Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Botanicals from the flowers of *Vernonia calvoana* and the leaves of *Senna spectabilis* showed anti-Klebsiella activity and potentiated the activity of antibiotics against multidrug-resistant phenotypes overexpressing efflux pumps

Derick J. Assonfack, Eric Cadet, Larissa Mpude, Ancela W. B. Yendze, Valaire Y. Matieta, Jenifer R. N. Kuete, Junior F. Megaptche, Idrios N. Bonsou, Michael F. Kengne, Armelle T. Mbaveng*, and Victor Kuete**

Abstract

Background: The alarming trend of infectious diseases in public health is mainly due to bacterial multidrug resistance, the emergence of which is partly caused by the inappropriate use of antibiotics. Bacteria of the *Klebsiella* genus have acquired a priority level of resistance to antibiotics, reducing their effectiveness. The aim of this study was to evaluate the anti-*Klebsiella* activity of methanol extracts of *Vernonia calvoana* flowers and *Senna spectabilis* leaves against multidrug-resistant *Klebsiella* phenotypes overexpressing efflux pumps.

Methods: The antibacterial activity of *Vernonia calvoana* and *Senna spectabilis* extracts alone, in combination with Phenylalanine-Arginine β-Naphthylamide (PAβN) and antibiotics, was assessed using the liquid medium microdilution method. Qualitative phytochemical composition was determined according to reference experimental protocols.

Results: Phytochemical screening of the different methanol extract revealed the presence of alkaloids, triterpenes, polyphenols, flavonoids and saponins. These extracts had inhibition spectra of 93.75%, with MICs ranging from 32 to 1024 µg/mL for *Vernonia calvoana* flower extract and from 16 to 1024 µg/mL for *Senna spectabilis* leaves. The excellent activity with a MIC value of 32 µg/mL against *K. oxytoca* isolate (KO95) and 64 µg/mL against *K. pneumonia* isolate (KP203) was observed in *Vernonia calvoana* flower extract. However, *Senna spectabilis* leaf extract showed an excellent activity, with MIC values of 16 µg/mL against *K. pneumoniae* (KP175) and 64 µg/mL against *K. pneumoniae* (K2) and *K. oxytoca* (KO107) strains. The anti-*Klebsiella* activity of *Vernonia calvaona* flower extract and *Senna spectabilis* leaf extract was improved by 87.5% and 100% respectively in the presence of PAβN, with activity improvement factor (AIF) values ranging from 2 to 256. Both extracts modulated the activity of antibiotics with activity modulator factor (AMF) values ranging from 2 to 128. The activity of ceftriaxone and tetracycline was enhanced to at least 75% at MIC/2 and MIC/4, imipenem and cefixime were modulated to 50% at MIC/2 and MIC/4 respectively, and levofloxacin to 62.5% at MIC/2.

Conclusion: Extracts from *Vernonia calvaona* flowers and *Senna spectabilis* leaves can be used alone or in combination with the usual antibiotics against *Klebsiella* phenotypes overexpressing efflux pumps, but further investigation is needed to identify the active compounds responsible for the observed activity.

Keywords: antibacterial; antibiotics; efflux pumps; multidrug resistance; *Klebsiella* species; *Senna spectabilis; Vernonia calvaona*

*Correspondence: *Tel.: +237 676542386; E-mail: armbatsa@yahoo.fr; ORCID: https://orcid.org/0000-0003-4178-4967 (Armelle T. Mbaveng); ** Tel.: +237 677355927; E-mail: kuetevictor@yahoo.fr; ORCID: http://orcid.org/0000-0002-1070-1236 (Victor Kuete)*

Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon

Other authors E-mails:
derickassonfack7@gmail.com (Derick J. Assonfack); ericcadet19@gmail.com (Eric Cadet); larissampude5@yahoo.com (Larissa Mpude); whitneyancela13@gmail.com (Ancela W. B. Yendze); yvmatieta@yahoo.com (Valaire Y. Matieta); jeniferkuete@gmail.com (Jenifer R. N. Kuete); bonichrist89@yahoo.com (Idrios N. Bonsou); megapfabrice@gmail.com (Junior F. Megaptche); fmkengne@yahoo.com (Michael F. Kengne)

Citation on this article: Assonfack DJ, Cadet E, Mpude L, Yendze AWB, Matieta VY, Kuete JRN, Megaptche JF, Bonsou IN, Kengne MF, Mbaveng AT, Kuete V. Botanicals from the flowers of Vernonia calvoana and the leaves of Senna spectabilis showed anti-Klebsiella activity and potentiated the activity of antibiotics against multidrug-resistant phenotypes overexpressing efflux pumps. Investigational Medicinal Chemistry and Pharmacology (2024) 7(3):101; Doi: https://dx.doi.org/10.31183/imcp.2024.00101.

Background

Infectious diseases are the greatest major threat causing a high number of deaths in public health across the world [1]. Those caused by drug-resistance are approximately responsible for 700,000 deaths each year [2]. It is estimated that antimicrobial resistance will cause about 10 million deaths by 2050 [3], this is because microbial infections usually fail to respond to treatment. In fact, the inappropriate use of antibiotics to control the spread of these infections, particularly those caused by bacteria, leads to the emergence of Multidrug-Resistance (MDR) phenotypes. Most of these phenotypes also include those of the genus *Klebsiella* mainly *Klebsiella pneumoniae* and *Klebsiella oxytoca* [4, 5]. The anti-biotherapy mostly used to fight this MDR has lost its effectiveness due to the resistant mechanisms developed by these bacteria towards antibiotics. According to the World Health Organization, these bacteria are classified as priority pathogens resistant to third-generation cephalosporins and carbapenems known as the best antibiotics to fight against MDR phenotypes [6]. One of the resistant mechanisms expressed by the *Klebsiella* species is the RND (Resistance Nodulation Division) efflux pumps family [7], including AcrAB-TolC which is the major and the most clinically important efflux pump in Gram-negative bacteria [8]. The expression of these pumps towards antibiotics leads to therapeutic failures [9]. Faced with these problems, there is an urgent need for alternatives to fight this multidrug resistance. Plant-derived bioactive compounds have displayed their potential through a direct antibacterial effect on pathogenic bacteria or by restoring the activity of usual antibiotics in combination [10-11-12]. Some previous investigations highlighted the direct or potentiated effect of these substances from food and medicinal plants from the African flora against the MDR phenotype [13-14]. Also, plant-derived drugs obtained from medicinal plants underwent preclinical tests and have been licensed in a particular country through clinical trials [15]. For instance, “Tokoro Combination” and “Akebia Formula” derived from *Dioscorea tokoro* and *Akebia sp.* respectively are being used as a drug from plants to fight against bacteria involved in urinary tract infections [16]. *Vernonia calvoana*, commonly called sweet bitter leaf, belongs to the family of Asteraceae. It is widely consumed as a vegetable in African countries and used in folk medicine to treat physiological disorders such as diabetes, measles, tuberculosis, and hyperlipidemia [17, 18]. This plant has been stated to be involved in the treatment of ovarian cancer [19]. Its hepatoprotective effects, hypo-lipidemic, and anti-diabetic activities have been demonstrated [20]. In addition, the antibacterial activity of methanol extract from the leaves of *Vernonia calvoana* against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* phenotypes was highlighted [21]. *Senna spectabilis* (DC.) Irwins and Barneby is a plant belonging to the Fabaceae family, widely used in Africa, Asia, Australia, and South and Latina America. Apart from growing as an ornamental plant in tropical and subtropical areas, *S. spectabilis* is one of the medicinal plants whose leaves are used in Cameroon by traditional healers by infusion to treat various diseases such as epilepsy, constipation, insomnia, malaria, dysenteries, headaches and anxiety [22, 23, 24]. This plant also possesses antimicrobial activity [25]. In effect, *Senna spectabilis* leaves were found to be active against *Salmonela typhi, Shigella flexineriae,* and *Shigella Dysenteriae* using a disc diffusion method [26]. In addition, the work of Arantes et al. [27] demonstrated that the extract of the leaves of this plant is effective against *Staphylococcus aureus* ATCC 6538 and *Streptococcus pyogenes* ATCC 19615 strains. In our continuous search for new natural products to fight against bacterial drug resistance, the present work was designed to evaluate the antibacterial activity of extract from *Vernonia calvaona* flowers and *Senna spectabilis* leaves and the effect of their association with usual antibiotics against clinical MDR strains and isolate of the *Klebsiella* species.

