Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Cellular modes of action of the methanol extract from the aerial parts of *Psychotria sycophylla* (K.Schum.) Petit (Rubiaceae) against multidrug-resistant bacteria

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

Abstract

Background: Infectious diseases caused by multidrug-resistant bacteria (MDR) remain a global health issue. The population uses several plants from the Cameroonian pharmacopeia to treat bacterial infections. The methanol extract from the aerial parts of *Psychotria sycophylla* (K.Schum.) Petit (Rubiaceae) (PSA) previously showed antibacterial activities against MDR bacteria. In the present study, the antibacterial activities of PSA were determined in the presence of an efflux pump inhibitor (EPI), phenylalanine-arginine β-naphthylamide (PAβN). The effects of PSA on the bacterial membrane and its ability to inhibit the bacterial biofilm were also assessed.

Methods: The evaluation of antibacterial activity of PSA in the presence of PAβN was evaluated using the microdilution method. The modification of the bacterial membrane permeability as well as the inhibition of biofilm were evaluated using spectrophotometric methods.

Results: When tested with PAβN, the inhibitory effects of PSA increased vis à vis all the tested bacteria. PSA also destroyed the bacterial membrane and showed significant inhibition of biofilms of *Escherichia coli* AG102 strain.

Conclusion: The reported data provide complementary information on the antibacterial effect of *Psychotria sycophylla* and might be helpful in formulating a phytomedicine to combat infections due to multidrug-resistant bacteria.

Keywords: Bacteria; modes of action; multidrug resistance; *Psychotria sycophylla*; Rubiaceae.

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

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

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

Citation on this article: Demgne OMF, Tchinda CF, Mbaveng AT, Guefack MGF, Beng VP, Kuete V. Cellular modes of action of the methanol extract from the aerial parts of Psychotria sycophylla (K.Schum.) Petit (Rubiaceae) against multidrug-resistant bacteria. Invest Med Chem Pharmacol. (2022) 5(2):66; Doi: https://dx.doi.org/10.31183/imcp.2022.00066

Background

Infectious diseases caused by multidrug-resistant bacteria remain a major public health problem worldwide, especially in developing countries where living conditions are very precarious [1, 2]. Despite the existence of several strategies to control bacterial infections, antibiotic therapy remains the most important one. Notwithstanding the impact of antibiotic therapy on human health, their inappropriate and excessive use by the population has led to selection pressure by bacteria [2, 3, 4], resulting in the emergence and re-emergence of numerous diseases responsible for the increase in the length of hospitalization, the cost of care and the mortality rate [5]. Moreover, the emergence of MDR bacterial strains is the main cause of therapeutic failure [6]. Among the known resistance mechanisms, active efflux through Resistance-Nodulation-Division (RND) and Major Facilitator Superfamily (MFS) pumps for Gram-negative and Gram-positive bacteria respectively [7] is well recognized. Regarding the growing concern of resistance globally, the search for new and effective antibacterial substances with low toxicity remains an absolute necessity. Cameroonian flora abounds with numerous pharmacologically active plants with multiple bioactive substances that previously demonstrated anti-inflammatory, antiproliferative, antiviral as well as antibacterial activities amongst others [8-29].

In our various strategies to combat MDR bacteria *via* the identification of antibacterial agents from traditional pharmacopeia, we have previously focused on *Psychotria sycophylla* (K.Schum.) Petit (Rubiaceae), used in traditional medicine for the treatment of various infections [30]. The antibacterial activity of the methanol extract of this plant as well as that of its isolated compounds has been in panels of Gram-negative bacteria and *Staphylococcus aureus strains* expressing resistant and MDR phenotypes [31]. In the present study, the activity of the crude methanol extract of this plant was further investigated against some MDR bacteria in the presence of Phenylalanine-Arginine-β-Naphthylamide (PAβN). Its effects were also determined on the bacterial membrane and the inhibition of biofilm in *Escherichia coli.*

Methods

Plant material and extraction

The aerial parts of *Psychotria sycophylla* (K.Schum.) Petit (Rubiaceae) collected in Dschang (West Region-Cameroon, in November 2016) and identified at the Cameroon National Herbarium (Voucher number: 35642/HNC) as processed to obtain the methanol extract (PSA) as earlier described [31].

