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Antibacterial and antibiotic resistance modifying activity of the extracts from *allanblackia gabonensis, combretum molle* and *gladiolus quartinianus* against Gram-negative bacteria including multi-drug resistant phenotypes

Aimé G. Fankam, Jules R. Kuiate and Victor Kuete[*]

Abstract

Background: Bacterial resistance to antibiotics is becoming a serious problem worldwide. The discovery of new and effective antimicrobials and/or resistance modulators is necessary to tackle the spread of resistance or to reverse the multi-drug resistance. We investigated the antibacterial and antibiotic-resistance modifying activities of the methanol extracts from *Allanblackia gabonensis, Gladiolus quartinianus* and *Combretum molle* against 29 Gram-negative bacteria including multi-drug resistant (MDR) phenotypes.

Methods: The broth microdilution method was used to determine the minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the samples meanwhile the standard phytochemical methods were used for the preliminary phytochemical screening of the plant extracts.

Results: Phytochemical analysis showed the presence of alkaloids, flavonoids, phenols and tannins in all studied extracts. Other chemical classes of secondary metabolites were selectively presents. Extracts from *A. gabonensis* and *C. molle* displayed a broad spectrum of activity with MICs varying from 16 to 1024 μg/mL against about 72.41 % of the tested bacteria. The extract from the fruits of *A. gabonensis* had the best activity, with MIC values below 100 μg/mL on 37.9 % of tested bacteria. Percentages of antibiotic-modulating effects ranging from 67 to 100 % were observed against tested MDR bacteria when combining the leaves extract from *C. molle* (at MIC/2 and MIC/4) with chloramphenicol, kanamycin, streptomycin and tetracycline.

Conclusion: The overall results of the present study provide information for the possible use of the studied plant, especially *Allanblackia gabonensis* and *Combretum molle* in the control of Gram-negative bacterial infections including MDR species as antibacterials as well as resistance modulators.
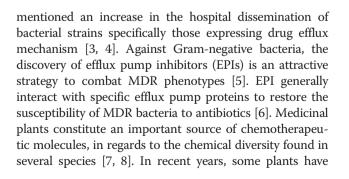
Keywords: Efflux pumps, Extracts, Gram-negative bacteria, MDR bacteria, Resistance modulators

Background

Infectious diseases caused by multi-drug resistant (MDR) Gram-negative bacteria are worldwide health concern, causing increasingly morbidity and mortality particularly in developing countries [1]. In Cameroon, previous studies showed high levels of resistance to commonly used antibiotics in Gram-negative bacilli [2]. Several reports also mentioned an increase in the hospital dissemination of bacterial strains specifically those expressing drug efflux mechanism [3, 4]. Against Gram-negative bacteria, the discovery of efflux pump inhibitors (EPIs) is an attractive strategy to combat MDR phenotypes [5]. EPI generally interact with specific efflux pump proteins to restore the susceptibility of MDR bacteria to antibiotics [6]. Medicinal plants constitute an important source of chemotherapeutic molecules, in regards to the chemical diversity found in several species [7, 8]. In recent years, some plants have

* Correspondence: kuetevictor@yahoo.fr
Department of Biochemistry, Faculty of science, University of Dschang,
P.O. Box 67, Dschang, Cameroon

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been successfully evaluated for their direct antibacterial action, and for their antibiotic-modulation activity [9–12]. In the present work, we hypothesized that herbal medicines traditionally used for the treatment of infectious diseases could contain molecules acting as antibacterial and/ or antibiotic-resistance modulators. This study was therefore designed to investigate the *in vitro* antibacterial and antibiotic-resistance modifying activities of the methanol extracts from *Allanblackia gabonensis* Pellegr. (Clusiaceae), *Gladiolus quartinianus* A. Rich (Iridaceae) and *Combretum molle* R. Br. ex G. Don (Combretaceae) against Gram-negative bacteria including multi-drug phenotypes. These plants are traditionally used to manage various ailments including bacterial related infections.

Methods

Plant materials and extraction

Medicinal plants used in this work were collected in different areas of Cameroon between January and April 2012. The plants were identified at the National Herbarium (Yaoundé, Cameroon), where voucher specimens were deposited under the reference numbers (Table 1). The air-dried and powdered plant material was weighed (300 g) and soaked in 1 L of methanol (MeOH) for 48 h at room temperature. The filtrate obtained through Whatman filter paper No. 1 was concentrated under reduced pressure in vacuum to obtain the crude extracts. All crude extracts were then kept at 4 °C until further uses.

