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Botanicals from *Aframomum letestuanum* Gagnep. (Zingiberaceae) can overcome the multidrug resistance of *Klebsiella* species overexpressing AcrAB-TolC efflux pumps

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Abstract

Background: Bacteria belonging to the genus *Klebsiella* have developed resistance mechanisms to clinically used antibiotics, leading to their loss of efficacy. In the present study, the *in vitro* antibacterial activity of methanol extracts from *Aframomum letestuanum*, as well as their modes of action against a panel of 14 bacterial species belonging to the genus *Klebsiella* including multidrug-resistant (MDR) phenotypes overexpressing efflux pumps were evaluated.

Methods: The broth microdilution method was used to assess the antibacterial activities of plant extracts (botanicals), while standard experimental protocols were used to study modes of action. Harborne's qualitative reference methods were used to identify the major groups of secondary metabolites present in the botanicals.

Results: Phytochemical screenings revealed that botanicals contain alkaloids, terpenoids, phenols, flavonoids, tannins, saponins, and anthocyanins. Botanicals from *A. letestuanum* seed pulp and seed inhibited the growth of all the bacteria tested, with MICs ranging from 16 to 512 µg/mL (pulp) and 64 to 2048 µg/mL (seed). The pulp extract had excellent activity, with MIC values of 16 µg/mL against K2, 32 µg/mL against Kp 80, and Kp 126, and 64 µg/mL against Kp55, Kp63, and ATCC11296. The seed extract displayed a MIC of 64 µg/mL against Kp58 and Kp2. *A. letestuanum* seed pulp extract inhibited bacterial growth in the exponential phase and induced inhibition of H^+-ATPase-dependent proton pumps in *K. pneumoniae* ATCC11296.

Conclusion: Botanicals from *A. letestuanum* are potential phytomedicines that deserve further investigation to develop novel drugs to overcome the multidrug resistance of *Klebsiella* species.

Keywords: *Aframomum letestuanum*; antibacterial; antibiotics; efflux pumps; multidrug resistance; *Klebsiella* species.

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Background

Infectious diseases are a major public health concern and are responsible for 17 million deaths worldwide each year. These infections represent approximately 30% of global mortality, with huge burdens, particularly in the developing countries of South Asia and sub-Saharan Africa. Among the bacteria responsible for human infections, *Klebsiella* species, more particularly *Kebsiella pneumoniae* and *Klebsiella oxytoca*, cause 7 to 14% of nosocomial pneumonias, 6 to 17% of urinary tract infections, and 3 to 20% of neonatal sepsis [1]. The mortality caused by bacterial infections has prompted the discovery of antibiotics that has contributed to improving human health. However, the success of antibacterial therapy is seriously hindered by the development of resistance of microorganisms to almost all classes of antibiotics. The resistance mechanisms developed by bacteria of the genus *Klebsiella* include reduction of cell membrane permeability, enzymatic inactivation of drugs, mutation of the cellular target, and active extrusion of drugs by efflux pump systems [2]. The World Health Organization (WHO) has recognized *Klebsiella* species among priority pathogens. The high rates of resistance to antibiotics used in the treatment of community-acquired and nosocomial infections due to the genus *Klebsiella* led to a reduction in the number of antibiotics that are still effective and usable in the care of patients [3]. The worrying rise in antibiotic resistance of *Klebsiella* species makes it necessary the search for novel antibacterial agents to effectively manage infections due to this bacterium. The role of medicinal plants as a good source of antibacterial agents has been demonstrated [4-14]. Several African medicinal plants, including dietary plants, have previously shown promising antibacterial activity against multidrug-resistant (MDR) bacteria [15-18]. In this work, we have targeted another African medicinal spice, *Aframomum letestuanum* Gagnep. (Zingiberaceae). The plant is traditionally used in Cameroon in cases of vomiting, nausea, skin infections, diarrhea, fever, toothache, inflammatory conditions, and stomach aches [19, 20]. This study was designed to evaluate the antibacterial activity and the effect of the association of botanicals from seed pulp and seed of this plant with antibiotics against MDR Klebsiella species. The mode of action of the botanical from the seed pulps was also determined.

Methods

Plant material and extraction

The seeds of *A. letestuanum* were collected in the city of Dschang in December 2022. The plant was identified by botanists from the National Herbarium of Cameroon (HNC) under the identification number 43134/HNC. The parts used were the seed pulp and seeds. The seeds and seed pulp of *A. letestuanum* were cleaned, dried away from the sun, and then ground. The powders obtained were macerated in 95% methanol in the proportions 1/3 (m/v) for 48 hours, the mixture was stirred 3 to 4 times each day to maximize the extraction yield. After maceration, the mixture was filtered using Whatman n°1, and the filtrate obtained was evaporated using a rotary evaporator (BÜCHI R-200) at 65°C (reduced pressure). The crude extracts were recovered in dry, sterile flasks and dried in an oven at 40°C for complete evaporation of the residual solvent. After drying, the extracts (botanicals) were stored at 4°C.

Chemicals and culture media

Chemicals used in the present study included the efflux pump inhibitor (EPI), the antibiotics, and bacterial growth revelator among others. The EPI, phenylalanine-arginine *β*-naphthylamide (PAβN) was used. para-Iodonitrotetrazolium chloride ≥ 97% (INT) was used as the bacterial growth indicator. Dimethyl sulfoxide (DMSO) served to solubilize plant extracts. Eight antibiotics, namely doxycycline (DOX), levofloxacin (LEV), imipenem (IMI), ciprofloxacin (CIP), ampicillin (AMP), ceftriaxone (CRO), tetracycline (TET), and vancomycin (VAN) were used. Mueller Hinton Agar (MHA) was used for the activation of bacterial strains and isolates; Mueller Hinton Broth (MHB) was used during microdilution as a nutrient medium for bacteria; Eosin methylene blue (EMB) was used as a specific and differential culture medium to confirm the purity of bacterial strains All chemicals and culture media were purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

Klebsiella species tested

The bacteria used in this work included fourteen (14) reference strains and clinical isolates of *Klebsiella* species amongst which twelve were *K. pneumoniae* and two were *K. oxytoca*. Their bacterial features are summarized in Table 1.

Determination of minimal inhibitory and bactericidal concentrations

The minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) of the studies botanicals and antibiotics alone, in the presence of PAβN (EPI) were determined using the broth microdilution method combined with the rapid INT colorimetric method as previously described in the similar experimental conditions [21-26]. Each experiment was repeated three times in triplicate.

Evaluation of the effect of the botanical from A. letestunuam seed pulp on the kinetics of bacterial growth

The kinetics of the growth *of K. pneumoniae* ATCC11296 in the presence of the botanical from the seed pulp of *A. letestunuam* was performed using spectrophotometric techniques, by measuring the optical densities (OD) as previously described [27-29] in the experimental conditions similar to those of Ngakam et al. [30] and Mapie Tiwa et al. [29].

Evaluation of the effect of the botanical from A. letestunuam seed pulp on the H^+-ATPases proton pumps

The effects of the botanical from *A. letestunuam* seed pulp were assessed on the H^+-ATPase-mediated proton pumps of *K. pneumoniae* ATCC11296, at 0.5×MIC, MIC, and 2×MIC as earlier described [31] in the similar experimental condition reported previously [29].

Determination of the antibiotic-potentiating effects of the crude extracts

The effects of the association of the botanicals at the sub-inhibitory concentrations of MIC/2 and MIC/4 with antibiotics (DOX, LEV, IMI, CIP, AMP, CRO, TET, and VAN) were determined against the MDR bacteria using the microdilution method combined with the rapid INT colorimetric method as previously described in the similar experimental conditions. Antibiotic-resistance modulating factor

(AMF) was calculated as the ratio of the MIC of the antibiotic alone versus MIC in combination with the plant extract. The potentiation effect was considered for AMF ≥ 2 [32].

Phytochemical screening of the botanicals

The phytochemical screening of the botanicals from the seeds and the seed pulp was done following the standard methods described for alkaloids, anthocyanins, flavonoids, phenols, saponins, tannins, and triterpenes [33, 34].