Methods

Plant material and extraction

The flowers of *Vernonia calvoana* and the leaves of *Senna spectabilis* were harvested in Dschang (West Region) and Mutengene (Sud West Region of Cameroon) respectively and were later identified at the National Herbarium of Cameroon (HNC) by Mr. TCHATCHOUANG NGANDOF Eric, comparing them with the reference samples preserved under code 42381/ HNC for *Vernonia calvoana* and 45740/ HNC for *Senna spectabilis.* The harvested plant parts were dried away from the sun. They were then crushed, and the powder obtained was soaked in methanol (in a 1:3 w/v) for 48 hours at room temperature, with shaking to enhance the extraction. The powder-solvent mixture was then filtered using Wattman No.1 paper. The resulting filtrates were concentrated using a BÜCHI R-200 rotary evaporator at 65°C and then dried at 45°C until the residual solvent completely evaporated. The resulting crude extract or botanicals were collected and stored in dark, sterile bottles at 4°C for future use.

Chemicals and culture media

The chemicals used include the bacterial growth indicator, *para*-Iodonitrotetrazolium chloride ≥97% (INT). Eight antibiotics amongst which some of them belong to the class of β-lactams: ampicillin (AMP), penicillin (PEN); carbapenem: imipenem (IMI); cephalosporins: cefixime (CFX), and ceftriaxone (CTX); fluoroquinolones: ciprofloxacin (CIP) and levofloxacin (LEV), and the tetracycline (TET) were used. The activation of the bacteria was done using the Mueller Hinton Agar (MHA) and the micro-dilution was done using the Mueller Hinton Broth (MHB) as a nutrient medium for bacteria. The purity of bacteria was confirmed using Eosin methylene blue (EMB) as a differential and specific culture medium. The Phenylalanine-Arginine β-Naphthylamide (PAβN) at 0.2% was used as efflux pump inhibitor (EPI). All chemicals were purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

Tested bacteria

The *Klebsiella* species tested include ten (10) reference strains and clinical isolates of *Klebsiella pneumoniae* and six (06) of *Klebsiella oxytoca* as reported in Table 1. Their features were earlier reported [28, 29, 30, 31].

Determination of minimal inhibitory and bactericidal concentrations

The determinations of the Minimal Inhibitory Concentrations (MIC) and the Minimal Bactericidal Concentrations (MBC) on the used bacteria strains and isolates were performed using a 96-well broth micro-dilution method combined with the rapid colorimetric INT test [32, 33]. Both plant extracts and the reference drug (imipenem) were respectively prepared at 8192 µg/mL and 512 µg/mL after being dissolved in DMSO-MHB. The bacterial inoculum used was prepared at 1.5×10^6 CFU/mL and the incubation conditions were

37°C for 18 hours. DMSO was used as the control solvent at a concentration less than 2.5%. MIC was defined as the lowest concentration of botanical exhibiting complete inhibition of bacterial growth after 18 to 24 hours of incubation, meanwhile, MBC was defined as the lowest concentration of a sample that did not induce a color change by adding INT after the following additional 48 hours of incubation [34-35]. Botanicals were also tested in the presence of PAβN which is an efflux pump inhibitor prepared at 100 µg/mL to evaluate the role of efflux pumps on the resistance of the bacteria to the samples [28]. Imipenem was used as a positive antibacterial control, meanwhile DMSO 2.5%+MHB and DMSO 2.5%+bacterial inoculum were respectively used as neutral and negative controls. Each experiment was repeated three times in triplicate.

Evaluation of the effect of efflux pumps on the antibacterial activity of the samples

The different extracts and the antibiotic (imipenem) were tested in the presence of PAβN as previously described [28]. The potentiation level of sample activity in the presence of PAβN was determined using the $MIC_{sample\ alone}/MIC_{sample\text{-}PA\beta N}$ combination ratio known as the activity improvement factors (AIFs). The bacteria tested included *K. pneumoniae* (K2, KP55, K24, KP175, and KP93), and *K. oxytoca* (KO249, KO096, and KO095). Each assay was repeated thrice.

Determination of the antibiotic-potentiating effects of the botanicals

The effect of the association of the botanicals with antibiotics was determined against *K. pneumoniae* K2, KP55, K24, KP175, KP93, and *K. oxytoca* KO249, KO96, and KO95. Previously, a preliminary assay was performed by evaluating the combination of the plant extracts at different sub-inhibitory concentrations (MIC/2, MIC/4, MIC/8, and MIC/16) with antibiotics on KP93, which then allowed the selection of the appropriate sub-inhibitory concentrations of MIC/2 and MIC/4 for further combination testing (Data not shown). Activity Modulation 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. A preliminary assay was also performed by evaluating a combination of the plant extracts at different sub-inhibitory concentrations (MIC/2, MIC/4, MIC/8, and MIC/16) with antibiotics on KP55 (Data not shown). Activity Modulation Factor (AMF) was then calculated as the ratio of the MIC of the antibiotic alone versus MIC in combination with the botanicals. The potentiation effect was considered for AMF ≥ 2 [36].

Phytochemical screening of botanicals

Phytochemical screening was done following the standard methods described for alkaloids, anthocyanins, flavonoids (Shinoda test), phenols, saponins, and triterpenes (Liebermann-Burchard test) [37, 38].

Interpretation of antibacterial data

Updated and rationally defined cutoff points of the antibacterial botanicals for Enterobacteria including *Klebsiella* species have been defined as follow: 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) [39]. This appreciation criterion was used to discuss the antibacterial activities of the studied samples. The bacteriostatic effect was considered when the ratio of the MBC/MIC was above 4 and the bactericidal effect when the MBC/MIC ratio was below or equal to 4 [40, 41].

Results

Antibacterial activity

The anti-*Klebsiella* activity of botanicals whose results are summarized in Table 3, was evaluated by determining the MICs and MBCs against three strains and thirteen isolates of *K. pneumoniae* and *K. oxytoca.* These results shows that the methanol extract from the flowers of *V. calvaona* had MICs ranging from 32 to 1024µg/mL, and from 16 to 1024µg/mL for the leaves of *S. spectabilis*, against *Klebsiella* strains and isolates. Both extracts displayed inhibitory activity spectra of 93.75% (15/16) against the strains and isolates studied in the present work. However, extract from the flowers of *V. calvaona* showed excellent anti-*Klebsiella* activity, with a MIC value of 32 µg/mL against *K. oxytoca* isolate (KO95) and a MIC value of 64 µg/mL against *K. pneumoniae* isolate (KP203), very good anti-*Klebsiella* activity with a MIC value of 128 µg/mL against *K. pneumoniae* (K2, KP55, K24, KP77 and KP126) and *K. oxytoca* (KO26) strains and isolates. We also noticed average anti-*Klebsiella* activity with a MIC value of 512 µg/mL against isolates of *K. pneumoniae* (KP81) and *K. oxytoca* (KO96, KO107, and KO55), and weak anti-*Klebsiella* activity with a MIC value of 1024 µg/mL against *K. pneumoniae* (ATCC11296 and KP175) and *K. oxytoca* (KO249).