Chemicals for antibacterial assays

Eight 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. The *p*-Iodonitrotetrazolium chloride 0.2 % (INT) and phenylalanine arginine β-naphthylamide (PAβN) (Sigma-Aldrich) were used as bacterial growth indicator and efflux pumps inhibitor respectively.

Microorganisms and growth conditions

Pathogenic bacteria used in the study were Gram-negative bacteria including MDR isolates (Laboratory collection) and reference strains (American Type Culture Collection) of *Escherichia coli* (ATCC8739, ATCC10536, AG100, AG100A, AG100A$_{Tet}$, AG102, MC4100 W3110), *Enterobacter aerogenes* (ATCC13048, CM64, EA27, EA3, 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) were used. Their features were previously reported [37]. They were maintained at 4 °C and sub-cultured on a fresh appropriate Mueller Hinton Agar (MHA) for 24 h before any antibacterial test.

Preliminary phytochemical investigation

The plant extracts were screened for the presence of major secondary metabolite classes such as alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, sterols and triterpenes according to common phytochemical methods previously described [38]. The tests were based on visual observation of the change in color or formation of precipitate after the addition of specific reagents.

Antibacterial assays

MICs and MBCs of the plant extracts and chloramphenicol were determined by microdilution method using rapid INT colorimetric assay [25, 39]. Briefly, the samples were first dissolved in 10 % Dimethyl-sulfoxide (DMSO)/Mueller Hinton Broth (MHB). The solution obtained was then added to MHB and serially diluted two fold (in a 96-well microplate). One hundred microliters of inoculum (1.5× 10^6 CFU/mL) prepared in MHB were then added. The plates were covered with a sterile plate sealer and then 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 less than 2.5 %, and did not affect the microbial growth. Wells containing MHB, 100 μL of inoculum, and DMSO at a final concentration of 2.5 % served as the negative control. The MIC of each sample was detected after 18 h of incubation at 37 °C following addition of 40 μL INT (0.2 mg/mL) and incubation at 37 °C for 30 min. Viable bacteria reduced the yellow dye to a pink. The MIC was defined as the lowest sample concentration that prevented this change and that resulted in the complete inhibition of bacterial growth. The MBC of the sample was determined by sub-culturing 50 μL of the suspensions from the wells which did not show any growth after incubation during MIC assays to 150 μl of fresh broth, and re-incubated at 37 °C for 48 h before revelation. The MBC was defined as the lowest concentration of sample which completely inhibited the growth of bacteria [40]. Each assay was performed in three independent tests in triplicate. The samples were also tested in the presence of phenylalanine arginine β-naphthylamide (PAβN) at a final concentration of 20 μg/mL as previously described [41] on nine MDR bacteria. All assays were performed three time in duplicate.

Antibiotic-modulation assay

To evaluate the antibiotic resistance modifying activity of the extracts, the MIC of antibiotic was determined in the presence or absence of the plant extracts. The 96-wells plate modulation method, as described by Stavri *et al.* [42] was used. Briefly, after serial dilutions of antibiotics (256–0.5 μg/mL), the plant extracts at their sub-inhibitory concentrations (MIC/2 and MIC/4; selected

