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Antibacterial and antibiotic-modulation activity of six Cameroonian medicinal plants against Gram-negative multi-drug resistant phenotypes

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Abstract

Background: Bacterial Infections involving multi-drug resistant (MDR) phenotypes constitute a worldwide health concern. The present work was designed to assess the antibacterial properties of the methanol extracts of six medicinal plants (*Anthocleista schweinfurthii*, *Nauclea latifolia*, *Boehmeria platyphylla*, *Caucalis melanantha*, *Erigeron floribundus* and *Zehneria scobra*) and the effects of their associations with antibiotics on MDR Gram-negative bacteria over-expressing active efflux pumps.

Methods: The antibacterial activities and the ability to potentiate antibiotic effects of the methanol extracts the tested plants were evaluated *in vitro* against twenty eight Gram-negative bacteria expressing MDR phenotypes, using broth microdilution method. The phytochemical screening of these extracts was also performed using standard methods.

Results: All tested extracts displayed moderate to low antibacterial activity on at least 14.3 % of the 28 tested bacteria, with MIC values ranged from 128 to 1024 µg/mL. The best antibacterial spectrum was observed with *Nauclea latifolia* bark extract. Extracts from *A. schweinfurthii* fruits, *N. latifolia* stem bark, *Z. scobra* and *N. latifolia* leaves showed synergistic effects with many antibiotics against MDR bacteria.

Conclusion: The overall results of the present study provide information for the possible use of the studied plants, especially *Nauclea latifolia* in the control of Gram-negative bacterial infections including MDR species as antibacterials as well as resistance modulators.

Keywords: Antibiotics, Antibacterial, Cameroon, Gram-negative bacteria, Multi-drug resistance, Synergy

Background

Infectious diseases still represent one of the major health concern worldwide [1]. According to the National Institute of Health, infectious diseases are the second cause of death and the leading cause of loss of productive life years worldwide. Bacterial infections are responsible of about 70 % of cases of death related to microorganisms [1]. The use of antibiotics and hygiene rules helped to fight infectious diseases in the past. However, they are becoming increasingly difficult to control as results of

the spread of resistant phenotypes. The resistance to antibiotics has increased in recent decades, mainly due of their inappropriate use [2]. Bacteria have developed several mechanisms of resistance including active efflux which plays an important role in multi-drug resistance (MDR), mainly in Gram-negative bacteria [3]. There is a need for the discovery of new active antimicrobials to combat MDR microorganisms. Amongst the new areas explored to overcome infectious diseases caused by MDR bacteria, medicinal plants seem to offer an ideal alternative since they are readily available source of bio-active agents and are well accepted by about 80 % of the world population. Many African medicinal plants and their metabolites were previously found active against

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Table 1 General informations and report on evidence of biological activities and chemistry of the studied plants