In the case of *Senna spectabilis* leaf extract, we noticed excellent anti-*Klebsiella* activity with a MIC value of 16 µg/mL against *K. pneumoniae* (KP175) and 64 µg/mL against *K. pneumoniae* (K2) and *K. oxytoca* (KO107) strains and isolates, and very good anti-*Klebsiella* activity with a MIC value of 128 µg/mL against *K. pneumoniae* isolate (KP77). Furthermore, this extract presented good anti-*Klebsiella* activity with a MIC value of 256 µg/mL against *K. pneumoniae* (ATCC11296 and KP24) and *K. oxytoca* (KO249) strains and isolates. In addition, we perceived average anti-*Klebsiella* activity with a MIC value of 512 µg/mL against isolates of *K. pneumoniae* (KP55, KP203 and KP126) and *K. oxytoca* (KO26 and KO55) and a weak anti-*Klebsiella* activity with a MIC value of 1024 µg/mL against *K. pneumoniae* (KP81) and *K. oxytoca* (KO96 and K95) strains and isolates. However, extracts from *V. calvaona* flowers and *S. spectabilis* leaves respectively showed bacteriostatic activity against *K. pneumoniae* (KP126 and KP175), and bactericidal activity against the rest of the strains and isolates tested.

PAβN increased the activity of both VCF and SSL.

The expression of efflux pumps pumps as well as their implication on the anti-*Klebsiella* activity of botanicals was demonstrated by using an efflux pump inhibitor (PAβN), with results conferred in Table 4. Extract from the flowers of *V. calvaona* was improved at 87.5% (7/8) against the strains and isolates tested, with activity improvement factors (AIF) ranging from 4 to 256 in the presence of PAβN. Similarly, *S. spectabilis* leaf extract was 100% enhanced against the various strains and isolates tested, with activity

improvement factors (AIF) ranging from 2 to 128 in the presence of the efflux pump inhibitor (EPI).

Antibiotic-activity modulation effects of VCF and SSL

The ability of botanicals to modulate the activity of antibiotics in association was assessed by determining their activity modulator factors (AMF) at sub-inhibitory concentrations. The results are summarized in Table 5 and Table 6. At MIC/2 and MIC/4, extract from the flowers of *V. calvaona* enhanced the activity of antibiotics, with activity modulator factors (AMF) ranging from 2 to 128 against strains and isolates tested. This potentiation was 100% at MIC/2 in the presence of ceftriaxone (CTX) and 75% at MIC/4 against various strains and isolates tested. Also, the activity of antibiotics ciprofloxacin (CIP), levofloxacin (LEV) at MIC/2, imipenem (IMI), and penicillin (PEN) at MIC/2 was improved at 87.5%, 87.5%, 62.5%, and 50% respectively against the strains and isolates tested. Moreover, the activity of tetracycline (TET) was enhanced at 62.5% at MIC/2 and at 50% at MIC/4 which was the same as levofloxacin (LEV) against *Klebsiella* phenotypes. Additionally, ampicillin (AMP) and cefixime (CFX) activity was enhanced at 50% at MIC/2 and 37.5% at MIC/4 against the strains and isolates tested. Similarly, at MIC/2 and MIC/4, *S. spectabilis* leave extract modulated the activity of antibiotics, with activity modulator factors (AMF) ranging from 2 to 128 for the strains and isolates tested. This potentiation was 75% at MIC/2 and MIC/4 in the presence of ceftriaxone (CTX) and tetracycline (TET) at MIC/2 against the tested bacteria. In addition, the activity of levofloxacin (LEV) and cefixime (CFX) was improved by 62.5% at MIC/2 and by 50% at MIC/4 against *Klebsiella* phenotypes. Imipenem (IMI) was improved by 50% at MIC/2 and by 37.5% at MIC/4. Furthermore, the activity of antibiotics ciprofloxacin (CIP), penicillin (PEN) at MIC/2, and ampicillin (AMP) were potentiated respectively by 25%, 25%, and 12.5% against the tested bacteria.

Phytochemical composition of the botanicals

Botanicals from the flowers of *V. calvaona* and the leaves *of S. spectabilis* both revealed the presence of triterpenes, polyphenols, flavonoids, alkaloids, and saponins.

Discussion

Infectious diseases are amongst the most frequently reported physiological disorders that increase mortality rates in developing countries. Globally, the perspectives of control and treatment of these diseases are slowly shifting from conventional drugs to drugs based on natural substances derived from plants, due to the rapid accessibility and predilection for organic products. This is particularly observed in sub-Saharan Africa, where the World Health Organization estimates that nearly 80% of the population uses plant-derived products as their primary care needs [42]. Indeed, these natural plant-derived compounds have shown promising results in combating the resistance of pathogenic bacteria, including those of the genus *Klebsiella* [28, 31, 43-54]. It is along the same line that the present work was carried out to determine the anti-*Klebsiella* activity and potentiating effect of extracts from *V. calvaona* flowers and *S. spectabilis* leaves against multidrug-resistant *Klebsiella* phenotypes overexpressing efflux pumps.

To assess the activity of both extracts, we used the classification method developed by Kuete [39] for Enterobacteriaceae. According to this classification, botanicals used in this study presented inhibitory activity spectra of 93.75%, with MICs ranging from 16 to 1024 µg/mL. Extract from the flowers of *V. calvaona* displayed excellent anti-*Klebsiella* activity with a MIC value of 32 µg/mL against *K. oxytoca* isolate (KO95) and a MIC value of 64 µg/mL against *K. pneumoniae* isolate (KP203), and a very good anti-*Klebsiella* activity with a MIC value of 128 µg/mL against *K. pneumoniae* strains and isolates tested (K2, KP55, K24, KP77, and KP126) and *K. oxytoca* (KO26). Similarly, extract from the leaves of *S. spectabilis* showed excellent activity with a MIC value of 16 µg/mL against *K. pneumoniae* (KP175) and 64 µg/mL against *K. pneumoniae* (K2) and *K. oxytoca* (KO107) strains and isolates, and very good anti-*Klebsiella* activity with a MIC value of 128 µg/mL against *K. pneumoniae* isolate (KP77). Previous investigations carried out in Nigeria reported the high antimicrobial potency of the leaf extract of *V. calvaona* against a range of pathogens, including Gram-negative resistant strains [21] as obtained in the present study. The good anti-*Klebsiella* activities obtained in the present work concerning the leaf extract of *S. spectabilis*, confirmed the report of certain investigators, who highlighted that the ethanol, ethyl acetate, and hexane extracts of *S. spectabilis* leaves had good antibacterial activity with a MIC value of 250 µg/mL against Gram-positive bacteria [27]. However, the weak activity obtained in the study carried out in Kenya by Mugweru et al. [26] using the aqueous extract of *S. spectabilis* leaves, with a diameter of inhibition of 9.2-15.8 mm against resistant Gram-negative bacteria, compared to the activity of the present work, could be due to the type of extract used. Recent investigations reported methanol extract to possess high antibacterial activity compared to aqueous extract [44]. In general, both extracts displayed MBC/MIC ratios lower than or equal to 4, clearly indicating their bactericidal effect [41].