Chemicals for antimicrobial assay

The chemicals used consisted of the reference antibiotics (RA) and the microbial growth indicator. The microbial growth indicator used in this work was *p*-iodonitrotrazolium chloride ≥97% (INT, Sigma-Aldrich). The reference antibiotics used were a phenolic Chloramphenicol (CHL) and a polypeptide, Polymyxin B (Poly B). The revelator of the biofilm formation was the purple crystal. Dimethylsulfoxide (DMSO) was used to dissolve the test samples. These molecules and reagents were obtained from SigmaAldrich (St. Quentin Fallavier, France).

Bacteria strains and culture media

The microorganisms used in this study included multidrug-resistant (MDR) bacteria expressing active efflux pumps. These bacteria consisted of a panel of 10 bacteria (Gram-negative and Gram-positive). They were various strains of *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Providencia stuartii,* and *Staphylococcus aureus.* These bacterial strains were provided by the American Type Culture Collection (ATCC) and the laboratory of UMR-MD1 of the University of Mediterranean, Marseille, France. The features of these bacteria were previously reported [19, 38]. They were maintained on agar plates at 4°C and subcultured onto appropriate fresh agar plates 24 hours before any antibacterial testing. Mueller Hinton agar (MHA; Sigma) was used for bacterial activation and Mueller Hinton broth (MHB; Sigma) was used for the determination of the minimal inhibitory concentration (MIC) of the test samples [32].

Cellular mode of action

Role of the efflux pumps in the resistance of bacteria to the test samples

The antibacterial activity of PSA and CHL (reference antibiotic) was evaluated according to the method described by Newton [33] with some modifications in the presence of the efflux pump inhibitor (EPI) at PAβN at 0.5 µg/mL. The determination of MICs of PSA was as previously described [32] with the difference that a 50 µL volume of a solution of the inhibitor was subsequently introduced, followed by a 50 µL volume of bacterial inoculum (of concentration 4×10^6 CFU/mL) for a final volume of 200 µL/well. The negative controls consisted of MHB and inhibitor on the one hand; inoculum, MHB, and 2.5% DMSO on the other; the neutral control consisted of MHB alone and the positive control consisted of CHL.

Effect of test sample on the bacterial membrane

The effect of the extract on the membrane was carried out by the method described by Leejae et al. [34]. Briefly, a bacterial suspension with an optical density (OD) of 2.0 was prepared from a fresh 18 h culture. Then, the bacterial cells were separated from the medium by centrifugation at 400 g, for 15 min, and washed twice in PBS buffer (pH 7.4) and re-suspended in the same buffer. The suspensions were treated with the extract at concentrations of 0.5 MIC, MIC, and 2 MIC. Polymyxin B was used as a positive control. The culture was incubated at 37°C for 60 min under agitation. Samples taken at times 0, 30, and 60 min of the experiment were centrifuged at 13400 g for 15 min. At each time and for each concentration of extract, the optical density will be measured at 260 nm with a spectrophotometer.