Species (family); Voucher number*	Traditional uses	Parts used traditionally	Area of plant collection	Bioactive or potentially bioactive components	Bioactivities
<i>Anthocleista schweinfurthii</i> Gilg. (Loganiaceae); 32389/HNC	Hernia, female sterility, stomach-ache in women, ovarian problems, venereal diseases, bronchitis, fever, purgative, malaria, hard abscesses anthelminthic, otitis, ophthalmia, pain, malaria, cancers, venereal diseases, bacterial diseases [21]	Stem bark, roots, Sap of young leaves, leaves	Bagangté, West region of Cameroon	Polyphenols, alkaloids, terpenes and steroids [21], schweinfurthiin 1, bauerenone 2, bauerenol 3, 1-hydroxy-3,7,8 trimethoxy-xanthone 4 and 1, 8-dihydroxy-3, 7 dimethoxy-xanthone 5 [35]	Antibacterial activity against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> [21]
<i>Boehmeria platyphylla</i> D. Don (Urticaceae); 27550/SRF/CAM	Stomachic [36] and dysentery [37], control bleeding [28], skin burns	Roots, leaves	Lebialem, South West region of Cameroon	Acetophenone (3,4-dimethoxy-w-(2'-piperidy1)) [27], cryptopleurine [28].	Nor reported
<i>Caucalis melanantha</i> (Hochst/Hien) (Apiaceae); 32891/ HNC	Evil eye [38], epilepsy [39], malaria, Stomachaches gastritis [40]	Leaves, roots, whole plant	Lebialem, South West region of Cameroon	α-Pinene, sabinene and terpinen-4-ol [31]	Antifungal activity [31]
<i>Erigeron floribundus</i> (H.BK) (Asteraceae); 5619S/RF/Cam	Skin disorders [32], Acquired immunodeficiency syndrome (AIDS) therapy [41], antipyretic, and anti-inflammatory [42], gastrointestinal tract infections	Whole plant	Lebialem, South West region of Cameroon	Saponins, flavonods, tannins, phenols, alkaloids and essential oils [42], Phenolics, olean-3-oleil-12,18 diene [43].	Analgesic and antiinflammatory [42]; antifungal activity against <i>Epidermophyton floccosum</i> , <i>Microsporium canis</i> , <i>M. gypseum</i> , <i>M. langeronii</i> , <i>Trichophyton mentagrophytes</i> , <i>T. rubrum</i> , <i>T. soudanense</i> and <i>Scopulariopsis brevicaulis</i> [44]
<i>Nauclea latifolia</i> Smith (Rubiaceae); 34577/HNC	Gonorrhoea [45], hypertension [46], gastrointestinal tract disorders [47], prolong menstrual flow [48]. stomach pain, constipation, fever, diarrhoea, piles dysentery [49].	Stem bark, leaves, roots, fruits	Bagangté, West region of Cameroon	Naucleamides A,B,C,D,E [50]	antimicrobial activity of methanol extract against <i>E. coli</i> , <i>S. dysenteriae</i> , <i>S. aureus</i> , <i>Bacillus subtilis</i> and <i>Aspergillus niger</i> [49]
<i>Zehneria scobra</i> (cf) Sondev (Cucurbitaceae); 19668/SRF/CAM	Fever, diarrhea, skin diseases, stomach pain, jaundice and kidney infection [51]	Leaves, frits, flowers, roots shoot	Bafou, West region of Cameroon	Gypenoside [51]	Antimicrobial activity of ethanol extract against <i>E-coli</i> , <i>Pseudomonas auruginosa</i> , <i>S. aureus</i> , <i>E. coli</i> [51], <i>Vibrio cholerae</i> , <i>Enterobacter aerogenes</i> , <i>Klebsiella pneumoniae</i> , <i>Salmonella paratyphi</i> , <i>Proteus mirabilis</i> , <i>Proteus vulgaris</i> , <i>Bacillus cereus</i> , <i>B. subtilis</i> and <i>Sterptococcus pneumonie</i> [52]

* HNC Cameroon National Herbarium, SRF Société des Réserves Forestières du Cameroun

MDR Gram-negative bacteria [4, 5]. Also the synergistic activities of some African medicinal plants with antibiotics against MDR Gram-negative bacteria were reported [5, 6]. It was demonstrated that several naturally occurring efflux pump inhibitors can restore the activity of antibiotics against MDR bacteria [7, 8]. The present study was therefore designed to investigate the antibacterial potential against MDR Gram-negative phenotypes expressing active efflux pumps of six Cameroonian medicinal plants used traditionally in the treatment of bacterial infections, namely *Anthocleista schweinfurthii* Gilg.(Loganiaceae), *Boehmeria platyphylla* D. Don (Urticaceae), *Caucalis melanantha* (Hochst/Hien) (Urticaceae), *Erigeron floribundus* (H.BK) (Asteraceae), *Nauclea latifolia* Smith (Rubiaceae) and *Zehneria scobra* (cf) Sondev (Cucurbitaceae).

Methods

Plant materials and extraction

The plant materials used in this study were collected on April 2013 in West and South West regions of Cameroon and identified by a specialist of the National Herbarium (Table 1). The plants included two trees namely *Anthocleista schweinfurthii* and *Nauclea latifolia*, and four herbs namely *Boehmeria platyphylla*, *Caucalis melanantha*, *Erigeron floribundus* and *Zehneria scobra*. The whole plant was collected for herbs whilst leaves, fruits and stem bark were collected for trees. Each plant material was dried at room temperature and powdered using a grinder. One hundred grams of each powder was then macerated in 1 L of pure methanol (MeOH) for 48 h and filtered through Whatman filter paper no.1. The filtrate obtained was concentrated under reduced pressure in a rotary evaporator to obtain the crude extract. All crude extracts were then kept at 4 °C until further uses.

Phytochemical screening

The major phytochemical classes such as phenols tannins, flavonoids, saponins, alkaloids, anthraquinones, cardiac glycosides, steroids and triterpenes (Table 2) were investigated according to the common described phytochemical methods [9–13].