Interpretation of antibacterial data

According to Kuete [35], the following threshold values are applied to botanicals: significant activity (MIC <100 µg/mL), moderate (100 <MIC ≤ 625 µg/mL), and low or negligible (MIC> 625 µg/mL). According to Tamokou et al. [36], the cutoff points for the antibacterial activity of botanicals from edible plants are as follows: highly active (MIC below 100 µg/mL), significantly active (100 ≤ MIC ≤ 512 µg/mL), moderately active (512 < MIC ≤ 2048 µg/mL), low activity (MIC > 2048 µg/mL), and considered not active (MIC > 10 mg/mL). However, updated and rationally defined cutoff points of the antibacterial botanicals have been defined, considering the various bacterial species [37-40]. For Enterobacteria including Klebsiella species: outstanding activity (MIC ≤8 µg/mL), excellent activity (8 < MIC ≤64 µg/mL), very good activity (64 < MIC ≤128 µg/mL), good activity (128 < MIC ≤256 µg/mL), average activity (256 < MIC ≤512 µg/mL), weak activity (512 < MIC ≤1024 µg/mL), and not active (MIC values >1024 µg/mL) [37]. Bactericidal activities are considered when the ratios MBC/MIC are below or equal to 2; MBC/MIC ratios above 2 define the bacteriostatic activities [41-44]. The above appreciation criteria have been used to discuss the antibacterial activities reported in the present study.

Results

Antibacterial activity of the botanicals from A. letestunuam

The antibacterial activity of the crude extracts was determined by evaluating the MIC and MBC of each sample against fourteen (14) strains and clinical isolates of *K. pneumoniae* and *K. oxytoca* (Table 2). The bactericidal or bacteriostatic effect of the different extracts was determined by determining the MBC/MIC ratio. Table 2 summarizes all the results obtained. It appears that the botanicals displayed antibacterial activities, with MIC values of between 16 µg/mL and 2048 µg/mL. The extract from the seed pulps of *A. letestuanum* inhibited the growth of all 14 tested bacteria with a range of MICs ranging from 16 to 512 µg/mL. This extract had excellent antibacterial activity against *K. pneumoniae* K2 with a MIC of 16 µg/mL. Other excellent activities of this pulp extract were obtained against *K. pneumoniae* KP80 and KP126 with a MIC of 32 µg/mL, against *K. pneumoniae* KP55, KP63, and *K pneumoniae* ATCC11296 with a MIC of 64 µg/mL. Very good activities were recorded with a MIC of 128 µg/mL against the *K. pneumoniae* KP22, KP24, and KP42. Good activities were observed against the *K. pneumoniae* KP46, KP58, *K. oxytoca* KO107, and KO96 strains. An average activity with a MIC of 512 µg/mL was observed against the strain *K. pneumoniae* KP44. A bactericidal activity was recorded with the extract from the seed pulps against *K. pneumoniae* KP80, KP42, KP46, and *K. oxytoca* KO107. Bacteriostatic activities were observed against the rest of the strains. The extract from the seeds of *A. letestuanum* also had a bacterial spectrum of activity of 100% with MIC values ranging from 64 to 2048 µg/mL. The excellent activities of the extract were recorded with a MIC of 64 µg/mL against *K. pneumoniae* KP58 and K2. This extract displayed very good activities of 128 µg/mL against *K. pneumoniae* KP22 and ATCC11296. Good activities of the extract were observed against *K. pneumoniae* KP80, KP24, KP126, KP63, and *K. oxytoca* KO107 with a MIC of 256 µg/mL; average activities of the extract were observed against *K. pneumoniae* KP55 and *K. oxytoca* KO96 strains with a MIC of 512 µg/mL; the extract was not active on the rest of the bacteria (MIC values ranging from 1024 to 2048 µg/mL). A bactericidal activity was recorded with the extract from the seeds against *K. pneumoniae* KP80, KP55, PK24, KP126, and *K. oxytoca* KO107. Bacteriostatic activities were observed against the rest of the strains.

PAβN enhanced the activity of botanicals from A. letestuanum

The resistance of the tested bacteria through efflux pumps was evaluated by determining the MICs of botanicals in the presence or absence of an efflux pump inhibitor (PAβN) (Table 3). In the presence of PAβN, the activity of the extracts of the seed pulps and seeds of *A. letestuanum* increased by 2- to 128-fold on 100% (11/11) of the tested bacteria. The highest increase of 128-fold was obtained with the seed extract combined with EPI against *K. pneumoniae* KP42. The improvement of the antibacterial activities of the extracts in the presence of PAβN indicates that the constituents of the botanicals are substrates of the bacterial efflux pumps.

Effect of botanicals from A. letestuanum seed pulp on bacterial growth kinetics

To identify at which phase of bacterial growth *A. letestuanum* exerts its anti-klebsiella effects, the growth kinetics of *K. pneumoniae* ATCC11296 in the presence of the methanol extract of *A. letestuanum* seed pulp was performed. Figure 1 depicts the curves of the growth kinetics in the absence and in the presence of the botanical and the positive control, CIP at ½MIC, MIC, and 2MIC. It is observed that the growth curve of *K. pneumoniae* ATCC11296 in the absence of any treatment, as well as that in the presence of the extract at the concentration MIC/2 show all the phases of bacterial growth except for the last phase: a latency phase (0 h - 2 h), an exponential phase (2 h - 10 h) and a stationary phase (10 h - 20 h). In the presence of the methanol extract of the seed pulp of *A. letestuanum* at MIC and 2MIC, a decrease in the exponential phase (02 h - 8 h) and an increase in the stationary phase (8 - 20 h) are observed. It can also be observed that there is a decrease in the exponential phase (2 h - 4 h) and a prolongation of the stationary phase (4 h - 20 h) for the growth curve in the presence of CIP.

Botanical from A. letestuanum seed pulp inhibits the H+-ATPases proton pumps

To verify the ability of the extract from the seed pulp of *A. letestuanum* to alter the functioning of the H^+-ATPase proton pumps in *K. pneumoniae* ATCC11296, the pH of the medium contained the bacterium at different times was measured in the presence or absence of the botanical from the seed pulp of *A. letestuanum*. Figure 2 depicts the effects of this extract on the H^+-ATPases proton pumps of *K. pneumoniae* ATCC11296 at MIC/2, MIC, and 2MIC. It can be observed that the pH of the medium containing *K. pneumoniae* ATCC11296 in the presence of the extract decreased time-dependently. However, pH of the medium

containing *K. pneumoniae* in the absence and in the presence of the extract at MIC/2 has a more pronounced decrease and reaches the lowest pH values (from pH 6.4 to 4.4 in the absence of the extract and to 4.6 in the presence of the extract at the concentration of MIC/2). The pH of the medium containing *K. pneumoniae* ATCC11296 in the presence of the extract at MIC decreases to reach pH 4.9; while the medium containing the extract at 2MIC experiences a slight decrease in pH during the first 10 minutes before remaining almost constant throughout the experiment to reach a finally reach pH 5.4. This is an indication the botanicals inhibit the H^+-ATPase proton pumps in *K. pneumoniae* ATCC11296.

The crude extracts from the seeds and seed pulp potentiated the activity of antibiotics

Botanicals from the seeds and seed pulp at their MIC/2 and MIC/4 were combined with antibiotics and tested against the studied bacteria (Tables 4 and 5). It appears that the antibacterial activities of the antibiotics increased in the presence of the extracts of *A. letestuanum* at MIC/2 and MIC/4, with the AMF ranging from 2 to 128. At MIC/2 and MIC/4, the extract of the seeds of *A. letestuanum* potentiated the activity of DOX, CRO, and TET against at least 80% of the MDR bacterial strains tested (Table 5); this extract also potentiated the activity of LEV, IMI, and CIP against at least 70% of the bacterial strains tested; the extract of the seeds of *A. letestuanum* potentiated the activity of VAN on at least 50% of the bacterial strains tested; at MIC/2, the activity of AMP was potentiated by the extract against 20% of the tested bacterial strains. The pulp extract of *A. letestuanum*, at MIC/2 and MIC/4, potentiated the activity of DOX, CRO, LEV, IMI, and CIP against at least 80% of the tested bacteria (Table 4); the activity of TET and VAN were potentiated by the extract against at least 50% of the tested bacteria. At MIC/2, the effect of AMP was potentiated by the extract against 10% of the tested bacteria.

Phytochemical composition of the botanicals

The phytochemical composition of botanicals from *A. letestuanum* revealed the presence of alkaloids, flavonoids, triterpenes, saponins, phenols, and anthocyanins in both seeds pulp and seed extracts, while tannins were present only in the seed pulp extract.