MDR phenotypes overexpressing efflux pumps can inhibit the direct effect of antibacterial agents, leading them to express low levels of activity. To bypass this phenomenon, the activity of botanicals can be improved by associating them with an efflux pump inhibitor (EPI). PAβN is an EPI that has been shown to inhibit the RND efflux system, particularly the tripartite AcrAB-TolC efflux pump [45-46]. In the present study, the resistance of the different bacteria used was elucidated using PAβN. The activity of botanicals was improved with activity improvement factors ranging from 2 to 256 in the presence of PAβN against *Klebsiella* strains and isolates tested. These results are similar to those reported in the previous work of certain investigators [31, 47]. The significant increase in activity of the various extracts in the presence of PAβN could justify the fact that phytochemicals found in these extracts are preferential substrates for efflux pumps. Furthermore, it was demonstrated that phytochemicals whose activity is being increased in the presence of PAβN are efflux pump substrates [28-29].

The effectiveness of antibacterial agents has reduced against MDR phenotypes. To improve their activity, these agents can be combined with natural substances derived from plants [48]. However, the activity of antibiotics has been reported to be modulated when combined with botanicals. In the present study, the various extracts potentiated the activity of antibiotics, with AMFs ranging from 2 to 128. They enhanced the activity of CTX by 75% at MIC/2 and MIC/4. The antibacterial activities of IMI and CFX were improved by 50%, while LEV was enhanced by 62.5% at MIC/2. Both extracts potentiated the activity of TET by 62.5% and 75% at MIC/2 and MIC/4 respectively. Previous work has shown that the secondary metabolite of the plant, when combined with antibiotics against *Klebsiella* species, destabilizes the cytoplasmic membrane of bacteria [49]. This could be attributed to terpenoids [49], and saponins [50] while flavonoids [51] and polyphenols [52] interfered with the antibiotic's efflux mechanism. For instance, certain flavonoids inhibit the growth of *K. pneumoniae* when associated with ampicillin [51]. Moreover, polyphenols including epigallocatechin gallate have been shown to modulate the activity of antibiotics including ciprofloxacin, tetracycline against *K. pneumoniae* isolates [52]. The synergistic effects of the various extracts with antibiotics against the tested strains and isolates suggest that they may be potential efflux pump inhibitors. Braga et al. [53] highlighted that plant extracts modulating the activity of at least 70% of antibiotics on at least 70% of bacteria strains are efflux pump inhibitors. Previous investigations reported numerous

classes of phytochemical compounds in the tested plant extracts. Both extracts contained triterpenes, polyphenols, flavonoids, alkaloids, and saponins [18, 26]. This indicates that the anti-*Klebsiella* activity including the potentiation of antibiotics activity obtained in this study could be due to the presence of these secondary metabolites. Finally, this study confirmed the importance of the flora of Africa as a good source of plant-based medicine [55-80].

Table 1. Bacterial features of the studied *Klebsiella pneumoniae* and *Klebsiella oxytoca* strains.

Bacteria strains and isolates	Features	References
Klebsiella pneumoniae		
ATCC11296	Reference strain	[37, 41]
K2	AcrAB-TolC	Laboratory collection of UNR-MD1, University of Marseille, France
KP55	Clinical isolate : MDR : TET^r ,AMP^r ,ATM^r, CEF^r	[37]
K24	Clinical isolate: TET^r, Chl^r, AMP^r, ATM^r	Laboratory collection
KP203	Clinical isolate: IMI^r, AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r, CIP^r, OFX^r, NAL^r, CTR^r, COL^r, PRL^r, PPT^r, TCC^r, TET^r, OXA^r, VAN^r, ATM^r, AMC^r, FOS^r	[31]
KP175	Clinical isolate: IMI^r, AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r,	[31]
KP77	Clinical isolate: IMI^r, AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r,	[31]
KP93	Clinical isolate: IMI^r, AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r,	[31]
KP126	Clinical isolate : MDR, AMP^r	Laboratory collection
KP81	Clinical isolate: AMX^r, FOX^r, CTX^r, CXM^r, COT^r, NAL^r, PRL^r, NIT^r.	Laboratory collection
Klebsiella oxytoca		
KO249	Clinical isolate: IMP^r, AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r, AMK^r, NAL^r, CTR^r, COL^r, PRL^r, PPT^r, TCC^r, TET^r, OXA^r, VAN^r, NIT^r, ATM^r, AMC^r, FOS^r	[31]
KO96	Clinical isolate : MDR : AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r, ERY^r, NAL^r, CTR^r, COL^r, PRL^r, TET^r, VAN^r, CIP^r, OFX^r, ATM^r, NIT^r, AMC^r, FOS^r.	Laboratory collection
KO107	Clinical isolate : MDR, ATM^r, DOX^r, MIT^r, CIP^r.	Laboratory collection
KO95	Clinical isolate : MDR : AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r, ERY^r, NAL^r, CTR^r, COL^r, PRL^r, TET^r, VAN^r, CIP^r, OFX^r, ATM^r, NIT^r, AMC^r, FOS^r.	[31]
KO26	Clinical isolate :MDR, AMC^r	Laboratory collection
KO55	Clinical isolate: AMX^r, CAZ^r, FOX^r, CTX^r, CXM^r, ERY^r, NAL^r, CTR^r, PRI^r, TET^r, VAN^r, CIP^r, OFX^r, NIT^r.	[31]

AMX^r, TET^r, AMP^r, ATM^r, CEF^r, ERY^r, CAZ^r, CIP^r, DOX^r, CTX^r, IMI^r, NOR^r, NAL^r, STR^r, PRL^r, NIT^r, FOX^r, COL^r, VAN^r, OFX^r, CMX^r, MIT^r, PPT^r, TCC^r resistant respectively to: Amoxicillin, tetracycline, ampicillin, aztreonam, cefepime, erythromycin, ceftazidine, ciprofloxacin, doxycycline, ceftriaxone, imipenem, norfloxacin, nalidixic acid, streptomycin, piperacillin, nitrofurantoin, cefoxitin, vancomycin, ofloxacin, cefmenoxime, methicillin, triclocarban MDR: Multidrug Resistant. *AcrAB-TolC, AcrAB:* efflux pumps, ATCC: *American type culture collection.*

Table 2. Phytochemical composition of methanol extracts from the flowers of *Vernonia calvaona (VCF)* and the leaves of *Senna spectabilis (SSL)*.

Secondary metabolites	***Botanicals***	
	VCF	**SSL**
Triterpenes	+	+
Polyphenols	+	+
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+
Anthocyanins	-	-

(+): present; (-): absent, VCF: *Vernonia calvaona* flowers, SSL: *Senna spectabilis* leaves.

Table 3. Minimum inhibitory and bactericidal concentrations of the extracts from the flowers of *V. calvaona* (VCF) and leaves of *S. spectabilis* (SSL), and IMI against the tested *Klebsiella* strains and isolates.