Chemicals for antibacterial assays

Seven commonly used antibiotics including tetracycline (TET), kanamycin (KAN), streptomycin (STR), ciprofloxacin (CIP), norfloxacin (NOR), chloramphenicol (CHL), ampicillin (AMP), erythromycin (ERY) (Sigma-Aldrich, St Quentin Fallavier, France) were used. *p*-Iodonitrotetrazolium chloride 0.2 % (INT) and phenylalanine arginine β -naphthylamide (PA β N) (Sigma-Aldrich) were used as bacterial growth indicator and efflux pumps inhibitor respectively.

Microorganisms and growth conditions

Pathogenic microorganisms used in the present study were Gram-negative bacteria including MDR isolates (Laboratory collection) and reference strains (American Type Culture Collection) of *Escherichia coli* (ATCC8739, ATCC10536, AG100, AG100A, AG100ATet, AG102, MC4100 W3110), *Enterobacter aerogenes* (ATCC13048, CM64, EA27, EA289, EA298, EA294), *Klebsiella pneumoniae* (ATCC11296, KP55, KP63, K24, K2), *Enterobacter cloacae* (ECCI69, BM47, BM67), *Pseudomonas aeruginosa* (PA01, PA124) and *Providencia stuartii* (ATCC29916, NEA16, PS2636, PS299645). The clinical strains were the laboratory collection from UMR-MD1, University of Marseille, France. Their features are reported in Additional file 1: Table S1. They were maintained at 4 °C and sub-cultured on a fresh appropriate Mueller Hinton Agar (MHA) for 24 h before any antibacterial test.

Table 2 Preliminary chemical composition of the studied plant extracts

Plant	Part used*	Phenols	Tannins	Flavonoids	Saponins	Alkaloids	Anthraquinones	Cardiac glycosides	Steroids	Triterpenes
<i>A. melanantha</i>	W	+	+	+	-	-	+	+	-	-
<i>A. Schweinfurthii</i>	B	+	+	-	-	-	+	+	-	+
	F	+	+	-	-	-	+	+	-	+
	L	+	+	-	-	-	-	+	-	-
<i>B. platyphylla</i>	W	+	+	-	-	-	+	+	-	-
<i>E. floribundus</i>	W	+	+	-	-	-	-	+	-	-
<i>N. latifolia</i>	B	+	+	-	-	-	+	+	+	+
	F	+	+	-	-	-	+	+	-	+
	L	+	+	-	-	-	+	+	+	+
<i>Z. scobra</i>	W	+	-	-	-	+	-	+	-	-

Extract were from [B stem bark, F fruits, L leaves, W whole plant]. (+): Present; (-): Absent

Table 3 Effect of sub-inhibitory concentrations of *Anthocleista schweinfurthii* fruits extract on the activities of first line antibiotics against Gram-negative MDR bacteria

Antibiotics and concentrations of extract	Bacterial strains and MIC values (µg/mL)							PBSS (%)
	<i>K. pneumoniae</i> K24	<i>P. stuartii</i> NAE16	<i>E. coli</i> AG100	<i>E. aerogenes</i> EA289	<i>P. aeruginosa</i> PA124	<i>E. aerogenes</i> CM64		
TET	0	64	64	16	8	16	32	-
	MIC/2	64	64(1) ^I	8(2) ^S	8(1) ^I	64(0.25) ^A	16(2) ^S	33.33
	MIC/5	64	64(1) ^I	8(2) ^S	8(1) ^I	64(0.25) ^A	16(2) ^S	33.33
NOR	0	≥64	1	≤0.25	-	128	2(1) ^I	-
	MIC/2	≥64	1(1) ^I	≤0.25	-	128 (1) ^I	2(1) ^I	0
	MIC/5	≥64	1(1) ^I	≤0.25	-	128 (1) ^I	2(1) ^I	0
STR	0	2	32	≥256	64	64	16	-
	MIC/2	2(1) ^I	16(2) ^S	≥256	8(8) ^S	32(2) ^S	16(1) ^I	50
	MIC/5	2(1) ^I	32(1) ^I	≥256	8(8) ^S	32(2) ^S	16(1) ^I	33.33
KAN	0	8	8	8	32	64	≤2	-
	MIC/2	8(1) ^I	8(1) ^I	2(4) ^S	2(16) ^S	32(2) ^S	≤2	50
	MIC/5	8(1) ^I	8(1) ^I	4(2) ^S	2(16) ^S	32(2) ^S	≤2	50
CHL	0	16	8	8	512	64	512	-
	MIC/2	8(2) ^S	(2) ^S	8(1) ^I	256(2) ^S	64(1) ^I	256(2) ^S	66.67
	MIC/5	8(2) ^S	(2) ^S	8(1) ^I	512(1) ^I	64(1) ^I	256(2) ^S	50
ERY	0	128	16(1) ^I	64	128	128	256	-
	MIC/2	64(2) ^S	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	128(2) ^S	33.33
	MIC/5	128(1) ^I	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	128(2) ^S	16.67
AMP	0	-	-	128	-	-	-	-
	MIC/2	-	-	32(4) ^S	-	-	-	16.67
	MIC/5	-	-	128(1) ^I	-	-	-	0