Table 1 Plants used in the present study and evidence of their bioactivities

Samples, family, and herbarium number[a]	Traditional treatment	Area of plant collection	Known bioactive or potentially active compounds	Screened biological activities of the crude extracts and known bioactive compounds
Allanblackia gabonensis Pellegr. (Clusiaceae); 17275SRF/Cam	Dysentery, cold, toothache [13]; pain, rheumatism, inflammations [14, 15].	Lebialem, South West region of Cameroon; (4°10′N 9°14′E/4.167°N 9.233°E)	Not reported	Crude extracts: Analgesic and anti-inlammatory effect of aqueous extract of the stem bark [14]; crude methanol fruits extracts (40 μg/mL) showed to inhibit growth of CCRF-CEM leukemia cells at about 50 % [16].
Gladiolus quartinianus A. Rich (Iridaceae); 17260/SRF/Cam	Infections of the skin, gut, urogenital system, and upper respiratory tract [17], gonorrhea, infectious conditions, constipation and dysentery [18].	Lebialem, South-West region of Cameroon; (4°10′N 9°14′E/4.167°N 9.233°E)	Not reported	Methanol crude extract was reported to possess moderate to significant anticancer activity (IC50: 29.60 to10.57 μg/mL) against drug-resistance cancer cell lines [16]
Combretum molle R. Br. ex G. Don (Combretaceae); 33311/ HNC	Fever, abdominal pains, convulsion, worm infections, human immunodeficiency virus (HIV)/acquiredimmune deficiency syndrome (AIDS) related infections [19]; hookworm, snake bite, leprosy, dysentery, general body swellings, arthritic and other inflammatory conditions, sterility, constipation [20, 21]; Parasitic, protozoan, infectious diseases [22], malaria [23]	University of Dschang, West region of Cameroon; (6°30′N 10°30′E/6.500°N 10.500°E)	Mollic acid glucoside [21]); β-D-glucopyranosyl 2α,3β,6β-trihydroxy-23-galloylolean-12-en-28-oate,combregenin, arjungenin, arjunglucoside I, and combreglucoside [24] .	Crude extracts were evaluated for: antibacterial activity [25–27]; antimycobacterial [28]; antifungal effects [29]; antimalarial [30]; anthelmintic activities [31]; anti-HIV by inhibition of ribonuclease-H [19]; Cytotoxic effects against T-24 bladder cancer cells [32]; Anti-inflammatory activity [24]; *in vitro* anticholinesterase and inhibitory effects on Rabbit Breathing [33]. Compounds: mollic acid glucoside (MAG) showed analgesic, anti-inflammatory properties in mice and rats [21], cardiovascular effect [34]; hypoglycaemic effect [35]; Punicalgin and CM-A, two isolated tannins were assessed for their anti-HIV activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) [36].

[a]Plants were identified at the Cameroon National Herbarium (HNC)

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after preliminary study; Table 2), were added and inoculation was done. The MIC was determined as described above. Modulation factors (MF), calculated as MIC $_\text{Antibiotic alone}$/MIC $_\text{Antibiotic alone + Extract}$; was used to express the modulating or synergy effects of the plant extracts.

Results

Phytochemical Screening of the plant extracts

The main classes of secondary metabolites for each extract were screened and the results are summarized in Table 3. Tannins, flavonoids, alkaloids and phenols were present in all tested extracts. Others classes of botanicals were selectively distributed in different plant extracts.

Antibacterial activity of the plant extracts

The results summarized in Table 4 show that all extracts were active on at least three of bacterial strains, with MIC values varying from 16 to 1024 µg/mL. Extracts from *Combretum molle* leaves (CML) and *Allanblackia gabonensis* displayed the most important spectrum of activity. Their inhibitory effects were observed on 72.41 % (27/29) for CML of the tested bacteria, 58.62 % (17/29) for leaves (AGL), 75.86 % (22/29) for flower (AGFl)

Table 2 MIC of antibiotics in combination with extracts at sub-inhibitory concentrations against *P. aeruginosa* PA124

Plant extracts [a]	Antibiotcs [b]								
		CHL	AMP	ERY	KAN	STR	TET	CIP	NFX
	ATB Alone	64	-	128	128	64	16	32	128
AGR	MIC/2	64(1)	- (NA)	128(1)	16**(2)**	32**(2)**	16(1)	32(1)	128(1)
	MIC/4	64(1)	- (NA)	128(1)	16(2)	64(1)	16(1)	32(1)	128(1)
	MIC/8	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
	MIC/16	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
AGB	MIC/2	64(1)	- (NA)	128(1)	128(1)	32**(2)**	8**(2)**	32(1)	128(1)
	MIC/4	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
	MIC/8	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
	MIC/16	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
AGL	MIC/2	64(1)	- (NA)	128(1)	16**(2)**	64(1)	16(1)	32(1)	128(1)
	MIC/4	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
	MIC/8	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
	MIC/16	64(1)	- (NA)	128(1)	128(1)	64(1)	16(1)	32(1)	128(1)
AGF	**MIC/2**	32**(2)**	- (NA)	128(1)	64**(2)**	32**(2)**	16(1)	32(1)	128(1)
	MIC/4	64(1)	- (NA)	128(1)	64**(2)**	32**(2)**	16(1)	32(1)	128(1)
	MIC/8	64(1)	- (NA)	128(1)	64**(2)**	64(1)	16(1)	32(1)	128(1)
	MIC/16	64(1)	- (NA)	128(1)	64**(2)**	64(1)	16(1)	32(1)	128(1)
AGFl	MIC/2	64(1)	- (NA)	128(1)	64**(2)**	64(1)	8**(2)**	32(1)	128(1)
	MIC/4	64(1)	- (NA)	128(1)	64**(2)**	64(1)	16(1)	32(1)	128(1)
	MIC/8	64(1)	- (NA)	128(1)	64**(2)**	64(1)	16(1)	32(1)	128(1)
	MIC/16	64(1)	- (NA)	128(1)	128	64(1)	16(1)	32(1)	128(1)
GQW	MIC/2	64(1)	- (NA)	128(1)	64**(2)**	32**(2)**	16(1)	32(1)	128(1)
	MIC/4	64(1)	- (NA)	128(1)	64**(2)**	64(1)	16(1)	32(1)	128(1)
	MIC/8	64(1)	- (NA)	128(1)	128	64(1)	16(1)	32(1)	128(1)
	MIC/16	64(1)	- (NA)	256(0.5)	128	64(1)	16(1)	32(1)	128(1)
CML	**MIC/2**	32**(2)**	- (NA)	64**(2)**	64**(2)**	32**(2)**	4**(4)**	16**(2)**	16**(2)**
	MIC/4	32**(2)**	- (NA)	64**(2)**	64**(2)**	32**(2)**	4**(4)**	16**(2)**	16**(2)**
	MIC/8	64(1)	- (NA)	128(–)	64**(2)**	64(1)	8**(2)**	32(1)	128(1)
	MIC/16	641)	- (NA)	128(–)	64**(2)**	64(1)	8**(2)**	32(1)	128(1)