Bacteria	*Botanicals*						Antibiotic		
	Vernonia calvaona			*Senna spectabilis*			Imipenem		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Klebsiella pneumoniae									
ATCC11296	1024	1024	1	256	<256		32	<32	nd
K2	128	256	2	**64**	512	nd	32	128	4
KP55	128	256	2	512	2048	4	128	128	1
K24	256	1024	4	256	512	2	64	128	4
KP203	**64**	128	2	512	2048	4	<1	16	nd
KP175	1024		nd	**16**	1024	64	16	128	8
KP77	128	512	4	128	256	2	>128	nd	nd
KP93	2048	>2048	nd	2048	>2048	nd	16	64	4
KP126	128	1024	8	512		nd	8	64	8
KP81	512		nd	1024		nd	32	>128	nd
Klebsiella oxytoca									
KO249	1024		nd	256		nd	>128	nd	nd
KO96	512		nd	1024		nd	32	128	4
KO107	512		nd	**64**	128	2	<1	128	nd
KO95	**32**	128	4	1024	2048	2	>128	nd	nd
KO26	128	512	4	512	>2048	nd	32	128	4
KO55	512	2048	4	512	>2048	nd	16	>128	nd

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, R: MBC/MIC ratio, nd: not determined

Table 4. Minimum inhibitory concentrations of the different extracts alone and in the presence of PAβN.

Bacteria	VCF			SSL			Imipenem		
	MIC alone	+PA*β*N	R	MIC alone	+PA*β*N	R	MIC alone	+Pa*β*N	R
Klebsiella pneumoniae									
K2	128	<8	16	64	16	4	32	<1	32
KP55	128	16	**8**	512	<8	**64**	128	<1	128
K24	256	256	1	256	128	2	64	8	8
KP175	1024	<8	**128**	16	<8	**2**	16	8	2
KP93	2048	<8	**256**	2048	16	**128**	16	<1	16
Klebsiella oxytoca									
KO249	1024	32	**4**	256	128	2	>128	16	8
KO96	512	<8	64	1024	<8	128	32	<1	**32**
KO95	32	<8	4	1024	32	**32**	>128	8	16

MIC alone: Minimum inhibitory concentration in the absence of the inhibitor, *+PAβ*N: Minimum inhibitory concentration in the presence of the inhibitor, R: *MIC/+PAβ*N ratio, nd: not determined, VCF: *Vernonia calvaona flowers,* SSL: *Senna spectabilis* leaves.

Table *5*. Effects of the combination of antibiotics and VCF against MDR bacteria.

ATB	Extract concentrations	Sub-inhibitory concentration (µg/mL) of *Vernonia calvaona* flower extract in the presence of antibiotics and Antibiotic-resistance modulating factor (AMF)								PBS
		Klebsiella pneumoniae					*Klebsiella oxytoca*			
		K2	KP55	K24	KP175	KP93	KO249	KO96	KO95	
CIP	0	64	64	8	64	64	16	1	32	
	MIC/2	<1**(64)**	8**(8)**	2**(4)**	<1**(64)**	<1**(64)**	4**(4)**	<1**(1)**	8**(4)**	87.5
	MIC/4	<1**(64)**	16**(4)**	2**(4)**	<1**(64)**	<1**(64)**	4**(4)**	<1**(1)**	8**(4)**	87.5
LEV	0	8	64	16	16	16	16	64	8	
	MIC/2	4**(2)**	16**(4)**	32**(0.5)**	<1**(16)**	<1**(16)**	<1**(16)**	<1**(64)**	<1**(8)**	87.5
	MIC/4	16**(0.5)**	16**(4)**	32**(0.5)**	<1**(16)**	<16**(1)**	4**(4)**	<1**(64)**	16**(0.5)**	50
TET	0	16	>128	128	64	64	>128	4	32	
	MIC/2	8**(2)**	8**(16)**	<1**(128)**	<1**(64)**	64**(1)**	32**(4)**	8**(0.5)**	>128**(0.25)**	62.5
	MIC/4	16**(2)**	64**(2)**	64**(2)**	128**(0.5)**	128**(0.5)**	32**(4)**	8**(0.5)**	>128**(0.25)**	50
CTX	0	16	128	64	>1024	256	128	32	64	
	MIC/2	<8**(2)**	<8**(16)**	<8**(8)**	<8**(128)**	64**(4)**	<8**(16)**	<8**(4)**	<8**(8)**	100
	MIC/4	16**(1)**	<8**(16)**	<8**(8)**	<8**(128)**	128**(2)**	32**(4)**	32**(1)**	32**(2)**	75
AMP	0	>1024	1024	>1024	>1024	1024	>1024	>1024	>1024	
	MIC/2	>1024**(1)**	<8**(128)**	<8**(128)**	<8**(128)**	<8**(128)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	50
	MIC/4	>1024**(1)**	8**(128)**	512**(2)**	<8**(128)**	1024**(1)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	37.5
PEN	0	>1024	128	>1024	16	1024	>1024	>1024	>1024	
	MIC/2	>1024**(1)**	<8**(128)**	256**(4)**	<8**(2)**	<8**(128)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	50
	MIC/4	>1024**(1)**	16**(64)**	512**(2)**	<8**(2)**	<8**(128)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	50
IMI	0	32	128	64	16	16	>128	32	>128	
	MIC/2	128**(0.25)**	<1**(128)**	<1**(64)**	<1**(16)**	<1**(16)**	4**(32)**	256**(0.125)**	>128**(1)**	62.5
	MIC/4	128**(0.25)**	32**(4)**	128**(0.5)**	<1**(16)**	<1**(16)**	64**(2)**	256**(0.125)**	>128**(1)**	62.5
CFX	0	32	1024	512	256	128	256	32	256	
	MIC/2	16**(0.5)**	<8**(128)**	<8**(64)**	1024**(0.25)**	<8**(16)**	128**(2)**	<8**(4)**	128**(2)**	50
	MIC/4	16**(0.5)**	16**(64)**	16**(32)**	1024**(0.25)**	<8**(16)**	512**(0.5)**	32**(1)**	256**(1)**	37.5

ATB: Antibiotics; MIC: Minimum Inhibitory Concentration; (): AIF (Activity Modulation Factor), PBS: percentage of bacteria with synergistic effects

Table 6. Effects of the combination of antibiotics and SSL against MDR bacteria.