(-): >256 µg/ml; (0): fold decrease in MIC values of the antibiotics after association with plants extract; S: Synergy, I: Indifference, A: antagonism, Antibiotics (CHL chloramphenicol, AMP ampicillin, KAN kanamycin, NOR norfloxacin, ERY erythromycin, TET tetracycline, STR streptomycin); PBSS percentage of bacteria strain on which synergism has been observed

Antibacterial assays

The MICs of the tested extracts were determined using a rapid INT colorimetric assay [14]. Briefly, test samples were first dissolved in dimethylsulfoxide/ Mueller Hinton Broth (DMSO/MHB). The solution obtained was then added to MHB and serially diluted two fold (in a 96-well microtiter plate). One hundred microliters of inoculums (1.5×10^6 CFU/ml) prepared in MHB were then added. The plates were covered, agitated with a shaker to mix the contents of the wells and incubated at 37 °C for 18 h. The final concentration of DMSO was 2.5 %, a concentration at which DMSO does not affect bacterial growth. Wells containing MHB, 100 µl of inoculum, and DMSO at a final concentration of 2.5 % served as the negative control. Chloramphenicol was used as reference antibiotic. The MICs of each extract were detected after 18 h of incubation at 37 °C after addition of 40 µl INT (0.2 mg/ml) and incubation at 37 °C for 30 min. Viable bacteria reduce INT with appearance of a pink dye. The MIC of each sample was defined as its lowest concentration that prevented this change and resulted in the complete inhibition of microbial growth. The Minimum Bactericidal Concentration

(MBC) was determined by sub-culturing samples from the wells with concentrations above or equal to the MIC on new plates of Mueller Hinton broth (MHB). The MBC was considered as the lowest concentration of the extract which prevented appearance of pink color after addition of INT. Each assay was performed in triplicate at three different days.

Antibiotic-modulation assay

To evaluate the antibiotic resistance modifying activity of the extracts, the MIC of antibiotics were determined in the presence or absence of the plant extracts using the broth microdilution technique as described above. After a preliminary assay on two MDR bacteria, *P. aeruginosa* PA124 and *E. aerogenes* CM64 (Additional file 1: Tables S3 and S4), extracts from *A. Schweinfurthii* fruits, *N. Latifolia* leaves and stem bark, and from the whole plant of *Z. scabra* were selected and tested at their MIC/2 and MIC/5 in combination with seven antibiotics (CHL, AMP, KAN, NOR, ERY, TET and STR) on six MDR bacterial strains (*P. aeruginosa* PA124, *E. aerogenes*

Table 4 Effect of sub-inhibitory concentrations of *Nauclea latifolia* stem bark extract on the activities of first line antibiotics against Gram-negative MDR bacteria

Antibiotics and concentrations of extract	Bacterial strains and MIC values ($\mu\text{g/mL}$)							PBSS (%)
	<i>K. pneumoniae</i> K24	<i>P. stuartii</i> NAE16	<i>E. coli</i> AG100	<i>E. aerogenes</i> EA289	<i>P. aeruginosa</i> PA124	<i>E. aerogenes</i> CM64		
TET	0	64	64	16	8	16	32	-
	MIC/2	64(1) ^I	64(1) ^I	8(2) ^S	8(1) ^I	16(1) ^I	16(2) ^S	33.33
	MIC /5	64(1) ^I	64(1) ^I	16(1) ^I	8(1) ^I	16(1) ^I	16(2) ^S	16.67
NOR	0	≥ 64	1	≤ 0.25	-	128	2(1) ^I	-
	MIC/2	≥ 64	1(1) ^I	≤ 0.25	-	128 (1) ^I	2(1) ^I	0
	MIC /5	≥ 64	1(1) ^I	≤ 0.25	-	128(1) ^I	2(1) ^I	0
STR	0	2	32	≥ 256	64	64	16	-
	MIC/2	2(1) ^I	32(1) ^I	≥ 256	32(2) ^S	64(1) ^I	8(2) ^S	16.67
	MIC /5	2(1) ^I	32(1) ^I	≥ 256	64(1) ^I	64(1) ^I	16(1) ^I	0
KAN	0	8	8	8	32	64	≤ 2	-
	MIC/2	8(1) ^I	8(1) ^I	4(2) ^S	16(2) ^S	64(1) ^I	≤ 2	33.33
	MIC /5	8(1) ^I	8(1) ^I	8(1) ^I	32(1) ^I	64(1) ^I	≤ 2	16.67
CHL	0	16	8	8	512	64	512	-
	MIC/2	16(1) ^I	4(2) ^S	8(1) ^I	512(1) ^I	64(1) ^I	512	16.67
	MIC /5	16(1) ^I	8(1) ^I	8(1) ^I	512(1) ^I	64(1) ^I	512	0
ERY	0	128	16(1) ^I	64	128	128	256	-
	MIC/2	128(1) ^I	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	128(2) ^S	16.67
	MIC /5	128(1) ^I	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	256(1) ^I	0
AMP	0	-	-	128	-	-	-	-
	MIC/2	-	-	256(2) ^I	-	-	-	0
	MIC /5	-	-	256(2) ^I	-	-	-	0