[a]: **Plan extracts:** (AGFl : *Allanblackia gabonensis* Flowers, AGF : *Allanblackia gabonensis* Fruits, AGL: *Allanblackia gabonensis* Leaves, AGB: *Allanblackia gabonensis* Stem barks, AGR: *Allanblackia gabonensis* Root barks, GQW: *Gladiolus quartinianus* Whole plant, CML: *Combretum molle* Leaves); [b]: **Antibiotics (**TET: tetracycline, KAN: kanamycin, STR: streptomycin, ERY: erythromycin, CHL: chloramphenicol, NFX: norfloxacin, CIP: ciprofloxacin, AMP: ampicillin); – **:** MIC not detected at up to 256 µg/mL; (**):** Modulation factor or gain of activity; NA: Not applicable, Values in bold represent the modulation factor ≥ 2, the selected extracts and its concentrations to be used on others MDR bacteria

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Table 3 Qualitative phytochemical composition of the plant extracts

Plant samples	Phytochemical composition								
	Triterpenes	Flavonoids	Alkaloids	Anthraquinones	Phenols	Anthocyanines	Saponins	Tannins	Steroids
Allanblackia gabonensis	-	+	+	+	+	+	_	+	-
	-	+	+	-	+	+	-	+	-
	+	+	+	+	+	+	+	+	-
	+	+	+	+	+	+	+	+	-
	+	+	+	-	+	+	+	+	-
Gladiolus quartinianus	+	+	+	-	+	+	-	+	+
Combretum molle	-	+	+	-	+	-	+	+	+

(+): present; (–): absent

and bark (AGB), 79.31 % (23/29) for bark and 86.20 % (25/29) for fruits (AGF) extracts from *Allanblackia gabonensis*. AGF was the best extract with MIC values below 100 μg/mL on 38 % (11/29) of the tested bacteria. CML mostly showed moderate activity with MIC values ranging from 128–512 μg/mL. The activity of CHL was comparable to that of plant extracts in certain cases. This was the case with AGF, AGFl and AGR against *K. pneumoniae* Kp55 (64 μg/mL); AGF against *K. pneumoniae* Kp53 (64 μg/mL), and AGFl against *P. stuartii* PS2636 (32 μg/mL). MICs values equal or above 1024 μg/mL were obtained with the extract from *G. quartinianus* (GQW). MBCs values were generally equal or below 1024 μg/mL (Table 4).

Antibacterial activity of the extracts in the presence of an Efflux Pumps Inhibitors

In the present work, extracts were combined with PAβN; However, no significant increase of the activities of the tested plant extracts was generally observed. Only AGL showed 4 times decrease of MICs against *E. coli* AG102 and *E. cloacae* BM67. In contrast, PAβN significantly improved the activity of the reference drug, CHL (more than 16 times) on MDR bacteria used (Table 5).