ATB	Extract concentrations	Sub-inhibitory concentration (µg/mL) of *Senna spectabilis* flower extract in the presence of antibiotics and Antibiotic-resistance modulating factor (AMF)								PBS
		Klebsiella pneumoniae					*Klebsiella oxytoca*			
		K2	KP55	K24	KP175	KP93	KO249	KO96	KO95	
CIP	0	64	64	8	64	64	16	1	32	
	MIC/2	<1**(64)**	64**(1)**	8**(1)**	64**(1)**	64**(1)**	16**(1)**	<1**(1)**	<1**(32)**	25
	MIC/4	<1**(64)**	64**(1)**	8**(1)**	64**(1)**	128**(0.5)**	16**(1)**	2**(0.5)**	<1**(32)**	25
LEV	0	8	64	16	16	16	16	64	8	
	MIC/2	4**(4)**	16**(4)**	16**(1)**	32**(0.5)**	128**(0.125)**	4**(4)**	2**(32)**	<1**(8)**	62.5
	MIC/4	4**(4)**	16**(4)**	16**(1)**	64**(0.25)**	128**(0.125)**	4**(4)**	2**(32)**	16**(0.5)**	50
TET	0	16	>128	128	64	64	>128	4	32	
	MIC/2	16**(2)**	64**(2)**	64**(2)**	64**(1)**	<1**(64)**	16**(8)**	<1**(0.25)**	<1**(32)**	75
	MIC/4	16**(2)**	64**(2)**	64**(2)**	128**(0.5)**	<1**(64)**	16**(8)**	64**(0.06)**	32**(1)**	62.5
CTX	0	16	128	64	>1024	256	128	32	64	
	MIC/2	16**(1)**	<8**(16)**	<8**(8)**	<8**(128)**	32**(8)**	16**(8)**	16**(0.5)**	<8**(8)**	75
	MIC/4	64**(0.25)**	32**(4)**	<8**(8)**	<8**(128)**	32**(8)**	16**(8)**	16**(0.5)**	<8**(8)**	75
AMP	0	>1024	1024	>1024	>1024	1024	>1024	>1024	>1024	
	MIC/2	>1024**(1)**	>1024**(1)**	1024**(1)**	>1024**(1)**	<8**(128)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	12.5
	MIC/4	>1024**(1)**	>1024**(1)**	1024**(1)**	>1024**(1)**	<8**(128)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	12.5
PEN	0	>1024	128	>1024	16	1024	>1024	>1024	>1024	
	MIC/2	>1024**(1)**	64**(2)**	1024**(1)**	>1024**(0.02)**	<8**(128)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	25
	MIC/4	>1024**(1)**	128**(1)**	1024**(1)**	>1024**(0.02)**	<8**(128)**	>1024**(1)**	>1024**(1)**	>1024**(1)**	12.5
IMI	0	32	128	64	16	16	>128	32	>128	
	MIC/2	128**(0.25)**	16**(8)**	16**(4)**	8**(2)**	<1**(16)**	>128**(1)**	64**(0.5)**	>128**(1)**	50
	MIC/4	128**(0.25)**	16**(8)**	64**(1)**	8**(2)**	<1**(16)**	>128**(1)**	128**(0.25)**	>128**(1)**	37.5
CFX	0	32	1024	512	256	128	256	32	256	
	MIC/2	16**(2)**	128**(8)**	128**(4)**	256**(1)**	<8**(16)**	16**(16)**	32**(1)**	256**(1)**	62.5
	MIC/4	16**(2)**	128**(8)**	512**(1)**	256**(1)**	<8**(16)**	128**(16)**	32**(1)**	256**(1)**	50

ATB: Antibiotics; MIC: Minimum Inhibitory Concentration; (): AIF (Activity Modulation Factor), PBS: percentage of bacteria showing synergistic effects

Conclusion

The present study aimed to demonstrate the anti-*Klebsiella* activity of methanol extract from the flowers of *V. calvaona* and the leaves of *S. spectabilis*. They displayed important anti-*Klebsiella* activity and potentiated the activity of usual antibiotics. Therefore, the extracts from the flowers of *V. calvaona* and the leaves of *S. spectabilis* can be used alone or in association with usual antibiotics to fight MDR *Klebsiella* phenotypes overexpressing efflux pumps. Although this study highlighted important results concerning antibacterial activity, further studies are needed to understand the mode of action and to purify active compounds responsible for the activity observed.

Abbreviations

AMP: Ampicillin
ATCC: American-Type Culture Collection
CIP: Ciprofloxacin
CTX: Ceftriaxone
DMSO: Dimethyl sulfoxide
DOX: Doxycycline
EMB: Eosin methylene blue
EPI: Efflux pump inhibitor
HNC: National Herbarium of Cameroon
IMI: Imipenem
INT: Para-Iodonitrotetrazolium chloride
LEV: Levofloxacin
MBC: Minimal Bactericidal Concentration
MDR: Multidrug-Resistant
MHA: Mueller Hinton Agar
MHB: Mueller Hinton
MIC: Minimal Inhibitory Concentrations
PAβN: Phenylalanine-arginine β-naphthylamide
RND: Resistance-Nodulation cell Division
SSL: *Senna spectabilis* leaves
TET: Tetracycline
VAN: Vancomycin

Authors' Contribution

DJA, EC, LM, AWBY, VYM, JRNK, INB, MFK, and JFM carried out the study; ATM and VK supervised the study; All authors read and approved the final version of the manuscript.

Acknowledgments

The authors are grateful to the Cameroon National Herbarium for identifying the plant.

Conflict of interest

The authors declare no conflict of interest.

Article history:

Received: 17 October 2024
Received in revised form: 10 December 2024
Accepted: 16 December 2024
Available online: 16 December 2024