(-): $>256 \mu\text{g/ml}$; 0: fold decrease in MIC values of the antibiotics after association with plants extract; S: Synergy, I: Indifference; A: antagonism, Antibiotics (CHL chloramphenicol, AMP ampicillin, KAN kanamycin, NOR norfloxacin, ERY erythromycin, TET tetracycline, STR streptomycin); PBSS percentage of bacteria strain on which synergism has been observed

EA289 and CM64, *E. coli* AG100, *P. stuartii* NAE16 and *K. pneumoniae* K24).

The reverse of Fractional Inhibitory concentration (1/FIC) was calculated as follows:

$$1/\text{FIC} = \text{MIC}_{\text{Antibiotic alone}}/\text{MIC}_{\text{Antibiotic in combination with}}$$

The interpretation was made as follows: Synergistic (≥ 2), Indifferent (1 to 0.5), or Antagonistic (≤ 0.25) [5, 15]. All assays were performed in triplicate and repeated thrice.

Results

Phytochemical composition of the tested extracts

The main classes of secondary metabolites for each extract were screened and the results are summarized in Table 2. It appears that all the plant extracts of this study possess at least 3 classes of screened secondary metabolites. Only three classes of the screened phytochemicals were detected in the extracts from *Z. scobra*, *E. floribundus* and *A. schweinfurthii* leaves. Extracts from *N. latifolia* leaves and stem bark contained six phytochemical

classes. All the extracts contained phenols and cardiac Glycosides.

Antibacterial activity

The results (Additional file 1: Table S2) showed that all extracts displayed antibacterial activity against at least 4/28 (14.3 %) tested bacterial strains, with MIC values ranged from 128 to 1024 $\mu\text{g/mL}$. Extracts from *A. schweinfurthii* leaves, fruits and bark exerted inhibitory effects respectively against 14/28 (50 %), 13/28 (46.4 %) and 8/28 (28.6 %) studied bacteria. The extracts from the fruits and leaves of *N. latifolia* were active respectively on 6/28 (21.4 %) and 7/28 (25 %) tested bacteria whilst the bark extract displayed the best spectrum of activity [active on 22/28 (78.6 %) tested bacteria]. Extracts of *B. platyphylla* and *Erigeron floribundus* also showed large spectra of antibacterial activity with MIC values recorded on 17/28 (60.7 %) and 21/28 (75 %) bacterial strains respectively. *Causalis melanantha* and *Z. scobra* extracts displayed low antibacterial spectra [MIC recorded respectively on 4/28 (14.3 %) and 7/28 (25 %) tested bacterial]. *P. aeruginosa* PA124 appeared to be the

Table 5 Effect of sub-inhibitory concentrations of *Nauclea latifolia* leaves extract on the activities of first line antibiotics against Gram-negative MDR bacteria