Antibiotic resistance modifying activities of the plant extracts

Tables 2, 6 and 7 highlights the potentiating effects of the extracts on the activity of eight commonly used antibiotics. The most important modulating effects were observed of association CML with aminoglycosides (kanamycin and streptomycin), the potentiation effects varying from 77.78 to 88.89 % and from 66.67 to 77.78 % at MIC/2 and MIC/4 respectively; and with tetracycline (100 % and 77.78 % at MIC/2 and MIC/4 respectively) (Table 6). The modulating effects also ranged between 50 to 67 %, with the extract from *A. gabonensis* fruits (AGF) when combined at (MIC/2) with the some antibiotics. At MIC/4, AGF showed synergy less than 50 % on the tested bacteria with all antibiotics (Table 7). The most significant

increases of antibiotic activity in the presence of plant extracts were noted with the association of streptomycin and CML and AGF on *E. coli* AG100A$_{Tet,}$ with more than 128 fold and 64-fold decreases of MIC respectively. No increase of activity was noted with ampicillin, a β-lactamine when it was combined with plant extracts.

Discussion

Medicinal plants are potential source of antimicrobial agents used in the treatment of infectious diseases [43, 44]. According to Kuete *et al.* [45, 46], the antibacterial activity of a plant extract is considered significant when the MICs are below 100 μg/mL, moderate when $100 \leq MIC \leq 625$ μg/mL and weak when MIC are above 625 μg/mL. Consequently, the antibacterial activities of the tested extracts particularly those from *A. gabonensis* (AGF, AGR, AGB and AGFl) and *C. molle* (CML) were generally moderated (Table 4). Significant activities were recorded with AGF, AGR, AGB, and AGFl respectively on 37.93 %, 24.14 %, 20.70 % and 17.24 %. This highlights the good antibacterial potential of the tested extracts. The overall activity recorded with most of the studied extracts could be considered significant, especially those from *A. gabonensis* and *C. molle* [47]. When analyzing carefully the MIC and MBC results for the extract, it can be noted that MBC ≤ 4 were obtained with these samples on most of the tested bacterial species, suggesting their killing effects [48].

PAβN is a potent inhibitor of RND systems like AcrAB-TolC in Enterobacteriaceae or MexAB-OprM in *P. aeruginosa* used in the present work [49, 50]; The activity observed when chloramphenicol was tested in the presence of PAβN increased significantly, confirming that the tested bacteria are good models of efflux pumps-expressing bacteria.

Reversal of multi-drug resistance appears today as another attempt to mitigate the spread of resistance in bacteria. In recent years, many plants extracts and secondary metabolites have been evaluated as modulators of the antibiotic activity in efflux pumps in MDR

Table 4 Minimal inhibitory concentration (MIC) and minimal bactericidal (MBC) of the plant extracts and CHL on the studied bacterial species

Bacteria used	Tested samples, MIC and MBC (µg/mL)															
	Allanblackia gabonensis										*Gladiolus quartinianus* (GQW)		*Combretum molle* (CML)		Chloramphenicol	
	Flowers (AGFl)		Fruits (AGF)		Leave (AGL)		Stem barks (AGB)		Root barks (AGR)							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli																
ATCC8739	**64**	512	**16**	256	512	-	**64**	256	**64**	256	1024	-	256	1024	2	32
ATCC10536	**64**	512	**32**	256	512	1024	**32**	128	**64**	256	1024	-	512	512	1	32
AG100	-	-	1024		-	-	1024	-	512	-	-	-	512	1024	8	128
AG100A	128	512	**64**	256	512	512	128	512	128	512	-	-	512	1024	0.5	64
AG100A_Tet	1024	-	512	-	-	-	1024	-	1024	-	-	-	1024	-	64	256
AG102	1024	-	1024	-	-	-	1024	-	1024	-	-	-	1024	-	32	>256
MC4100	256	512	128	1024	512	-	256	512	512	-	1024	-	256	1024	32	>256
W3110	256	-	256	512	512	1024	**64**	512	256	1024	-	-	512	1024	8	128
E. aerogenes																
ATCC13048	128	1024	**16**	128	256	1024	**32**	512	**32**	512	1024	-	1024	-	8	64
CM64	-	-	1024	-	-	-	-		-		-	-	1024	-	>256	>256
EA27	512	1024	128	512	512	-	128	512	128	512	-	-	1024	1024	64	256
EA3	1024	-	-	-	1024	-	512	-	512	-	-	-	1024	-	128	>256
EA289	1024	-	1024	-	-	-	-	-	1024	-	-	-	1024	-	128	>256
EA298	1024	-	-	-	-		-	-	-	-	-	-	1024	-	64	>256
EA294	128	256	**64**	512	256	1024	**64**	512	128	1024	1024	-	128	512	16	64
E. cloacae																
ECCI69	-	-	1024	-	-	-	-	-	1024	-	-	-	-	-	>256	>256
BM47	-	-	1024	-	-	-	1024	-	-	-	-	-	-	-	>256	>256
BM67	-	-	-	-	-	-	-	-	-	-	-	-	1024	-	>256	>256
K. pneumoniae																
ATCC11296	128	1024	**16**	128	256	1024	**32**	256	**32**	256	1024	-	512	1024	4	32
KP55	**64**	256	**64**	256	1024	-	256	1024	**64**	512	-	-	512	1024	64	256
KP63	128	256	**64**	256	256	1024	128	512	128	256	-	-	512	-	64	256
K24	-	-	1024		-		1024	-	1024	-	-	-	1024	-	32	256
K2	128	1024	**32**	128	512	1024	128	256	**64**	256	-	-	256	1024	16	256