Discussion

The search for naturally occurring phytomedicine against microbial infections, parasitic and viral infections, and malignant cells including the MDR phenotypes from the flora of Africa has been very successful in the last two decades [6, 13, 45-66]. To tackle the multidrug resistance of bacteria of the genus *Klebsiella*, the clinical isolates overexpressing active efflux pumps were selected. Resistance-Nodulation-Division (RND) efflux pumps are one of the most important determinants of multidrug resistance (MDR) in Gram-negative bacteria [67-69]. The EPI, PAβN has been shown to inhibit the RND pumps, namely AcrAB-TolC in Enterobacteria and MexAB-OprM in *Pseudomonas aeruginosa* [70-73]. In the present study, the activity of the reference antibiotic, IMI increased in the presence of PAβN against the tested strains of *Klebsiella* species (Table 3), clearly confirming that these bacteria actively express efflux pumps belonging to AcrAB-Tolc. According to the recent classification scale of the antibacterial activities of plant extracts proposed by Kuete [37], the extract of the seed pulp of *A. letestuanum* showed excellent activity against *K. pneumoniae* K2, KP80, KP126, ATCC 11296, KP55, and KP63 (Table 2). The extract from the seeds of *A. letestuanum* also displayed excellent activity against *K. pneumoniae* KP58 and K2. These results agree with many previous works, in particular, the work of Nguenang et al. [20] which showed significant antibacterial activity of the leaves of *A. letestuanum* on several bacterial species including *K. pneumoniae*; These data also corroborate those of Ekamgue et al. [74] and Tiotsop et al. [75] who had highlighted the good activities of *A. letestuanum* against *Staphylococcus aureus* and *P. aeruginosa* respectively. The work of Olajuyigbe et al. [76] indicated that plants of the genus *Aframomum* are known for their antibacterial activities, and this was attributed to the presence of terpenoids like aframodial [77]. Indeed, aframodial has been reported to actively inhibit the growth of *Salmonella enteriditis*, *Pseudomonas fragi*, P. fluorescens, *Proteus vulgaris*, *Staphylococcus aureus*, *Aspergillus flavus*, *Streptococcus pyogens, A. parasiticus, A. ochraceus* and *A. niger* in biological assays of *Aframomum* extracts [78]. It was also reported by Djeussi et al. [16] that plants of the genus *Aframomum* are active against *Enterobacter aerogenes* EA294. Phytochemical screening of the extracts of *A. letestuanum* seed pulps and seeds revealed the presence of groups and classes of secondary metabolites known for their antibacterial properties, including alkaloids, polyphenols, flavonoids, tannins, triterpenes, saponins, and anthocyanins. Indeed, the work of Marzoqi [79] has shown that polyphenols bind to polysaccharides and proteins and thus inhibit their actions resulting in the interruption of energy metabolism. The kinetics of bacterial growth is specific to each bacterium. The bacterial growth curve generally comprises 4 phases: the latent phase, the exponential phase, the stationary phase, and the decay phase. Each phase corresponds to biochemical processes necessary for the proper development of the bacterial cell. The inhibition of these biochemical processes leads to an elongation of these phases, thus inhibiting the growth of the said bacterium. In the present work, the shortening of the exponential phase and the prolongation of the stationary phase indicates the inhibitory action of the extract of the seed pulp of *A. letestuanum* at the MIC and 2MIC at these different phases. This indicates that it probably acts by reducing bacterial multiplication, thus making the stationary phase early. The prolongation of the latter could explain the inhibition of bacterial growth by the extract due to a limitation of nutrients, thus leading to the death of bacteria and the accumulation of metabolic waste [80, 81]. The pulp extract of *A. letestuanum* was also tested to highlight its possible effect on the functioning of the H^+-ATPase proton pumps of *K. pneumoniae* ATCC11296. The proton pump is a trans-membrane protein (active membrane transporter), moving protons against their concentration gradient thanks to the energy released by the hydrolysis of an adenosine triphosphate (ATP) molecule. ATPase proton pumps play different roles in bacterial cells. One of the most important roles is to regulate the pH of the cytoplasm of Gram-negative bacteria. Inhibition of these pumps causes a decrease in H^+-protons in the extracellular medium which becomes less acidic [82]. The increase in environmental pH in the presence of an antibacterial substance could lead to an inhibitory effect on the H^+-ATPase-dependent proton pump of the substance. This could compromise the survival of bacteria since the amount of energy produced will be very low for their metabolism, growth, and multiplication [83]. The methanol extract of the seed pulp of *A. letestuanum* tested induces the inhibition of bacterial growth by disruption of the H^+-ATPase-dependent proton pumps in *K. pneumoniae* ATCC11296, thus suggesting that these pumps constitute one of the targets of antibacterial action of the extract

from the seed pulps of *A. letestuanum*. In general, botanicals improved the activity of all antibiotics against at least one MDR bacterial strain. The extract of the seeds of *A. letestuanum* potentiated the activity of DOX, CEF, and TET against at least 80% of the tested MDR bacteria; this extract also potentiated the activity of the antibiotics LEV, IMI, and CIP against at least 70% of the bacteria tested. The seed pulp extract of *A. letestuanum* potentiated the activity of DOX, CRO, LEV, IMI, and CIP against at least 80% of the tested bacteria. The results corroborate those of Ekamgue et al. [74] and Tiotsop et al. [75] which showed that the plant *A. letestuanum* potentiated the activity of the antibiotics DOX, TET, AMP, LEV, IMI, and CRO against *Staphylococcus aureus* and *Pseudomonas aerogenes*, respectively. The potentiating effect observed in these extracts could be explained by the presence of flavonoids. In effect, Wiyogo et al. [84] showed that certain flavonoids associated separately with certain antibiotics such as AMP inhibit the activities of *K. pneumoniae* ESBL. Dey et al. [85] have demonstrated that polyphenols namely epigallocatechingallate can increase the activity of CIP and TET on strains of *K. pneumoniae* producing β-lactamase, mainly by interfering with the efflux mechanism antibiotic. Based on the work of Braga et al. [86], substances capable of potentiating the activity of antibiotics on more than 70% of a panel of MDR bacteria overexpressing efflux pumps are potential efflux pump inhibitors. Therefore, botanicals from the seed and seed pulp of *A. letestuanum* are potential sources of efflux pump inhibitors.

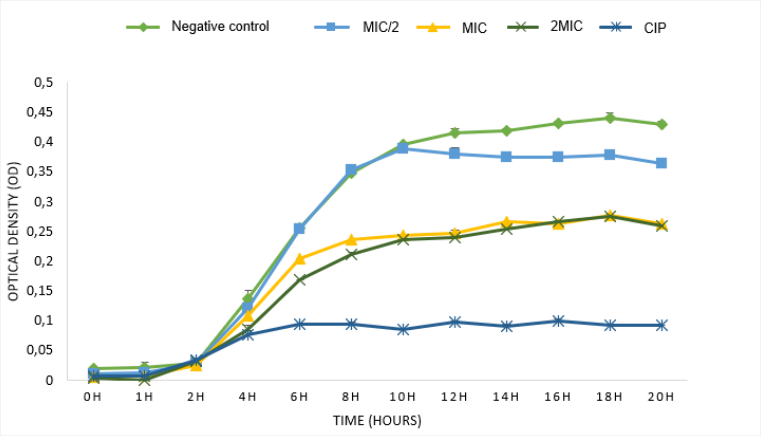

Figure 1. Effects of the methanol extract of *Aframomum letestuanum* seeds polyps on the growth kinetics of *K. pneumoniae* ATCC 11296

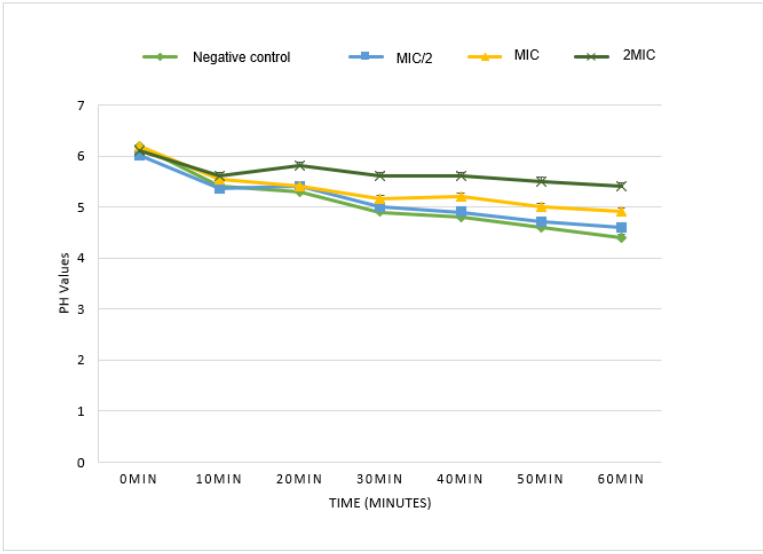

Figure 2. Effect of *A. letestuanum* pulp extract on ATP-ases-H^+ proton pumps of *K. pneumoniae* ATCC11296

Table 1. Characteristics of strains and isolates of *K. pneumoniae* and *K. oxytoca* used.