Antibiotics and concentrations of extract	Bacterial strains and MIC values (µg/mL)						PBSS (%)	
	<i>K. pneumoniae</i> K24	<i>P. stuartii</i> NAE16	<i>E. coli</i> AG100	<i>E. aerogenes</i> EA289	<i>P. aeruginosa</i> PA124	<i>E. aerogenes</i> CM64		
TET	0	64	64	16	8	16	32	-
	MIC/2	64(1) ^I	32(2) ^S	8(2) ^S	2(4) ^S	16(1) ^I	32(1) ^I	50.00
	MIC /5	64(1) ^I	32(2) ^S	8(2) ^S	4(2) ^S	16(1) ^I	32(1) ^I	50.00
NOR	0	≥64	1	≤0.25	-	128	2(1) ^I	-
	MIC/2	≥64	1(1) ^I	≤0.25	-	128 (1) ^I	2(1) ^I	0
	MIC /5	≥64	1(1) ^I	≤0.25	-	128 (1) ^I	2(1) ^I	0
STR	0	2	32	≥256	64	64	16	-
	MIC/2	2(1) ^I	16(2) ^S	32(≥8) ^S	16(4) ^S	64(1) ^I	2(8) ^S	50.00
	MIC /5	2(1) ^I	32(1) ^I	32(≥8) ^S	32(2) ^S	64(1) ^I	8(2) ^S	50.00
KAN	0	8	8	8	32	64	≤2	-
	MIC/2	8(1) ^I	8(1) ^I	2(4) ^S	16(2) ^S	64(1) ^I	≤2	33.33
	MIC /5	8(1) ^I	8(1) ^I	4(2) ^S	32(1) ^I	64(1) ^I	≤2	16.67
CHL	0	16	8	8	512	64	512	-
	MIC/2	16(1) ^I	4(2) ^S	8(1) ^I	256(2) ^S	64(1) ^I	512	33.33
	MIC /5	16(1) ^I	4(2) ^S	8(1) ^I	256(2) ^S	64(1) ^I	512	33.33
ERY	0	128	16(1) ^I	64	128	128	256	-
	MIC/2	128(1) ^I	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	256(1) ^I	0
	MIC /5	128(1) ^I	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	256(1) ^I	0
AMP	0	-	-	128	-	-	-	-
	MIC/2	-	-	64(2) ^S	-	-	-	16.67
	MIC /5	-	-	128(1) ^I	-	-	-	0

(-) : >256 µg/ml; 0: fold decrease in MIC values of the antibiotics after association with plants extract; S: Synergy, I: Indifference; A: antagonism, Antibiotics (*CHL* chloramphenicol, *AMP* ampicillin, *KAN* kanamycin, *NOR* norfloxacin, *ERY* erythromycin, *TET* tetracycline, *STR* streptomycin); PBSS percentage of bacteria strain on which synergism has been observed

most resistant bacteria strain with the sensitivity observed only towards *N. latifolia* bark extract.

Antibiotic resistance modifying activities of the plant extracts

Preliminary results obtained in two most resistant strains, *P. aeruginosa* PA124 and *E. aerogenes* CM64 (results presented in Additional file 1: Tables S3 and S4) allowed selecting the following extracts: *A. schweinfurthii* fruits, *N. latifolia* leaves and bark and *Z. scobra* as well as the appropriate sub-inhibitory concentrations of MIC/2 and MIC/5 for further studies. From the results summarised in Tables 3, 5, 5 and 6, it appears that all the four extracts improved the activities of antibiotics, from 2 to more than 64 folds. The highest activities were observed with *A. schweinfurthii* fruits (Table 3) and *Z. scobra* (Table 6). *A. schweinfurthii* fruits potentiated the activities of TET on 66.7 % and 50 % of the bacteria strains at MIC/2 and MIC/5 respectively. It also increased the activity of KAN (MIC/2 and MIC/5) and STR (MIC/2) in 50 % of the tested bacterial strains while *Z. scobra* improved the activities of STR on 66.7 % and

50 % of the bacteria strains at MIC/2 and MIC/5 respectively. *Z. scobra* also improved the activity of CHL on 50 % of the tested bacterial strains at the two sub-inhibitory concentrations of MIC/2 and MIC/5 (Table 6). Synergistic effects (50 % of antibiotic activity potentiating at MIC/2 and MIC/5) were observed with *N. Latifolia* leaves extract (Table 5) on TET and STR. The highest rate of improvement of antibiotic activity by *N. Latifolia* stem bark extract was rather noticed on TET and KAN with a rate of 33.3 %. Among the four extracts, this later displayed the lowest antibiotic potentiating effect. Moreover, no synergistic effect was observed with NOR, while synergy between the studied extracts and antibiotics were observed with Ampicillin, with a rate of only 16.67 % (Table 5).