Determination of the effect of test samples on the biofilm formation

Inhibition of biofilm formation

The anti-biofilm activity was assessed in 96-well flat-bottom plates using a method adapted by Chaib et al. [35] with some modifications. The *E. coli* bacterial strain (AG102) was treated in microdilution plates with PSA at concentrations of 4096 µg/mL - 128 µg/mL respectively and then incubated at 37°C for 18 h under orbital shaking (REMI) at 7 g. Next, the planktonic cells were removed, and the plates were rinsed with sterile distilled water and stained for 15 minutes by incorporating 200 µL of crystal violet into

each well. Staining with acetic acid (30%), followed by measurement of the absorbance of the crystal violet solution at 595 nm was performed after the biofilms were stained and rinsed with sterile distilled water. The tests were repeated three times and the minimum biofilm inhibitory concentrations 50% ($MBIC_{50}$) were deduced using the biofilm inhibition percentages for each test calculated in relation to the untreated control values, according to the following formula:

$$\% \text{ inhibition} = [OD(\text{untreated value}) - OD(\text{treated value}) / OD(\text{untreated value})] \times 100$$

% inhibition = Percentage inhibition; Untreated value = Optical density of the biofilm alone; Treated value = Optical density of the biofilm in the presence of the test specimen.

Effect of test samples on formed biofilm

The effect of PSA on formed biofilm was studied using the method previously described by Chaib et al. [35] with slight modifications. The bacterial culture of *E. coli* AG102 was previously prepared in liquid medium (MHB) and incubated at 37°C for 18 hours under orbital shaking Incubator, REMI at 7 g. After biofilm formation, the planktonic cells were removed, and the plates were treated with crude extract of *P. sycophylla* at varying concentrations between 4096 µg/mL - 128 µg/mL respectively. Subsequently, the plates were re-incubated again at 37°C for 18 hours under Orbital Shaking Incubator, REMI (7 g) and rinsed with sterile distilled water, then stained for 15 minutes by incorporating 200 µL of crystal violet into each well. Discolouration with acetic acid (30%), followed by measurement of absorbance of the violet crystal solution at 595 nm was carried out after the biofilms were stained and rinsed with sterile distilled water. The tests were repeated three times and the biofilm eradication concentrations (BEC) were deduced using the biofilm eradication percentages for each test which were calculated in relation to the untreated control values, according to the following formula:

$$\% \text{ eradication} = [OD(\text{untreated value}) - OD(\text{treated value}) / OD(\text{untreated value})] \times 100$$

Eradication % = Eradication percentage; Untreated value = Optical density of the preformed biofilm; Treated value = Optical density of the preformed biofilm in the presence of the test specimen.

Results

Role of PAβN on the resistance of bacteria to test samples

Table 1 shows that PAβN improved the activity of PSA on all tested bacteria (AIF ≥ 2). Indeed, except for strain PS2636 towards PSA in the presence of PAβN, a clear improvement of the antibacterial activity of PSA when associated with PAβN was noted. PAβN tested at up to 128 µg/mL does not show any growth inhibitory effect on the selected bacteria. In general, the activity obtained with PSA in combination with the EPI was clearly better than that with CHL associated with the inhibitor. The AIF determined for PSA was 90% greater than or equal to that of CHL. Bacterial strain PS2636 was indifferent to the combination of *PSA* with PAβN. However, the MIC of *PSA* + PAβN was the same as that in the absence of PAβN (MIC= 4 µg/mL). The activity of PSA was exponentially improved in the presence of PAβN compared to other bacterial strains tested with AIFs up to 128 (on ATCC11296). All other bacterial strains were more than sensitive to the combination of the test substance with PAβN. PSA had improved activity in the presence of PAβN with AIFs ranging from 2 to 128. The activity of CHL was selectively enhanced with AIFs between 2 and 32. *K. pneumoniae* strains (ATCC11296 and KP55) showed no improvement in their susceptibility to the combination of CHL and PAβN.

Effect of PSA on the bacterial membrane

The leakage of intracellular constituents from *E. coli* AG102 cytoplasm after exposure to different concentrations of PSA was assessed by measuring the absorbance of the filtrate at 260 nm. Irreversible damage to the bacterial membrane could be explained by the presence of cytoplasmic elements in the culture broth. The results obtained after treatment of AG102 bacterial cells with our different sample concentrations (2 x MIC, MIC, and 0.5 x MIC/2) and determination of the proteins in the culture media are shown in Figure 1. The results show a concentration-dependent increase in optical density compared to the control after 12 hours. The observation was more remarkable at higher concentrations. The activity of PSA at 0.5 x MIC and MIC was greater than that of polymixin B.