Bacterial species	**Bacterial strains or isolates**	Characteristics	**Reference**
Klebsiella pneumoniae	Kp 55	Clinical isolate: TET^r , AMP^r, ATM^r, CEF^r	[71, 87]
	Kp 63	Clinical isolate TET^r, Chl^r, AMP^r, ATM^r	[88, 89]
	ATCC11296	Reference strain	[71]
	Kp 22	Clinical isolate MDR, LEV, ATM^r, DOX^r , IMI^r , NIT^r	Laboratory Collection
	Kp 58	Clinical isolate MDR, MIN^r, AMP^r	Laboratory Collection
	K 2	AcrAB-TolC	Laboratory Collection, of UNR-MD1, University of Marseille, France [29]
	Kp 44	Clinical isolate MDR, ATM^r; DOX^r, MiN^r	Laboratory Collection
	Kp 126	Clinical isolate MDR, AMP^r	Laboratory Collection
	Kp 46	Clinical isolate TET^r AMP^r, ATM^r, CEF^r	Laboratory Collection
	Kp 80	Clinical isolate MDR, AMP^r	Laboratory Collection
	Kp 24	Clinical isolate TET^r, CHL^r, AMP^r, ATM^r	Laboratory Collection
	Kp 42	Clinical isolate MDR, MiN^r, AMP^r	Laboratory Collection
Klebsiella oxytoca	Ko 107	Clinical isolate MDR, ATM^r, DOX^r, MIN^r, CIP^r	Laboratory Collection
	Ko96	Clinical isolate MDR, ATM^r, MIN^r, AMP^r,GEN^r	Laboratory Collection

OFX^r, TET^r, AMP^r, ATM^r,CEF^r, CIP^r, IMI^r, CHL^r, MIN^r , DOX^r, GEN^r, NIT^r LEV^r resistance respectively to: Ofloxacin, tetracycline Ampicillin, Aztreonam, CEFixin ciprofloxacin, imipenem, chloramphenicol, minocycline doxycycline, gentamicin, nitrofurantoin, MDR : multidrug-resistant. Kp: *Klebsiella pneumoniae;* Ko: *Kebsiella oxytoca*

Table 2. Antibacterial activities of crude extracts of *Aframomum letestuanum*

Bacterial species	**Bacterial strains or isolates**	**Tested samples, MIC and MBC (MIC and MBC in µg/mL), and their ratio**								
		Seeds extract			**Seed pulp extract**			**ATB (Imipenem)**		
		MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Klebsiella pneumoniae	KP80	256	1024	4	32	128	4	4	4	1
	KP55	512	2048	4	64	1024	16	8	16	2
	KP24	256	1024	4	128	2048	16	4	16	4
	KP126	256	1024	4	32	1024	32	4	16	4
	KP58	64	-	nd	256	-	nd	4	4	1
	KP2	64	1024	16	16	1024	64	4	4	1
	KP22	128	1024	8	128	1024	8	4	8	2
	ATCC11296	128	2048	16	64	2048	32	4	8	2
	KP44	1024	-	nd	512	-	nd	8	8	1
	KP42	2048	-	nd	128	256	2	4	16	4
	KP46	2048	-	nd	256	512	2	4	4	1
	KP63	256	-	nd	64	-	nd	4	4	1
Klebsiella oxytoca	KO96	512	-	nd	256	-	nd	4	4	1
	KO107	256	512	2	256	512	2	4	4	1

R: MBC/MIC ratio; (-) : > 1024 or inactive; (nd): not determined; MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; IMI: imipenem; ATB: Antibiotic.

Table 3. Minimum inhibitory concentrations of the crude extracts in the presence of PAβN

Bacterial species	**Bacterial strains or isolates**	**Tested samples, MIC in the absence or presence of EPI (in µg/mL), and their ratio**								
		Seeds extract			**Seed pulp extract**			**ATB (Imipenem)**		
		MIC alone	MIC+PAβN	R	MIC alone	MIC+PAβN	R	MIC alone	MIC+PAβN	R
Klebsiella pneumoniae	KP55	512	256	2	64	<16	4	8	1/2	16
	KP24	256	<16	<6	128	<16	< 8	4	1	4
	KP126	256	256	1	32	<16	< 2	4	2	2
	KP58	64	32	2	256	64	< 4	4	1	4
	ATCC11296	128	32	4	64	<16	< 4	4	1	4
	KP46	2048	512	4	256	256	1	4	1/2	8
	KP63	256	<16	< 16	64	<16	< 16	4	<1/4	< 16
	KP44	1024	<16	< 64	512	<16	32	8	8	1
	KP42	2048	<16	< 128	128	<16	< 8	4	1/2	8
Klebsiella oxytoca	KO107	256	16	16	256	128	2	4	1	4
	KO96	512	<16	< 32	256	<16	< 16	4	1	4

R: MIC alone *vs* MIC with PAβN ratio; MIC alone: Minimum Inhibitory Concentration; MIC+PAβN: Minimum Inhibitory Concentration in the presence of PAβN; ATB: Antibiotic

Table 4. MICs (µg/mL) of antibiotics in the absence and presence of the seed pulp extract of *Aframomum letestuanum*.

ATB	Extract concentration	MIC of antibiotics in the presence of extract and Antibiotic-resistance modulating factor (AMF)										PSP (%)
		K. pneumoniae								*K. oxytoca*		
		KP24	ATCC11296	KP46	KP44	KP42	KP63	KP58	KP126	KO107	KO96	
DOX	0	1/8	4	8	1/2	1/2	8	1/4	1/4	1/2	8	
	MIC/2	<1/16(2)	1/16(64)	1(8)	<1/16(8)	1/16(8)	1/2(16)	1/8(2)	1/8(2)	<1/16(8)	1/8(64)	100%
	MIC/4	< 1/16(2)	1/8(32)	1(8)	<1/16(8)	1/16(8)	1/4(32)	1/16(4)	1/16(4)	<1/16(8)	1/4(32)	100%
LEV	0	1	1	4	1/4	4	8	1/2	1/2	1/4	1/2	
	MIC/2	< 1/16(16)	< 1/16(16)	1/16(64)	<1/16(4)	1/16(64)	8(1)	1/8(4)	1/8(4)	1/16(4)	1/4(2)	90%
	MIC/4	1/4(4)	1/8(8)	1/16(64)	<1/16(4)	1/16(64)	8(1)	1/8(4)	1/8(4)	1/8(2)	1/2(1)	80%%
IMI	0	4	4	4	8	4	4	4	4	4	4	
	MIC/2	1/16(64)	1/8(32)	<1/4(16)	<1/4(32)	<1/4(16)	4(1)	1/4(16)	1/4(16)	<1/2(8)	1/16(64)	90%
	MIC/4	1/8(64)	1/2(16)	<1/8(16)	<1/16(32)	1/4(4)	8(1)	1/4(8)	1/4(8)	<1/8(8)	1/8(64)	90%
CIP	0	1	1	2	1/8	4	4	1/2	1/2	1/4	2	
	MIC/2	< 1/16(16)	1/8(8)	1/2(4)	<1/16(2)	1/8(32)	8(1/2)	1/16(8)	1/16(8)	<1/16(4)	1/8(16)	90%
	MIC/4	< 1/16(16)	1/16(16)	1/2(4)	<1/16(2)	1/4(16)	4(1)	1/16(8)	1/16(8)	<1/16(4)	1/2(4)	90%
AMP	0	>64	>64	>64	>64	>64	>64	256	>64	>64	256	
	MIC/2	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	128(2)	>64(1)	>64(1)	256(1)	10%
	MIC/4	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	256(1)	>64(1)	>64(1)	256(1)	0%
CRO	0	16	8	8	16	8	32	16	64	8	16	
	MIC/2	<1/2(32)	1(8)	<1/2(16)	<1/2(32)	<1/2(16)	2(16)	<1/2(32)	4(16)	<1/2(16)	16(1)	90%
	MIC/4	<1/2(32)	1/2(16)	<1/2(16)	<1/2(32)	2(4)	4(8)	<1/2(32)	2(32)	<1/2(16)	4(4)	100%
TET	0	2	1	8	8	1	1	1	1	2	8	
	MIC/2	2(1)	1/8(8)	8(1)	8(1)	1/8(8)	1/4(4)	1/2(2)	1/8(8)	<1/16(32)	1/16**(128)**	70%
	MIC/4	2(1)	1/16(16)	8(1)	8(1)	1/8(8)	1/4(4)	1/2(2)	1/8(8)	1/2(4)	1/16**(128**)	70%
VAN	0	2	2	>64	1	>64	4	32	32	1	32	
	MIC/2	16(1/8)	16(1/8)	>64(1)	<1/2(2)	>64(1)	<1/2(8)	32(1)	1(32)	<1/2(2)	8(4)	50%
	MIC/4	16(1/8)	16(1/8)	>64(1)	<1/2(2)	>64(1)	2(2)	64(1/2)	1/2(64)	<1/2(2)	2(16)	50%