Discussion

Medicinal plants are potential source of antimicrobial agents used in the treatment of infectious diseases [16]. According to Rios and Recio [17], and Kuete et al. [17], the antibacterial activity of a plant extract is considered significant when the MICs are below 100 µg/mL. The

activity is considered moderate when $100 \leq \text{MIC} \leq 625 \mu\text{g/mL}$ and weak when MIC are above $625 \mu\text{g/mL}$ [17]. Therefore, the antibacterial activities reported in the present study can mostly be regarded as moderate or low. This could be explained by the fact that the tested bacteria are mostly MDR phenotypes. In fact, *P. aeruginosa* and MDR Enterobacteriaceae (*K. pneumoniae*, *E. aerogenes*, *E. cloacae* and *P. stuartii* and *E. coli*) tested in the present study have been classified as antimicrobial-resistant organisms of concern in healthcare facilities [18–20]. The previously reported activities of *A. schweinfurthii* include antibacterial inhibitory effects of n-hexane, dichloromethane, ethyl acetate and methanol extracts from leaves and stem bark against *Staphylococcus aureus* ATCC 33591 and *E. coli* ATCC 27195 [21]. The MIC values obtained in the present study were respectively 62.5 and 125 $\mu\text{g/ml}$ against *S. aureus* and *E. coli*. Such values were higher than those previously documented, highlighting the MDR feature of the studied bacteria. MBC values were obtained in few cases (Additional file 1: Table S2). A keen look of data (Additional file 1: Table S2) indicates that, in most of the cases, the tested extract exerted

bacteriostatic effects with a ratio MBC/MIC above 4. The overall antibacterial activity of the tested extracts could be due their phytochemical composition. However, the presence of a specific class of second metabolite could not guarantee the antibacterial activity of the plant, as this will depend on nature of the compounds, its concentration as well as the possible interactions with other constituents of the extract. It is also surprising that saponins, known to possess antibacterial activities were not detected in the tested extracts; However, this does means that the extract were completely devoid of this class of secondary metabolite; One of the most understandable explanation should that saponins could be present in very little amounts in the tested extract, and therefore could not be detected using the qualitative phytochemical methods. Some cardiac glycosides such as bufalin, ouabain, digoxin are toxic meanwhile many of them have therapeutic uses and these primarily involve the treatment of cardiac failure [22–24]. Their utility results from an increased cardiac output by increasing the force of contraction. By increasing intracellular calcium, cardiac glycosides increase calcium-induced calcium release and thus contraction [23, 24]. The

Table 6 Effect of sub-inhibitory concentrations of *Zehneria scobra* extract on the activities of first line antibiotics against Gram-negative MDR bacteria

Antibiotics and concentrations of extract	Bacterial strains and MIC values ($\mu\text{g/mL}$)						PBSS (%)	
	<i>K. pneumoniae</i> K24	<i>P. stuartii</i> NAE16	<i>E. coli</i> AG100	<i>E. aerogenes</i> EA289	<i>P. aeruginosa</i> PA124	<i>E. aerogenes</i> CM64		
TET	0	64	64	16	8	16	32	-
	MIC/2	64(1) ^I	64(1) ^I	8(2) ^S	8(1) ^I	16(1) ^I	32(1) ^I	16.67
	MIC/5	64(1) ^I	64(1) ^I	8(2) ^S	8(1) ^I	16(1) ^I	32(1) ^I	16.67
NOR	0	≥ 64	1	≤ 0.25	-	128	2(1) ^I	-
	MIC/2	≥ 64	1(1) ^I	≤ 0.25	-	128 (1) ^I	2(1) ^I	0
	MIC/5	≥ 64	1(1) ^I	≤ 0.25	-	128 (1) ^I	2(1) ^I	0
STR	0	2	32	≥ 256	64	64	16	-
	MIC/2	2(1) ^I	16(2) ^S	≥ 256	8(8) ^S	32(2) ^S	4(4) ^S	66.67
	MIC/5	2(1) ^I	16(2) ^S	≥ 256	16(4) ^S	32(2) ^S	4(4) ^S	50
KAN	0	8	8	8	32	64	≤ 2	-
	MIC/2	8(1) ^I	8(1) ^I	4(2) ^S	4(8) ^S	64(1) ^I	≤ 2	33.33
	MIC/5	8(1) ^I	8(1) ^I	8(1) ^I	8(4) ^S	64(1) ^I	≤ 2	16.67
CHL	0	16	8	8	512	64	512	-
	MIC/2	8(2) ^S	(2) ^S	8(1) ^I	512(1) ^I	64(1) ^I	256(2) ^S	50
	MIC/5	8(2) ^S	8(1) ^I	8(1) ^I	512(1) ^I	64(1) ^I	256(2) ^S	50
ERY	0	128	16(1) ^I	64	128	128	256	-
	MIC/2	128(1) ^I	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	128(2) ^S	16.67
	MIC/5	128(1) ^I	16(1) ^I	64(1) ^I	128(1) ^I	128(1) ^I	256(1) ^I	0
AMP	0	-	-	128	-	-	-	-
	MIC/2	-	-	128(1) ^I	-	-	-	0
	MIC/5	-	-	128(1) ^I	-	-	-	0

(-) : $>256 \mu\text{g/ml}$; 0: fold decrease in MIC values of the antibiotics after association with plants extract; S: Synergy. I: Indifference; A: antagonism. Antibiotics (CHL chloramphenicol, AMP ampicillin, KAN kanamycin, NOR norfloxacin, ERY erythromycin, TET tetracycline, STR streptomycin); PBSS percentage of bacteria strain on which synergism has been observed

traditional use of the studied plants could suggest that their cardiac glycoside could be not toxic and have very low toxic effects.