References

1. Fongang H, Mbaveng AT, Kuete V. 2023. Chapter One - Global burden of bacterial infections and drug resistance. *Advances in Botanical Research*. 106:1-20. https://doi.org/10.1016/bs.abr.2022.08.001.
2. O'neil J. 2016. Tackling drug-resistant infections globally: final report and recommendations the review on antimicrobial resistance. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf. Accessed on November 10, 2024
3. de Kraker ME, Stewardson AJ, Harbarth S. 2016. Will 10 million people die a year due to antimicrobial resistance by 2050? *PLoS Medicine*. 13(11): e1002184.
4. Odonkor ST, Addo KK. 2011. Bacteria resistance to antibiotics: recent trends and challenges. *Int J Biol Med Res*. *2*(4): 1204-1210.
5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. *18*(3): 268-281.
6. WHO (World Health Organization). Bacterial Priority Pathogens List, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva, https://www.who.int/publications/i/item/9789240093461; 2024. Accessed on November 10, 2024
7. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, Luisi BF. 2018. Multidrug efflux pumps: structure, function and regulation. *Nat Rev Microbiol.* 16(1): 523–539
8. Jang s. 2023. AcrAB−TolC, a major efflux pump in Gram negative bacteria: toward understanding its operation mechanism. *BMB reports*. 56(6): 326.
9. Lomovskaya O, Bostian KA. 2006. Practical applications and feasibility of efflux pump inhibitors in the clinic—a vision for applied use. *Biochem Pharmacol*. *71*(7): 910-918.
10. 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(1): 1-9.
11. Demgne OMF, Damen F, Fankam AG, Guefack MGF, Wamba BE, Nayim P, Kuete V. 2021. Botanicals and phytochemicals from the bark of *Hypericum roeperianum* (Hypericaceae) had strong antibacterial activity and showed synergistic effects with antibiotics against multidrug-resistant bacteria expressing active efflux pumps. *J Ethnopharmacol*. 277(1): 114257.
12. Cadet E, Assonfack DJ, Yendze AWW, Mpude L, Matieta VY, Kuete JRN, Megaptche JF, Bonsou IN, Kuete V, Mbaveng AT. 2024. Antibacterial activity and antibiotic-potentiating effects of methanol extracts from Ocimum basilicum and Sarcocephalus latifolius against multidrug-resistant Gram-negative bacteria overexpressing efflux pumps. *Invest Med Chem Pharmacol.* 7(2):97.
13. Fankam AG, Kuete V, Voukeng IK, Kuiate JR, Pages JM. 2011. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. *BMC Complement Altern Med*.*11*(1): 1-11.
14. Matieta VY, Kuete V, Mbaveng AT. 2023. Anti-*Klebsiella* and antibiotic potentiation activities of the methanol extracts of seven Cameroonian dietary plants against multidrug-resistant phenotypes over-expressing AcrAB-TolC efflux pumps. *Invest Med Chem Pharmacol. 6*(1): 73.
15. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. 2019. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobl Resist Infection Cont*. *8*(1): 1-28.
16. Khameneh B, Eskin NM, Iranshahy M, Fazly Bazzaz BS. 2021. Phytochemicals: a promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics*. *10*(9): 1044.
17. Ejoh RA, Nkonga DV, Inocent G, Moses MC. 2007. Nutritional components of some non-conventional leafy vegetables consumed in Cameroon. *Pak J Nutr*. *6*(6): 712-717.
18. Igile GO, Iwara IA, Mgbeje BIA, Uboh FE, Ebong PE. 2013. Phytochemical, proximate and nutrient composition of *Vernonia calvaona* Hook (*Asterecea*): A green-leafy vegetable in Nigeria. *J Food Res*. *2*(6): 1.
19. Mbemi AT, Sims JN, Yedjou CG, Noubissi FK, Gomez CR, Tchounwou PB. 2020. *Vernonia calvoana* shows promise towards the treatment of ovarian cancer. *Int J Mol Sci*. 21(12): 4429.
20. Iwara IA, Igile GO, Uboh FE, Eyong EU, Ebong PE. 2015. Hypoglycemic and hypolipidemic potentials of extract of *Vernonia calvoana* on alloxan-induced diabetic albino wistar rats. *Eur J Med Plants*. 8(2): 78-86.
21. Ati B, Iwara I, Bassey A, Igile G, Duke E, Ebong P. 2016. Antimicrobial Activity of Leaf Extract–Fractions of *Vernonia calvoana* against Selected Stock Cultures in Microbiology Laboratory, Cross River University of Technology, Calabar. *Int J Curr Microbiol App Sci*. *5*(5): 512-20.
22. Jothy SL, Torey A, Darah I, Choong YS, Saravanan D, Chen Y, Sasidharan S. 2012. *Cassia spectabilis (*DC) Irwin et Barn: A promising traditional herb in health improvement. *Molecules*. 17(9): 10292-10305.
23. Bum EN, Nkantchoua GN, Njikam N, Taiwe GS, Ngoupaye GT, Pelanken MM, Rakotonirina SV. 2010. Anticonvulsant and sedative activity of leaves of *Senna spectabilis* in mice. Int J Pharmacol. 6(2): 123-128.
24. Nsonde-Ntandou GF, Ndounga M, Ouamba JM, Gbeassor M, Etou-Ossebi A, Ntoumi F, Abena AA. 2005. Ethnobotanical survey, chemical screening and effective treatment of certain plants used in traditional medicine to treat malaria in Brazzaville. *Phytothérapie*. *3*(1): 13-18.
25. Chukeatirote E, Hanpattanakit P, Kaprom A, Tovaranonte J. 2007. Antimicrobial activity of *Senna spectabilis* and *S. tora*. J Plant Sci. 2(1): 123-126.
26. Mugweru FG, Nyamai DW, Arika WM, Mworia JK, Ngugi MP, Njagi E, Kisangau P. 2016. In vivo safety of aqueous extracts of *Maytemus putterlickoides*, *Senna spectabilis* and *Olinia usambarensis* on mice models. *J Clin Toxicol*. 6:3.
27. Arantes VP, dos Santos LF, da Silva DK, da Silva GO, Costa GM. 2016. Estudo comparativo da atividade antibacteriana de extratos vegetais de *Senna spectabilis*, *Rosmarinus officinalis* e *Eugenia uniflora* frente à cepa padrão de *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538 e *Streptococcus pyogenes* ATCC 19615. *Arq. ciências saúde UNIPAR*. 20(3): 151-158.
28. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Alibert-Franco S, Ngadjui BT, Pages JM. 2010. Efflux pumps are involved in the defense of Gram-negative bacteria against the natural products isobavachalcone and diospyrone. *Antimicrob Agent Chemother*. 54(5):1749-1752.
29. Kuete V, Alibert-Franco S, Eyong KO, Ngameni B, Folefoc GN, Nguemeving JR, Tangmouo JG, Fotso GW, Komguem J, Ouahouo BMW, Pagès JM. 2011. Antibacterial activity of some natural products against bacteria expressing a multidrug resistant phenotype. *International Journal of Antimicrobial Agents.* 37(2):156-161.
30. Kengne MF, Tsobeng OD, Dadjo BS, Kuete V, Mbaveng AT, 2024. Multidrug Resistant Enteric Bacteria from Cancer Patients Admitted in Douala Laquintinie Hospital, Littoral Region of Cameroon. *Can J Infect Dis Med Microbiol*. 2024(1): 2084884.
31. Kengne GF, Matieta VY, Tiwa SM, Ngakam R, Megaptche JF, Nayim P, Mbaveng AT. 2024. Botanicals from *Aframomum letestuanum* Gagnep. (Zingiberaceae) can overcome the multidrug resistance of *Klebsiella* species overexpressing AcrAB-TolC efflux pumps. *Invest Med Chem Pharmacol.* 7(1): 88.
32. Eloff JN. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med.* 64(8):711-713
33. Kuete V, Mbaveng AT, Tsaffack M, Beng VP, Etoa FX, Nkengfack AE, Meyer JJ, Lall N. 2008. Antitumor, antioxidant and antimicrobial activities of *Bersama engleriana* (Melianthaceae). *J Ethnopharmacol*. *115*(3): 494–501.
34. Ekamgue B, Mbaveng AT, Kuete V. 2023. Anti-staphylococcal and antibioticpotentiating activities of botanicals from nine Cameroonian food plants towards multidrug-resistant phenotypes. *Invest Med Chem Pharmacol.* 6(1):75.
35. Moungoue Ngwaneu LS, Mbaveng AT, Nayim P, Wamba BEN, Youmbi LM, Bonsou IN, Ashu F, Kuete V. 2022. Antibacterial and antibiotic potentiation activity of *Coffea arabica* and six other Cameroonian edible plants against multidrug-resistant phenotypes. *Invest Med Chem Pharmacol.* 5(2):68
36. Coutinho HD, Vasconcellos A, Freire-Pessoa HL, Gadelha CA, Gadelha TS. Almeida-Filho GG. 2010. Natural products from the termite Nasutitermes corniger lower aminoglycoside minimum inhibitory concentrations. *Pharmacogn Mag.* 6(1):1-4.
37. Harbone JB. 1973. Phytochemical methods: A guide to modern techniques of plant analysis. London, Chapman and Hall Ltd. 116.
38. Kuete V. 2013. Medicinal Plant Research in Africa: Pharmacology and Chemistry In: Pharmacology and Chemistry. Edited by Kuete V, 1 edn. Oxford: Elsevier.
39. Kuete V. 2023. Potential of African medicinal plants against Enterobacteria: Classification of plants antibacterial agents. *Advances in Botanical Research.* 106(1): 151-335. https://doi.org/10.1016/bs.abr.2022.08.006.
40. Carbonnelle B, Denis F, Marmonier A, Pinon G, Vague R. 1987. Medical bacteriology: Usual techniques. Paris: SIMEP.
41. Kuete V, Dongfack MD, Mbaveng AT, Lallemand MC, Van-Dufat HT, Wansi JD, Seguin E, Tillequin F, Wandji J. 2010. Antimicrobial activity of the methanolic extract and compounds from the stem bark of Drypetes tessmanniana. *Chin J Integr Med.* 16(4):337-343.
42. Chooto K. 2004. Herbs have a place in Modern Healthcare. *Daily Nation.*
43. Fankam AG, Kuiate JR, Kuete V. 2014. Antibacterial activities of *Beilschmiedia obscura* and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes. *BMC Complement Altern Med*. *14(1)*: 1-9.
44. Ibrahim N, Kebede A. 2020. In vitro antibacterial activities of methanol and aqueous leave extracts of selected medicinal plants against human pathogenic bacteria. *Saudi J Biol Sci*. *27*(9): 2261-2268.
45. Pagès JM, Amaral L. 2009. Mechanisms of drug efflux and strategies to combat them: challenging the efflux pump of Gram-negative bacteria. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*. *1794*(5): 826-833.
46. Opperman TJ, Kwasny SM, Kim HS, Nguyen ST, Houseweart C, D'Souza S, Bowlin TL. 2014. Characterization of a novel pyranopyridine inhibitor of the AcrAB efflux pump of *Escherichia coli*. *Antimicrobl Agents Chemother*. *58*(2): 722-733.
47. Assonfack DJ, Mpude L, Cadet E, Yendze AWW, Matieta VY, Kuete JRN, Megaptche JF, Bonsou IN, Kengne MF, Kuete V, Mbaveng AT. Unveiling the anti-Klebsiella activity of methanol extracts from Hallea ciliata leaves and barks against multidrug-resistant strains overexpressing AcrAB-TolC efflux pumps. *Invest Med Chem Pharmacol (2024) 7(3):98*.
48. Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, Nabavi SM. 2017. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiol Res*. 196(1): 44-68.
49. Fadli M, Saad A, Sayadi S, Chevalier J, Mezrioui NE, Pagès JM, Hassani L. 2012. Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against nosocomial infection–bacteria and their synergistic potential with antibiotics. *Phytomedicine*. 19(5): 464-471.
50. Delcour A. 2009. Outer membrane permeability and antibiotic resistance. Biochimica et biophysica acta (BBA)-proteins and proteomics. 1794(5): 808816.

