: Kanamycin; MBC: Minimal Bactericidal Concentration; MDR: Multidrug resistant; MHB: Mueller Hinton Broth; MIC: Minimal Inhibitory Concentration; NOR: Norflocaxin; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P.*

stuartii: *Providencia stuartii*; *P. aureus*: *Staphylococcus aureus*; STR: Streptomycin; TET: Tetracycline.

Authors' Contribution

OMFD and CFT carried out the study and designed the experiments; CFT and VK wrote the manuscript; ATM, VK and VPB supervised the work; VK provided the bacterial strains and facilities for antibacterial assays; all authors read and approved the final manuscript.

Acknowledgments

Authors are thankful to the Cameroon National Herbarium (Yaoundé) for the plant identification.

Conflict of interest

The authors declare no conflict of interest

Article history:
Received: January 26, 2022
Received in revised form: March 08, 2022
Accepted: March 12, 2022
Available online: March 12, 2022

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