To the best of our knowledge, the present work describes for the first time the antibacterial activity of *B. platyphylla*. This activity could be due to the presence of the detected phytochemicals. In fact, antibacterial compounds such as acetophenone [25] and cryptopleurine [26–28] were previously isolated from *B. Platyphylla*. The antibacterial activity of *C. melanantha* and *E. floribundus* is also reported here for the first time. However these plants were previously reported for their antifungal activities [29–32]. The antibacterial activities of extracts from *Zehneria scobra* and *Nauclea latifolia* [33, 34] were reported on some bacteria: The present study therefore provides additional information on the activity of these plants against MDR Gram-negative phenotypes.

The synergistic effects between antibiotics and the tested plants are also reported here for the first time. The observed synergistic effects could be due to possible interaction between plant constituents and the tested antibiotics. As the strains used in this study are known to actively expressed efflux pumps, one of the possible explanations for the observed synergistic effects could be the ability of the constituents of the extracts to act as efflux pumps inhibitor. This can explain why the effect of antibiotics with intracellular targets such as STR, CHL and KAN increased contrary to that of beta-lactamine (AMP) acting in the cell wall (Tables 3, 4, 5 and 6).

Conclusion

The overall results of the present study provides baseline information for the possible use of the tested plants, especially *A. schweinfurthii*, *N. Latifolia*, *B. platyphylla* and *E. floribundus* in the control of infections due to Gram-negative bacteria. The present study indicates that the tested plant extracts alone could not be used efficiently to tackle MDR bacterial infections. However, it was demonstrated that extracts from *A. schweinfurthii* fruits and *Z. scobra* could be used in combination with some antibiotics to fight bacterial multi-drug resistance.

Availability of data and materials

The datasets supporting the conclusions of this article are presented in this main paper and supporting material. Plant materials used in this study have been identified at the Cameroon National Herbarium where voucher specimens are deposited.

Consent for publication

Not applicable in this section.

Ethic approval and consent to participate

Not applicable in this section.

Additional file

Additional file 1: Table S1. Bacterial strains and features. **Table S2.** Minimal inhibitory concentration (MIC) and minimal bactericidal (MBC) of the plant extracts and CHL on the studied bacteria. **Table S3.** Effects of different concentrations of plant extracts on the MIC ($\mu\text{g/ml}$) of antibiotics against *P. aeruginosa* PA124. **Table S4.** Effects of different concentrations of plant extracts on the MIC ($\mu\text{g/ml}$) of antibiotics against *E. aerogenes* CM64 (DOC 320 kb)

Abbreviations

A. schweinfurthii: *Anthocleista schweinfurthii*; AMP: ampicillin; ATCC: American type culture collection; *B. platyphylla*: *Boehmeria platyphylla*; *C. melanantha*: *Caucalis melanantha*; CFU: colony forming unit; CHL: chloramphenicol; CIP: ciprofloxacin; DMSO: dimethylsulfoxide; *E. floribundus*: *Erigeron floribundus*; *E. aerogenes*: *Enterobacter aerogenes*; *E. cloacae*: *Enterobacter cloacae*; *E. coli*: *Escherichia coli*; ERY: erythromycin; FIC: fractional inhibitory concentration; INT: *p*-iodonitrotetrazolium chloride; *K. pneumoniae*: *Klebsiella pneumoniae*; KAN: kanamycin; MBC: minimum bactericidal concentration; MDR: multi-drug resistant; MeOH: methanol; MHA: Mueller Hinton agar; MHB: Mueller Hinton broth; MIC: minimum inhibitory concentration; *N. latifolia*: *Nauclea latifolia*; NOR: norfloxacin; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P. stuartii*: *Providencia stuartii*; PA β N: phenylalanine arginine β -naphthylamide; STR: streptomycin; TET: tetracycline; *Z. scobra*: *Zehneria scobra*.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DED, VK and BN designed the study; DED and JAKN carried the experiments and wrote the manuscript; VK provided the bacterial strains and chemicals for antibacterial assays; all the authors read and approved the final manuscript.

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