Table 4 Minimal inhibitory concentration (MIC) and minimal bactericidal (MBC) of the plant extracts and CHL on the studied bacterial species *(Continued)*

P. stuartii																
ATCC29916	**64**	512	**16**	128	256	512	128	512	**64**	256	-	-	512	512	8	128
NEA16	1024	-	1024	-	-		-	-	-	-	-	-	1024	-	64	256
PS2636	**32**	512	**64**	128	512	-	256	256	128	512	-	-	512	-	32	256
PS299645	128	1024	128	-	128	512	256	512	128	512	-	-	512	512	32	256
P. aeruginosa																
PA01	512	1024	512	512	512		512	1024	512	-	1024	-	512	1024	32	256
PA124	-	-	-		-		-		-	-	-	-	1024	-	64	>256

- - : MIC and MBC not detected at up to 1024 μg/mL, Values in bold represent significant antibacterial activity of the plant extract

Table 5 MIC of the samples in the absence and presence of PAβN on the selected MDR bacterial species

MDR Bacteria used	Samples and MIC (μg/mL)															
	Allanblackia gabonensis										*Gladiolus quartinianus* (GQW)		*Combretum molle* (CML)		chloramphenicol	
	Flowers (AGFl)		Fruits (AGF)		Leaves (AGL)		Stem bark (AGB)		Root barks (AGR)							
	Alone	+ PAβN	alone	+ PAβN	Alone	+ PAβN	alone	+ PAβN	Alone	+ PAβN	Alone	+ PAβN	Alone	+ PAβN	Alone	+ PAβN
E. coli																
AG100A$_{Tet}$	1024	1024	1024	512	-	-	1024	1024	1024	1024	-	-	1024	1024	**64**	**≤2**
AG102	1024	1024	1024	512	**-**	**512**	1024	1024	124	1024	-	-	1024	1024	**32**	**≤2**
E. aerogenes																
CM64	-	-	1024	1024	-	-	-	-	-	-	-	-	1024	1024	**256**	**16**
EA289	1024	1024	1024	1024	-	1024	-	-	1024	1024	-	-	1024	512	**128**	**8**
E. cloacae																
ECCl69	-	-	1024	1024	-	-	-	-	-	-	-	-	-	-	>256	128
BM67	-	1024	-	-	**-**	**512**	-	-	-	-	-	-	1024	1024	**>256**	**32**
K. pneumoniae																
K24	-	1024	1024	1024	-	-	1024	1024	1024	1024	-	-	1024	1024	**32**	**2**
P. stuartii											-					
NEA16	-	-	1024	1024	-	-	-	1024	-	1024	-	-	1024	1024	**64**	**8**
P. aeruginosa																
PA124	-	-	-	-	-	-	-	-	-	-	-	-	1024	1024	**64**	**8**

Table 6 Resistance modulating effect of the methanol leaves extract from *Combretum molle* at its sub-inhibitory concentrations on selected