Anti-biofilm activity

From the results obtained, it appears that PSA reduced or even inhibited the formation of biofilms of *E. coli* AG102 strain. In the absence of PSA, the optical density values are much higher than those reported by the strain in the presence of the active substance. The absorbance obtained in the presence of the AG102 strain with decreasing concentrations of PSA reflects an inhibition (Figure 2). PSA inhibited biofilm formation with inhibition percentages ranging from 90.25% to 96.7%. The biofilm inhibiting activity of PSA was concentration dependent. From the results depicted in Figure 3, PSA has remarkable destructive activities on the already formed biofilm. In general, comparing the eradication percentages obtained from the control wells (containing formed biofilms) with those of the test wells (containing PSA), the values are lower than those in the test wells. Indeed, PSA effectively eradicated the formed biofilms with eradication percentages ranging from 78.52% to 85.35%. This observed eradication was higher at higher concentrations.

Discussion

MDR bacteria can over-express efflux pump systems to expel antibacterial molecules from the cell and thus prevent them from inhibitory activities. The restoration of bacterial susceptibility using EPIs to allow a threshold concentration of antibacterials to be reached, capable of inducing bacterial cell death, is the best-known means of combating this type of resistance today. PAβN is an efflux pump inhibitor that is believed to act on the RND family of pumps [36]. Regarding the results obtained from the association of PSA with PAβN (AIF ≥ 2) (Table 1), it also appears that the activity of the active ingredients of PSA is a substrate of bacterial efflux pumps and should be combined with EPI for more efficiency. These results corroborate with previous work [37-38] which demonstrated that efflux pumps decrease the intracellular concentration of chemicals and consequently their activity. The enhanced activity noted with MDR bacteria overexpressing efflux pumps clearly confirms that the constituents of PSA are the substrates for efflux pumps, having intracellular targets [39-40].

The structural and chemical diversity of phytochemicals in plants allows them to have various targets on the bacterial cell structure including the membrane, cell wall, metabolism, and/or molecular targets (proton pumps, proteins, DNA/RNA) *via* different modes of action. Active molecules targeting the bacterial wall must find complementary receptors for binding and suitable for action; whereas those acting inside the cell must be able to both cross the membrane and find target elements in the cell. The intracellular compartment consists of nucleic acids and their derivatives, but also of the proteins that are the main components of the cell. An increase in absorbance at 260 nm of the extracellular medium is an indication of the presence of nucleic acids and their derivatives and therefore reflects a loss of membrane integrity [41 - 42]. The results of this study revealed a concentration-dependent increase in absorbance at 260 nm compared to the control, suggesting damage to the cytoplasmic membrane (Figure 1). This irreversible alteration of the membrane could explain the bactericidal effect of PSA at high concentrations. Thus, the cell membrane constitutes a likely site of action for bactericidal substances [43].

Some bacteria could organize themselves into structured communities surrounded by a polysaccharide matrix (biofilm) produced by the bacteria themselves and attached to a support. The formation of biofilms by secretion of a matrix (proteins, DNA, and extra-polysaccharide) or by adhesion to medical devices, implants, and damaged tissues is the main cause of nosocomial infections. This matrix could hinder or delay the entry of antibiotics and therefore hinders the activity of antibiotics and consequently causes therapeutic failures and the emergence of resistant phenotypes. From the data recorded in this work about the formation and eradication of biofilms, it can be deduced that PSA not only reduced or even inhibited the formation of biofilms by the *E. coli* AG102 strain, but also destroyed the biofilms that had already been formed (Figures 2 and 3). These results lead us to assert that PSA exhibits excellent efficiency in inhibiting biofilm formation and is capable of rapidly and effectively disrupting biofilm architecture. The ability of an antimicrobial agent to penetrate and disperse the biofilm confirms its potency and efficacy because the biofilm layer is an impermeable barrier to many antibiotics [44]. This effectiveness would be closely linked to the richness in secondary metabolites of PSA. Indeed, several authors have demonstrated the effectiveness of terpenes, polyphenols, and flavonoids in inhibiting the formation of biofilms, their stability once formed, but also on the functioning of the molecules involved in Quorum Sensing [20, 45, 46].