51. Wiyogo IO, Endraswari PD, Setiawati Y. 2021. Antibacterial activity of ethanol extract of Kemuning (Murraya Paniculata) against *Klebsiella pneumoniae* ESBL by in vitro test. *Indonesian J Trop Infect Dis*. *9*(2): 102-107.
52. 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.
53. 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.
54. 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.
55. Kuete V, Mbaveng AT, Zeino M, Fozing CD, Ngameni B, Kapche GD, Ngadjui BT, Efferth T. 2015. Cytotoxicity of three naturally occurring flavonoid derived compounds (artocarpesin, cycloartocarpesin and isobavachalcone) towards multi-factorial drug-resistant cancer cells. *Phytomedicine*. 22(12):1096-1102.
56. Dzoyem JP, Kuete V, Eloff JN. 2014. 23 - Biochemical Parameters in Toxicological Studies in Africa: Significance, Principle of Methods, Data Interpretation, and Use in Plant Screenings. In: *Toxicological Survey of African Medicinal Plants.* edn. Edited by Kuete V: Elsevier, pp.659-715.
57. Sandjo LP, Kuete V, Tchangna RS, Efferth T, Ngadjui BT. 2014. Cytotoxic benzophenanthridine and furoquinoline alkaloids from *Zanthoxylum buesgenii* (Rutaceae). *Chem Cent J.* 8(1):61.
58. Kuete V. 2014. 22 - Physical, Hematological, and Histopathological Signs of Toxicity Induced by African Medicinal Plants. In: *Toxicological Survey of African Medicinal Plants.* edn. Edited by Kuete V: Elsevier; pp. 635-657.
59. Mbaveng AT, Hamm R, Kuete V. 2014. 19 - Harmful and protective effects of terpenoids from african medicinal plants. In: *Toxicological Survey of African Medicinal Plants.* edn. Edited by Kuete V: Elsevier; pp. 557-576.
60. 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;pp. 261-300.
61. Kuete V, Fokou FW, Karaosmanoğlu O, Beng VP, Sivas H. 2017. Cytotoxicity of the methanol extracts of *Elephantopus mollis, Kalanchoe crenata* and 4 other Cameroonian medicinal plants towards human carcinoma cells. *BMC Complement Altern Med.* 17(1):280.
62. 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.
63. Kuete V, Tabopda TK, Ngameni B, Nana F, Tshikalange TE, Ngadjui BT. 2010. Antimycobacterial, antibacterial and antifungal activities of *Terminalia superba* (Combretaceae). *S Afr J Bot.* 76(1):125-131.
64. Wamba BEN, Nayim P, Mbaveng AT, Voukeng IK, Dzotam JK, Ngalani OJT, Kuete V. 2018. *Syzygium jambos* displayed antibacterial and antibiotic-modulating activities against resistant phenotypes. *Evid Based Complement Alternat Med.* 2018:5124735.
65. Kuete V, Sandjo L, Seukep J, Maen Z, Ngadjui B, Efferth T. 2015. Cytotoxic compounds from the fruits of *Uapaca togoensis* towards multi-factorial drug-resistant cancer cells. *Planta Med.* 81(1):32-38.
66. 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.
67. 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.
68. 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.
69. Kuete V, Ango PY, Yeboah SO, Mbaveng AT, Mapitse R, Kapche GD, Ngadjui BT, Efferth T. 2014. Cytotoxicity of four *Aframomum* species (*A. arundinaceum, A. alboviolaceum, A. kayserianum and A. polyanthum*) towards multi-factorial drug resistant cancer cell lines. *BMC Complement Altern Med.* 14:340.
70. Fankam AG, Kuiate JR, Kuete V. 2017. Antibacterial and antibiotic resistance modulatory activities of leaves and bark extracts of *Recinodindron heudelotii* (Euphorbiaceae) against multidrug-resistant Gram-negative bacteria. *BMC Complement Altern Med.* 17(1):168.
71. Mbaveng AT, Manekeng HT, Nguenang GS, Dzotam JK, Kuete V, Efferth T. 2018. Cytotoxicity of 18 Cameroonian medicinal plants against drug sensitive and multi-factorial drug resistant cancer cells. *J Ethnopharmacol.* 222:21-33.
72. 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.
73. Djeussi DE, Sandjo LP, Noumedem JA, Omosa LK, B TN, Kuete V. 2015. Antibacterial activities of the methanol extracts and compounds from *Erythrina sigmoidea* against Gram-negative multi-drug resistant phenotypes. *BMC Complement Altern Med.* 15(1):453.
74. 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 JTrad Med.* 3:211-223.
75. Kuete V, Djeussi DE, Mbaveng AT, Zeino M, Efferth T. 2016. Cytotoxicity of 15 Cameroonian medicinal plants against drug sensitive and multi-drug resistant cancer cells. *J Ethnopharmacol.* 186:196-204.
76. Nganou BK, Mbaveng AT, Fobofou SAT, Fankam AG, Bitchagno GTM, Simo Mpetga JD, Wessjohann LA, Kuete V, Efferth T, Tane P. 2019. Furoquinolines and dihydrooxazole alkaloids with cytotoxic activity from the stem bark of *Araliopsis soyauxii*. *Fitoterapia*. 133:193-199.
77. Adem FA, Kuete V, Mbaveng AT, Heydenreich M, Ndakala A, Irungu B, Efferth T, Yenesew A. 2018. Cytotoxic benzylbenzofuran derivatives from *Dorstenia kameruniana*. *Fitoterapia.* 128:26-30.
78. Mbaveng AT, Chi GF, Nguenang GS, Abdelfatah S, Tchangna Sop RV, Ngadjui BT, Kuete V, Efferth T. 2020. Cytotoxicity of a naturally occuring spirostanol saponin, progenin III, towards a broad range of cancer cell lines by induction of apoptosis, autophagy and necroptosis. *Chem Biol Interact.* 326:109141.
79. Adem FA, Kuete V, Mbaveng AT, Heydenreich M, Koch A, Ndakala A, Irungu B, Yenesew A, Efferth T. 2019. Cytotoxic flavonoids from two *Lonchocarpus* species. *Nat Prod Res.* 33(18):2609-2617.
80. Kuete V. 2024. The best African plant-derived antibacterial products for clinical perspectives: The state-of-the-art. Invest Med Chem Pharmacol. 7(2):94.