MIC: Minimum Inhibitory Concentration; (): AMF (Antibiotic-resistance modulating Factor); PSP (%): percentage of strain where potentiation effect was observed; ATB: Antibiotic; LEV: Levofloxacin; VAN: Vancomycin; CIP: Ciprofloxacin; TET: Tetracycline; DOX: Doxycycline; IMI: Imipenem; Ceftriaxone: CRO; AMP: Ampicillin.

Table 5. MICs (µg/mL) of antibiotics in the absence and presence of the seeds extract of *Aframomum letestuanum*.

ATB	Extract concentration	MIC of antibiotics in the presence of extract and Antibiotic-resistance modulating factor (AMF)										PSP (%)
		K. pneumoniae								*K. oxytoca*		
		KP24	ATCC11296	KP46	KP44	KP42	KP63	KP58	KP126	KO107	KO96	
DOX	0	1/8	4	8	1/2	1/2	8	1/4	4	1/2	8	
	MIC/2	<1/16(2)	1/16(64)	1/2(16)	<1/16(8)	<1/16(8)	4(2)	1/16(4)	1/8(32)	1/4(2)	1/16(128)	100%
	MIC/4	<1/16(2)	1/16(64)	1/2(16)	1/8(4)	1/8(4)	2(4)	1/16(4)	1/16(64)	1/4(2)	1/16(128)	100%
LEV	0	1	1	4	1/4	4	8	1/2	1	1/4	1/2	
	MIC/2	1/2(2)	1/16(16)	1/16(64)	<1/16(4)	1/16(64)	8(1)	1/16(8)	1/16(16)	1/4(1)	< 1/16(8)	80%
	MIC/4	1/2(2)	1/16(16)	1/16(64)	1/4(1)	1/16(64)	8(1)	1/16(8)	1/8(8)	1/4(1)	1/4(2)	70%
IMI	0	4	4	4	8	4	4	4	4	4	4	
	MIC/2	4(1)	1/16(64)	1/4(16)	<1/4(32)	1/2(8)	4(1)	1/4(16)	1(4)	2(2)	1/4(16)	80%
	MIC/4	8(1)	1/8(64)	1/8(16)	4(0,5)	1/8(8)	8(1)	1/8(16)	1/2(8)	1/2(2)	2(4)	70%
CIP	0	1	1	2	1/8	4	4	1/2	2	1/4	2	
	MIC/2	1/4(4)	1/16(16)	1 (2)	< 1/16(2)	1/8(32)	4(1)	1/16(8)	1/8(16)	1/4(1)	1/16(32)	80%
	MIC/4	1/4(4)	1/16(16)	2(1)	<1/16(2)	1/8(32)	4(1)	1/8(4)	1/16(32)	1/4(1)	1/16(32)	70%
AMP	0	>64	>64	>64	>64	>64	>64	256	>64	>64	256	
	MIC/2	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	128(2)	>64(1)	>64(1)	128(2)	20%
	MIC/4	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	>64(1)	256(1)	>64(1)	>64(1)	256(1)	0%
CRO	0	16	8	8	16	8	32	16	64	8	16	
	MIC/2	16(1)	8(1)	<1/2(16)	<1/2(32)	<1/2(16)	2(16)	<1/2(32)	4(16)	<1/2(16)	1/2(32)	80%
	MIC/4	16(1)	8(1)	<1/2(16)	8(2)	<1/2(16)	4(8)	1(16)	4(16)	<1/2(16)	8(2)	80%
TET	0	2	1	8	8	1	1	1	1	2	8	
	MIC/2	1(2)	1/16(16)	8(1)	8(1)	1/8(8)	1/4(4)	1/16(16)	1/16(16)	1(2)	1/16(128)	80%
	MIC/4	1(2)	1/16(16)	8(1)	8(1)	1/4(4)	1/2(2)	1/16(16)	1/16(16)	1(2)	1/8(64)	80%
VAN	0	2	2	>64	1	>64	4	32	32	1	32	
	MIC/2	2(1)	2(1)	>64(1)	<1/2(2)	>64(1)	2(2)	4(8)	2(16)	<1/2(2)	1/2(64)	60%
	MIC/4	8(1/4)	8(1/4)	>64(1)	<1/2(2)	>64(1)	2(2)	32(1)	2(16)	<1/2(2)	1/4(128)	50%

MIC: Minimum Inhibitory Concentration; (): AMF (Antibiotic-resistance modulating Factor); PSP (%): percentage of strain where potentiation effect was observed; ATB: Antibiotic; LEV: Levofloxacin; VAN: Vancomycin; CIP: Ciprofloxacin; TET: Tetracycline; DOX: Doxycycline; IMI: Imipenem; Ceftriaxone: CRO; AMP: Ampicillin.

Conclusion

In the present work, it was demonstrated that the botanicals from seeds and seed pulp of *A. letestuanum* are promising antibacterial agents. The methanol extract of the seed pulp exerted its antibacterial effect at the exponential phase probably through the alteration of the bacterial H^+-ATPase- proton pumps. The phytochemicals from the seeds and seed pulp are substrates for efflux pumps; The botanicals improved the activity DOX, CRO, LEV, IMI, TET, and CIP against at least 70% of the bacteria tested. Finally, the botanicals from the seeds and seed pulp of *A. letestuanum* are potential sources of effective antibacterial phytomedicine alone and in combination with antibiotics against MDR *Klebsiella* species.