Antibiotics[a]	Extract concentrations	MDR Bacteria used									% Modulating effect[b]
		E. coli			*E. aerogenes*			*K. pneumoniae*	*P. stuartii*	*P. aeruginosa*	
		AG100	AG102	AG100_{Tet}	CM64	EA289	EA298	K24	NEA16	PA124	
AMP	0	-	-	-	-	-	-	-	-	-	
	MIC/2	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	0
	MIC/4	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	0
CHL	0	8	32	64	256	128	64	64	64	64	
	MIC/2	8(1)	16(**2**)	32(**2**)	256(1)	64(**2**)	8(**8**)	64(1)	16(**4**)	32(**2**)	**66.65**
	MIC/4	8(1)	32(1)	64(1)	256(1)	64(**2**)	32(**2**)	64(1)	32(**2**)	32(**2**)	44.44
CIP	0	≤0.5	≤0.5	128	64	128	1	128	1	32	
	MIC/2	≤0.5(NA)	≤0.5	64(**2**)	64(1)	128(1)	1(1)	64(**2**)	1(1)	16(**2**)	33.33
	MIC/4	≤0.5(NA)	≤0.5	64(**2**)	64(1)	128(1)	1(1)	64(**2**)	1(1)	16(**2**)	33.33
NFX	0	≤0.5	≤0.5	256	-	128	8	-	4	128	
	MIC/2	≤0.5(NA)	≤0.5	128(**2**)	64(**>4**)	128(1)	8(1)	-(NA)	4(1)	64(**2**)	33.33
	MIC/4	≤0.5(NA)	≤0.5	256(1)	64(**>4**)	128(1)	8(1)	-(NA)	4(1)	64(**2**)	22.22
KAN	0	64	4	8	2	16	32	4	16	128	
	MIC/2	4(**16**)	4(1)	4(**2**)	≤0.5(**>4**)	2(**8**)	32(1)	2(**2**)	4(**4**)	64(**2**)	**77.78**
	MIC/4	8(**8**)	4(1)	4(**2**)	1(**2**)	4(**4**)	32(1)	2(**2**)	8(**2**)	64(**2**)	**77.78**
STR	0	>64	-	-	4	16	32	4	32	64	
	MIC/2	>64(NA)	256(**≥2**)	4(**>64**)	1(**4**)	4(**4**)	16(**2**)	1(**4**)	8(**4**)	32(**2**)	**88.89**
	MIC/4	>64(NA)	-	128(**>2**)	2(**2**)	4(**4**)	32(1)	2(**2**)	16(**2**)	32(**2**)	**66.67**
ERY	0	32	64	**-**	-	64	16	128	16	128	
	MIC/2	8(**4**)	64(1)	256(>1)	-(NA)	64(1)	4(**4**)	128(1)	4(**4**)	64(**2**)	44.44
	MIC/4	8(**4**)	64(1)	-(NA)	-(NA)	64(1)	16(1)	128(1)	8(**2**)	128(1)	22.22
TET	0	2	8	32	16	32	4	16	4	16	
	MIC/2	≤0.5(**>4**)	2(**4**)	8(**4**)	4(**4**)	8(**4**)	2(**2**)	8(**2**)	2(**2**)	4(**4**)	**100**
	MIC/4	1(**2**)	8(1)	32(1)	8(**2**)	8(**4**)	2(**2**)	8(**2**)	2(**2**)	4(**4**)	**77.78**

a: **Antibiotics** (TET: tetracycline, KAN: kanamycin, STR: streptomycin, ERY: erythromycin, CHL: chloramphenicol, NFX: norfloxacin, CIP: ciprofloxacin, AMP: ampicillin; **-** : MIC not detected at up to 256 μg/mL**; ()**: Modulation factor or gain of activity; **b :** Percentage of Antibiotic's modulation Activity by the Plant extracts; Indications in bold represent the modulation factor ≥ 2, antibiotics having a percentage of modulating effect greather than 50 %; NA: Not applicable

Table 7 Resistance modulating effect of the Fruits methanol extract from *Allanblackia gabonensis* at its sub-inhibitory concentrations on selected MDR bacteria