Abbreviations

AMP: Ampicillin
ATCC: American-Type Culture Collection
ESBL: Broad Spectrum (or Extended) β-Lactamases
CIP: Ciprofloxacin
MBC: Minimal Bactericidal Concentration
MIC: Minimal Inhibitory Concentration
CRO: Ceftriaxone
DMSO: Dimethylsulfoxide
DOX: Doxycycline
AMF: Antibiotic-resistance modulating factor
HNC: National Herbarium of Cameroon
IMI: Imipenem,
INT: Para-iodonitrotetrazolium chloride
EPI: Efflux pump inhibitors
LEV: Levofloxacin
MDR: Multidrug-resistant
MHA: Mueller Hinton Agar

MHB: Mueller Hinton Broth
WHO: World Health Organization
RND: Resistance-Nodulation-Division
TET: Tetracycline
VAN: Vancomycin

Authors' Contribution

GKF, VYM, RN, SMT, JFM, and PN carried out the study; ATM and VK supervised the study; All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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References

1. Janda J, Abbott S. 2006. The Genera *Klebsiella* and *Raoultella*. *The Enterobacteria.* 2:115-129.
2. Duval M, Cossart P. 2019. *[Un nouveau mécanisme de résistance aux antibiotiques-Le recyclage des ribosomes]*. *Medecine/Sciences.* 35(8-9):613-615.
3. WHO. 2020. Antimicrobial resistance. *https://wwwwhoint/news-room/fact-sheets/detail/antimicrobial-resistance*. Accessed on December 12, 2022. .
4. Nguemeving JR, Azebaze AG, Kuete V, Eric Carly NN, Beng VP, Meyer M, Blond A, Bodo B, Nkengfack AE. 2006. Laurentixanthones A and B, antimicrobial xanthones from *Vismia laurentii*. *Phytochemistry.* 67(13):1341-1346.
5. Nono EC, Mkounga P, Kuete V, Marat K, Hultin PG, Nkengfack AE. 2010. Pycnanthulignenes A-D, antimicrobial cyclolignene derivatives from the roots of *Pycnanthus angolensis*. *J Nat Prod*, 73(2):213-216.
6. Kuete V, Sandjo LP. 2012. Isobavachalcone: an overview. *Chin J Integr Med.* 18(7):543-547.
7. Kuete V, Efferth T. 2010. Cameroonian medicinal plants: pharmacology and derived natural products. *Front Pharmacol.* 1:123.
8. Omosa LK, Midiwo JO, Mbaveng AT, Tankeo SB, Seukep JA, Voukeng IK, Dzotam JK, Isemeki J, Derese S, Omolle RA, Efferth T, Kuete V. 2016. Antibacterial activity and structure-activity relationships of a panel of 48 compounds from kenyan plants against multidrug resistant phenotypes. *SpringerPlus.* 5:901.
9. Tchinda CF, Voukeng KI, Beng VP, Kuete V. 2016. Antibacterial activities of the methanol extracts of *Albizia adianthifolia, Alchornea laxiflora, Laportea ovalifolia* and three other Cameroonian plants against multi-drug resistant Gram-negative bacteria *Saudi J Biol Sci.* 24:950-955.
10. Kuete V, Tangmouo JG, Penlap Beng V, Ngounou FN, Lontsi D. 2006. Antimicrobial activity of the methanolic extract from the stem bark of *Tridesmostemon omphalocarpoides* (Sapotaceae). *J Ethnopharmacol.* 104(1-2):5-11.
11. Ngameni B, Kuete V, Simo IK, Mbaveng AT, Awoussong PK, Patnam R, Roy R, Ngadjui BT. 2009. Antibacterial and antifungal activities of the crude extract and compounds from *Dorstenia turbinata* (Moraceae). *S Afr J Bot.* 75(2):256-261.
12. Kuete V, Wiench B, Hegazy ME, Mohamed TA, Fankam AG, Shahat AA, Efferth T. 2012. Antibacterial activity and cytotoxicity of selected Egyptian medicinal plants. *Planta Med.* 78(2):193-199.
13. Komguem J, Meli AL, Manfouo RN, Lontsi D, Ngounou FN, Kuete V, Kamdem HW, Tane P, Ngadjui BT, Sondengam BL *et al.* 2005. Xanthones from Garcinia smeathmannii (Oliver) and their antimicrobial activity. *Phytochemistry.* 66(14):1713-1717.
14. Kuete V, Wansi JD, Mbaveng AT, Kana Sop MM, Tadjong AT, Beng VP, Etoa FX, Wandji J, Meyer JJM, Lall N. 2008. Antimicrobial activity of the methanolic extract and compounds from *Teclea afzelii* (Rutaceae). *S Afr J Bot.* 74(4):572-576.
15. Voukeng IK, Beng VP, Kuete V. 2016. Antibacterial activity of six medicinal Cameroonian plants against Gram-positive and Gram-negative multidrug resistant phenotypes. *BMC Complement Altern Med.* 16(1):388.
16. Djeussi DE, Noumedem JA, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkuete AH, Kuete V. 2013. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complement Altern Med.* 13(1):164.
17. Dzotam JK, Touani FK, Kuete V. 2016. Antibacterial activities of the methanol extracts of *Canarium schweinfurthii* and four other Cameroonian dietary plants against multi-drug resistant Gram-negative bacteria. *Saudi J Biol Sci.* 23:565-570.
18. Manekeng HT, Mbaveng AT, Nguenang GS, Seukep JA, Wamba BEN, Nayim P, Yinkfu NR, Fankam AG, Kuete V. 2018.Anti-staphylococcal and antibiotic-potentiating activities of seven Cameroonian edible plants against resistant phenotypes. *Invest Med Chem Pharmacol.* 1:7.
19. Kuete V. 2023. Chapter Twelve - Ethnopharmacology, phytochemistry and pharmacology of potent antibacterial medicinal plants from Africa. *Advances in Botanical Research* 107:353-660. https://doi.org/10.1016/bs.abr.2022.08.022.
20. Nguenang GS, Mbaveng AT, Fankam AG, Manekeng HT, Nayim P, Wamba BEN, Kuete V. 2018. *Tristemma hirtum* and five other Cameroonian edible plants with weak or no antibacterial effects modulate the activities of antibiotics against gram-negative multidrug-resistant phenotypes. *ScientificWorldJournal.* 2018:7651482.
21. Fankam AG, Kuiate JR, Kuete V. 2015. 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. *BMC Complement Altern Med.* 15:206.
22. Kuete V, Betrandteponno R, Mbaveng AT, Tapondjou LA, Meyer JJ, Barboni L, Lall N. 2012. Antibacterial activities of the extracts, fractions and compounds from *Dioscorea bulbifera*. *BMC Complement Altern Med.* 12:228.
23. Mbaveng AT, Sandjo LP, Tankeo SB, Ndifor AR, Pantaleon A, Nagdjui BT, Kuete V. 2015. Antibacterial activity of nineteen selected natural products against multi-drug resistant Gram-negative phenotypes. *Springerplus.* 4:823.
24. Noumedem JA, Mihasan M, Kuiate JR, Stefan M, Cojocaru D, Dzoyem JP, Kuete V. 2013. *In vitro* antibacterial and antibiotic-potentiation activities of four edible plants against multidrug-resistant Gram-negative species. *BMC Complement Altern Med.* 13:190.
25. Touani FK, Seukep AJ, Djeussi DE, Fankam AG, Noumedem JA, Kuete V. 2014. Antibiotic-potentiation activities of four Cameroonian dietary plants against multidrug-resistant Gram-negative bacteria expressing efflux pumps. *BMC Complement Altern Med.* 14:258.
26. Seukep JA, Fankam AG, Djeussi DE, Voukeng IK, Tankeo SB, Noumdem JA, Kuete AH, Kuete V. 2013. Antibacterial activities of the methanol extracts of seven Cameroonian dietary plants against bacteria expressing MDR phenotypes. *Springerplus.* 2:363.
27. Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J Appl Microbiol.* 88(1):170-175.
28. Guefack M-GF, Messina NDM, Mbaveng AT, Nayim P, Kuete JRN, Matieta VY, Chi GF, Ngadjui BT, Kuete V. 2022. Antibacterial and antibiotic-potentiation activities of the hydro-ethanolic extract and protoberberine alkaloids from the stem bark of *Enantia chlorantha* against multidrug-resistant bacteria expressing active efflux pumps. *J Ethnopharmacol.* 296:115518.
29. Mapie Tiwa S, Matieta VY, Ngakam R, Kengne Fonkou G, Megaptche JF, Nayim P, Mbaveng AT, Kuete V. 2024. Antibacterial potential and modes of action of methanol extracts of *Elephantopus mollis* Kunth (Asteraceae) against multidrug-resistant Gram-negative bacteria overexpressing efflux pumps. *Invest Med Chem Pharm.* 7(1):86.
30. Ngakam R, Matieta VY, Kengne Fonkou G, Mapie Tiwa S, Megaptche JF, Nayim P, Mbaveng AT, Kuete V. 2024. Antibacterial potential and modes of action of methanol extracts of flowers and leaves of *Vernonia glabra* (Steetz) Vatke (Asteraceae) against multidrug-resistant Gram-negative bacteria overexpressing efflux pumps. *Invest Med Chem Pharmacol.* 7(1):87.
31. Ekamgue B, Mbaveng AT, Seukep AJ, Matieta VY, Kuete JRN, Megaptche JF, Guefack MGF, Nayim P, Kuete V. 2023. Exploring *Mangifera indica* (Anacardiaceae) leaf and bark methanol extracts as potential adjuvant therapy in the management of multidrug-resistant *Staphylococcus aureus*. *Invest Med Chem Pharm.* 6(2):84.
32. Kovač J, Gavarić N, Bucar F, Smole Možina S. 2014. Antimicrobial and resistance modulatory activity of Alpinia katsumadai seed phenolic extract, essential oil and post-distillation extract. *Food Technol Biotechnol.* 52(2):248-254.
33. Harbone J. 1973. Phytochemical methods: a guide to modern techniques of plant analysis. London: Chapman & Hall.
34. Kuete V. 2013. Medicinal Plant Research in Africa In: *Pharmacology and Chemistry*. Edited by Kuete V, 1 edn. Oxford: Elsevier.
35. Kuete V. 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Med.* 76(14):1479-1491.
36. Tamokou JDD, Mbaveng AT, Kuete V. 2017. Chapter 8 - Antimicrobial Activities of African Medicinal Spices and Vegetables. In: *Medicinal Spices and Vegetables from Africa.* edn.: Academic Press: 207-237.
37. Kuete V. 2023. Chapter Six - Potential of African medicinal plants against Enterobacteria: Classification of plants antibacterial agents. *Advances in Botanical Research* 106:151-335. https://doi.org/10.1016/bs.abr.2022.1008.1006.
38. Tankeo SB, Kuete V. 2023. Chapter Seven - African plants acting on Pseudomonas aeruginosa: Cut-off points for the antipseudomonal agents from plants. *Advances in Botanical Research* 106: 337-412. https://doi.org/10.1016/bs.abr.2022.08.007.
39. Tchinda CF, Kuete V. 2023. Chapter Nine - Potential of African flora to combat tuberculosis and drug resistance of Mycobacteria: Rationale classification of antimycobacterial agents from a natural source. *Advances in Botanical Research* 106: 523-598. https://doi.org/10.1016/bs.abr.2022.08.009.

40. Wamba BEN, Mbaveng AT, Kuete V. 2023. Chapter Eight - Fighting Gram-positive bacteria with African medicinal plants: Cut-off values for the classification of the activity of natural products. *Advances in Botanical Research* 106: 413-522. https://doi.org/10.1016/bs.abr.2022.08.008.
41. Mims C, Playfair J, Roitt I, Wakelin D, Williams R. 1993. Antimicrobials and chemotherapy. *In: Mims CA, et al Eds, Med Microbiol Rev.* 35:1-34.
42. Nayim P, Mbaveng AT, Wamba BEN, Fankam AG, Dzotam JK, Kuete V. 2018. Antibacterial and antibiotic-potentiating activities of thirteen Cameroonian edible plants against gram-negative resistant phenotypes. *ScientificWorldJournal.* 2018:4020294.
43. Tchana ME, Fankam AG, Mbaveng AT, Nkwengoua ET, Seukep JA, Tchouani FK, Nyassé B, Kuete V. 2014. Activities of selected medicinal plants against multi-drug resistant Gram-negative bacteria in Cameroon. *Afr Health Sci.* 14(1):167-172.
44. Mbaveng AT, Kuete V, Nguemeving JR, Beng VP, Nkengfack AE, Marion Meyer JJ, Lall N, Krohn K. 2008. Antimicrobial activity of the extracts and compounds from *Vismia guineensis* (Guttiferae). *Asian J Trad Med.* 3:211-223.
45. Kuete V, Sandjo LP, Djeussi DE, Zeino M, Kwamou GM, Ngadjui B, Efferth T. 2014. Cytotoxic flavonoids and isoflavonoids from *Erythrina sigmoidea* towards multi-factorial drug resistant cancer cells. *Invest New Drugs.* 32:1053–1062.
46. Tekwu EM, Askun T, Kuete V, Nkengfack AE, Nyasse B, Etoa FX, Beng VP. 2012. Antibacterial activity of selected Cameroonian dietary spices ethno-medically used against strains of *Mycobacterium tuberculosis*. *J Ethnopharmacol.* 142(2):374-382.
47. Kuete V, Ngameni B, Mbaveng AT, Ngadjui B, Meyer JJ, Lall N. 2010. Evaluation of flavonoids from *Dorstenia barteri* for their antimycobacterial, antigonorrheal and anti-reverse transcriptase activities. *Acta Trop.* 116(1):100-104.
48. Kuete V, Tangmouo JG, Marion Meyer JJ, Lall N. 2009. Diospyrone, crassiflorone and plumbagin: three antimycobacterial and antigonorrhoeal naphthoquinones from two *Diospyros* spp. *Int J Antimicrob Ag.* 34(4):322-325.
49. Dzoyem JP, Nkuete AH, Kuete V, Tala MF, Wabo HK, Guru SK, Rajput VS, Sharma A, Tane P, Khan IA *et al.* 2012. Cytotoxicity and antimicrobial activity of the methanol extract and compounds from *Polygonum limbatum*. *Planta Med.* 78(8):787-792.
50. Kuete V, Ngameni B, Wiench B, Krusche B, Horwedel C, Ngadjui BT, Efferth T. 2011. Cytotoxicity and mode of action of four naturally occuring flavonoids from the genus *Dorstenia*: gancaonin Q, 4-hydroxylonchocarpin, 6-prenylapigenin, and 6,8-diprenyleriodictyol. *Planta Med.* 77(18):1984-1989.
51. Mbaveng AT, Kuete V, Efferth T. 2017. Potential of Central, Eastern and Western Africa medicinal plants for cancer therapy: spotlight on resistant cells and molecular targets. *Front Pharmacol.* 8:343.
52. Kuete V, Efferth T. 2015. African flora has the potential to fight multidrug resistance of cancer. *BioMed Res int.* 2015:914813.
53. Kuete V, Nkuete AHL, Mbaveng AT, Wiench B, Wabo HK, Tane P, Efferth T. 2014. Cytotoxicity and modes of action of 4′-hydroxy-2′,6′-dimethoxychalcone and other flavonoids toward drug-sensitive and multidrug-resistant cancer cell lines. *Phytomedicine.* 21(12):1651-1657.
54. Kuete V, Wiench B, Alsaid MS, Alyahya MA, Fankam AG, Shahat AA, Efferth T. 2013. Cytotoxicity, mode of action and antibacterial activities of selected Saudi Arabian medicinal plants. *BMC Complement Altern Med.* 13:354.
55. Mbaveng AT, Bitchagno GTM, Kuete V, Tane P, Efferth T. 2019. Cytotoxicity of ungeremine towards multi-factorial drug resistant cancer cells and induction of apoptosis, ferroptosis, necroptosis and autophagy. *Phytomedicine.* 60:152832.
56. Mbaveng AT, Zhao Q, Kuete V. 2014. 20 - Harmful and protective effects of phenolic compounds from african medicinal plants. In: *Toxicological Survey of African Medicinal Plants.* edn. Edited by Kuete V: Elsevier: 577-609.
57. Mbaveng AT, Fotso GW, Ngnintedo D, Kuete V, Ngadjui BT, Keumedjio F, Andrae-Marobela K, Efferth T. 2018. Cytotoxicity of epunctanone and four other phytochemicals isolated from the medicinal plants *Garcinia epunctata* and *Ptycholobium contortum* towards multi-factorial drug resistant cancer cells. *Phytomedicine.* 48:112-119.
58. Kuete V, Dzotam JK, Voukeng IK, Fankam AG, Efferth T. 2016. Cytotoxicity of methanol extracts of *Annona muricata, Passiflora edulis* and nine other Cameroonian medicinal plants towards multi-factorial drug-resistant cancer cell lines. *Springerplus.* 5(1):1666.
59. Kuete V, Mbaveng AT, Sandjo LP, Zeino M, Efferth T. 2017. Cytotoxicity and mode of action of a naturally occurring naphthoquinone, 2-acetyl-7-methoxynaphtho[2,3-b]furan-4,9-quinone towards multi-factorial drug-resistant cancer cells. *Phytomedicine.* 33:62-68.
60. Kuete V, Sandjo LP, Kwamou GM, Wiench B, Nkengfack AE, Efferth T. 2014. Activity of three cytotoxic isoflavonoids from Erythrina excelsa and *Erythrina senegalensis* (neobavaisoflavone, sigmoidin H and isoneorautenol) toward multi-factorial drug resistant cancer cells. *Phytomedicine.* 21(5):682-688.
61. Kuete V. 2014. 21 - Health Effects of Alkaloids from African Medicinal Plants. In: *Toxicological Survey of African Medicinal Plants.* edn. Edited by Kuete V: Elsevier: 611-633.
62. Kuete V, Sandjo LP, Mbaveng AT, Seukep JA, Ngadjui BT, Efferth T. 2015. Cytotoxicity of selected Cameroonian medicinal plants and *Nauclea pobeguinii* towards multi-factorial drug-resistant cancer cells. *BMC Complement Altern Med.* 15:309.
63. Poumale HMP, Hamm R, Zang Y, Shiono Y, Kuete V. 2013. 8 - Coumarins and Related Compounds from the Medicinal Plants of Africa. In: *Medicinal Plant Research in Africa.* edn. Edited by Kuete V. Oxford: Elsevier: 261-300.