Antibiotics[a]	Extract concentrations	MDR Bacteria used									% Modulating effect[b]
		E. coli			*E. aerogenes*			*K. pneumoniae*	*P. stuartii*	*P. aeruginosa*	
		AG100	AG102	AG100A$_{Tet}$	CM64	EA289	EA298	K24	NEA16	PA124	
AMP	0	-	-	-	-	-	-	-	-	-	
	MIC/2	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	0
	MIC/4	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	-(NA)	0
CHL	0	8	32	64	256	128	64	64	64	64	
	MIC/2	8(1)	32(1)	64(1)	256(1)	128(1)	32(**2**)	32(**2**)	64(1)	32(**2**)	33.33
	MIC/4	8(1)	32(1)	64(1)	256(1)	128(1)	64(1)	64(1)	64(1)	64(1)	0
CIP	0	≤0.5	≤0.5	128	64	128	1	128	1	32	
	MIC/2	≤0.5(NA)	≤0.5(NA)	64(**2**)	32(**2**)	128(1)	2(0.5)	128(1)	1(1)	32(1)	33.33
	MIC/4	≤0.5(NA)	≤0.5(NA)	64(**2**)	64(1)	128(1)	2(0.5)	128(1)	1(1)	32(1)	11.11
NFX	0	≤0.5	≤0.5	256	-	128	8	-	4	128	
	MIC/2	1(≤0.5)	≤0.5(NA)	256(1)	128(**>2**)	128(1)	8(1)	-(NA)	4(1)	128(1)	11.11
	MIC/4	1(≤0.5)	≤0.5(NA)	256(1)	-(NA)	128(1)	8(1)	-(NA)	4(1)	128(1)	0
KAN	0	64	4	8	2	16	32	4	16	128	
	MIC/2	2(**32**)	4(1)	2(**4**)	≤0.5(**≥4**)	1(**16**)	32(1)	2(**2**)	16(1)	64(**2**)	**66.67**
	MIC/4	4(**16**)	4(1)	4(**2**)	2(1)	4(**4**)	32(1)	4(1)	16(1)	64(**2**)	44.44
STR	0	>64	-	-	4	16	32	4	32	64	
	MIC/2	>64(NA)	-(NA)	2(**>128**)	2(**2**)	2(**8**)	32(1)	4(1)	16(**2**)	32(**2**)	55.56
	MIC/4	>64(NA)	-(NA)	8(**>32**)	2(**2**)	4(**4**)	32(1)	4(1)	32(1)	32(**2**)	44.44
ERY	0	32	64	-	-	64	16	128	16	128	
	MIC/2	16(**2**)	64(1)	256(>1)	-(NA)	64(1)	16(1)	128(1)	16(1)	128(1)	11.11
	MIC/4	16(**2**)	64(1)	-(NA)	-(NA)	64(1)	16(1)	128(1)	16(1)	128(1)	0
TET	0	2	8	32	16	32	4	16	4	16	
	MIC/2	1(**2**)	4(**2**)	8(**4**)	4(**4**)	4(**8**)	4(1)	16(1)	2(**2**)	16(1)	**66.67**
	MIC/4	1(**2**)	8(1)	8(**4**)	4(**4**)	8(**4**)	4(1)	16(1)	4(1)	16(1)	44.44

[a]: **Antibiotics** (TET: tetracycline, KAN: kanamycin, STR: streptomycin, ERY: erythromycin, CHL: chloramphenicol, NFX: norfloxacin, CIP: ciprofloxacin, AMP: ampicillin; **-** : MIC not detected at up to 256 µg/mL**; ()**: Modulation factor or gain of activity; [b] **:** Percentage of antibiotic's modulation activity by the Plant extracts; Indications in bold represent the modulation factor ≥ 2 and antibiotics having a percentage of modulating effect greater than 50 %; NA: Not applicable

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bacteria [9–11, 37, 51–54]. Herein, we demonstrated that a beneficial effect of the combination of the extracts from the leaves of *C. molle* (CML) and fruits of *A. gabonensis* (AGF) with CHL, KAN and STR could be achieved. Synergistic or modulating effects of the plant extracts particularly *C. molle* extract with antibiotics were noted on more than 70 % of the tested MDR bacteria, suggesting that some of their constituents can act as efflux pump inhibitors [51]. These constituents might act by blocking the efflux pumps located in the cell membrane in the tested bacteria, preventing the extrusion of antibiotics in the cytoplasm and therefore restoring their activity as observed in this study [55, 56]. This is the first time to report the potential of the studied extracts, particularly those from the leaves of *C. molle* (CML) and the fruits of *A. gabonensis* (AGF) to reverse antibiotic resistance in MDR bacteria.

Conclusion

This study provides informative data on the antimicrobial potential of the tested plant extracts and suggests that extracts from *Allanblackia gabonensis* could be a source of natural antibacterial products whilst *Combretum molle* leaves extract could contain both antibacterial substances and antibiotic-modulation agents. These data indicate that these plants can be used to fight bacterial infections and especially those involving MDR phenotypes.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
AGF carried out the study; AGF and VK wrote the manuscript; JRK and VK supervised the work; VK designed the experiments, provided the bacterial strains and chemicals; all authors read and approved the final manuscript.

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