64. Ngounou FN, Manfouo RN, Tapondjou LA, Lontsi D, Kuete V, Penlap V, Etoa FX, Dubois MAL, Sondengam BL. 2005. Antimicrobial diterpenoid alkaloids from *Erythrophleum suaveolens* (guill. & perr.) brenan. *Bull Chem Soc Ethiop.* 19(2):221-226.
65. Omosa LK, Midiwo JO, Kuete V. 2017. Chapter 19 - Curcuma longa. In: *Medicinal Spices and Vegetables from Africa.* edn. Edited by Kuete V: Academic Press: 425-435.
66. Dzoyem JP, Tchuenguem RT, Kuiate JR, Teke GN, Kechia FA, Kuete V. 2014. *In vitro* and *in vivo* antifungal activities of selected Cameroonian dietary spices. *BMC Complement Altern Med.* 14:58.
67. Fernando DM, Kumar A. 2013. Resistance-Nodulation-Division Multidrug Efflux Pumps in Gram-Negative Bacteria: Role in Virulence. *Antibiotics (Basel).* 2(1):163-181.
68. Pradel E, Pages JM. 2002. The AcrAB-TolC efflux pump contributes to multidrug resistance in the nosocomial pathogen *Enterobacter aerogenes*. *Antimicrob Agents Chemother.* 46(8):2640-2643.
69. Chollet R, Chevalier J, Bryskier A, Pagès J-M- 2004. The AcrAB-TolC pump is involved in macrolide resistance but not in telithromycin efflux in *Enterobacter aerogenes* and *Escherichia coli*. *Antimicrob Agents Chemother.* 48(9):3621-3624.
70. Lamers RP, Cavallari JF, Burrows LL. 2013. The efflux inhibitor phenylalanine-arginine beta-naphthylamide (PAβN) permeabilizes the outer membrane of gram-negative bacteria. *PLoS One.* 8(3):e60666.
71. Kuete V, Alibert-Franco S, Eyong KO, Ngameni B, Folefoc GN, Nguemeving JR, Tangmouo JG, Fotso GW, Komguem J, Ouahouo BMW *et al*. 2011. Antibacterial activity of some natural products against bacteria expressing a multidrug-resistant phenotype. *Int J Antimicrob Ag.* 37(2):156-161.
72. Ambadiang MMM, Atontsa BCK, Tankeo SB, Nayim P, Wamba BEN, Bitchagno GTM, Mpetga JDS, Penlap VB, Kuete V. 2020. Bark extract of *Cassia sieberiana* DC. (Caesalpiniaceae) displayed good antibacterial activity against MDR gram-negative phenotypes in the presence of phenylalanine-arginine *β*-naphthylamide. *BMC Complement Med Ther.* 20(1):342.
73. Lorenzi V, Muselli A, Bernardini AF, Berti L, Pages JM, Amaral L, Bolla JM. 2009. Geraniol restores antibiotic activities against multidrug-resistant isolates from Gram-negative species. *Antimicrob Agents Chemother.* 53(5):2209-2211.
74. Ekamgue B, Mbaveng AT, Kuete V. 2023. Anti-staphylococcal and antibiotic-potentiating activities of botanicals from nine Cameroonian food plants towards multidrug-resistant phenotypes. *Invest Med Chem Pharmacol.* 6(1):75.
75. Tiotsop RS, Kuete V, Mbaveng AT. 2023. Botanicals from ten selected Cameroonian edible plants displayed Anti-pseudomonas effects and potentiated the activities of antibiotics against multidrug-resistant phenotypes over-expressing MexAB-OprM efflux pumps. *Invest Med Chem Pharmacol.* 6(1):74.
76. Olajuyigbe OO, Adedayo O, Coopoosamy RM. 2020. Antibacterial activity of defatted and nondefatted methanolic extracts of *Aframomum melegueta* K. Schum. against multidrug-resistant bacteria of clinical importance. *ScientificWorldJournal.* 2020:4808432.
77. Ayafor JF, Tchuendem MH, Nyasse AB, Tillequin F, Anke H. 1994. Aframodial and other bioactive diterpenoids from *Aframomum* species. *Pure Appl Chem.* 66:2327-2330.
78. Adegoke GO, Skura BJ. 1994. Nutritional profile and antimicrobial spectrum of the spice *Aframomum danielli* K. Schum. *Plant Foods Hum Nutr.* 45(2):175-182.
79. Sharara DT, Al-Marzoqi AH, Hussein HJ. 2021. *In vitro* antibacterial efficacy of the secondary metabolites extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from hemodialysis fluid. *Ann Romanian Soc Cell Biol.* 25(6):9267-9274.
80. Prescott LM, Willey JM, Sherwood L, Woolverton CJ. 2018. [Microbiologie]. *De Boeck Supérieur 4th edition*.
81. Delhalle L, Daube G, Adolphe Y, Crevecoeur S, Clinquart A. 2012. *[Les modèles de croissance en microbiologie prévisionnelle pour la maîtrise de la sécurité des aliments (synthèse bibliographique)]. Biotechnol Agron Soc Environ.* 16:369-381.
82. Kobayashi H. 1985. A proton-translocating ATPase regulates pH of the bacterial cytoplasm. *J Biol Chem.* 260(1):72-76.
83. Youmbi LM, Atontsa BCK, Tankeo SB, Wamba NEB, Nayim P, Nganou KB, Bitchagno GTM, Simo KI, Mpetga JDS, Penlap VB. 2020. Antibacterial potential and mechanism of action of botanicals and phytochemicals from *Stachytarpheta cayennensis* (Verbenaceae) against Gram-negative multidrug-resistant phenotypes expressing efflux pumps. *Invest Med Chem Pharmacol.* 3(1):35.
84. Wiyogo IO, Endraswari PD, Setiawati Y. 2021. Antibacterial activity of ethanol extract of Kemuning (*Murraya Paniculata*) against Klebsiella pneumoniae ESBL by in vitro test. *Indones J Trop Infect Dis.* 9(2):101-106.
85. Dey D, Ghosh S, Ray R, Hazra B. 2016. Polyphenolic secondary metabolites synergize the activity of commercial antibiotics against clinical isolates of β-lactamase-producing *Klebsiella pneumoniae*. *Phytother Res.* 30(2):272-282.
86. Braga LC, Leite AA, Xavier KG, Takahashi JA, Bemquerer MP, Chartone-Souza E, Nascimento AM. 2005. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Can J Microbiol.* 51(7):541-547.
87. Dzotam JK, Kuete V. 2017. Antibacterial and antibiotic-modifying activity of methanol extracts from six cameroonian food plants against multidrug-resistant enteric bacteria. *BioMed Res Int.* 17:1583510.
88. Dzotam JK, Simo IK, Bitchagno G, Celik I, Sandjo LP, Tane P, Kuete V. 2018. *In vitro* antibacterial and antibiotic modifying activity of crude extract, fractions and 3′,4′,7-trihydroxyflavone from *Myristica fragrans* Houtt against MDR Gram-negative enteric bacteria. *BMC Complement Altern Med.* 18(1):15.
89. Seukep JA, Sandjo LP, Ngadjui BT, Kuete V. 2016. Antibacterial and antibiotic-resistance modifying activity of the extracts and compounds from *Nauclea pobeguinii* against Gram-negative multi-drug resistant phenotypes. *BMC Complement Altern Med.* 16:193.