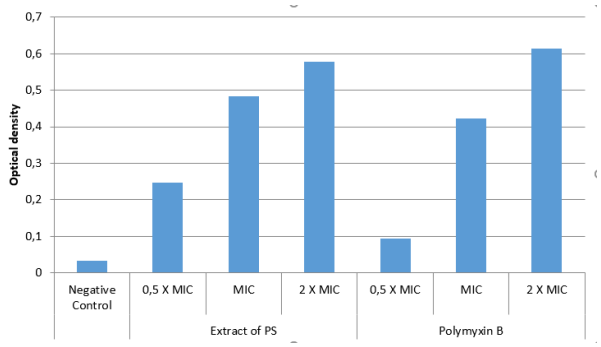

Figure 1. Optical density of the medium inoculated with *E. coli* AG102 and treated with the methanol extract of *P. sycophylla.*
MIC : Minimal Inhibitory Concentration ; PS : *Psychotria sycophylla ; Extract of PS or PSA: methanol extract of the aerial part of Psychotria sycophylla.*

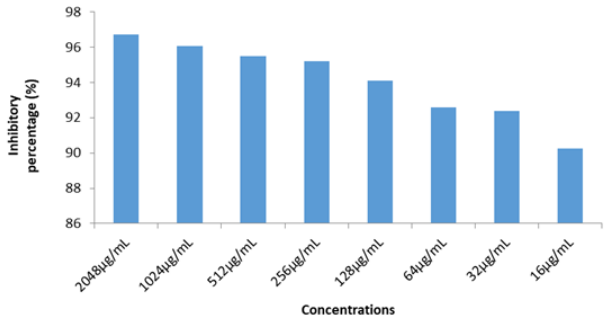

Figure 2. Inhibition in the formation of biofilm of *E. coli* AG102 treated by PSA

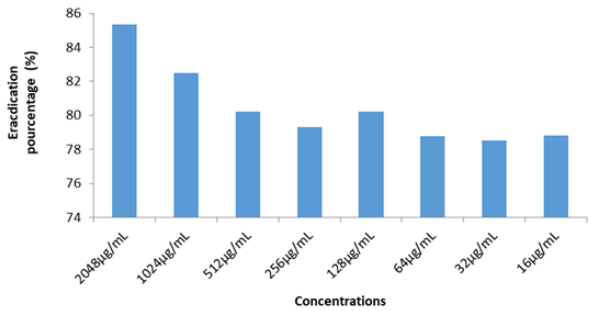

Figure 3. Eradication of biofilm formed by AG102 by PSA

Table 1. Antibacterial activity of PSA in absence and presence of PAβN

Bacterial strains	PSA		CHL		PAβN
	-	+	-	+	
Escherichia coli					
ATCC8739	512	64 (8)	8	2 (4)	>128
AG102	256	64 (4)	64	4 (16)	>128
Klebsiella pneumoniae					
ATCC11296	256	2 (128)	8	8 (1)	>128
KP55	128	16 (8)	32	32 (1)	>128
Enterobacter aerogenes					
ATCC13048	256	16 (8)	8	8 (8)	>128
CM64	512	32 (16)	128	4 (32)	>128
Providencia stuartii					
ATCC29916	256	64 (4)	16	8 (2)	>128
PS2636	4	4 (1)	32	4 (8)	>128
Staphylococcus aureus					
ATCC25923	256	8 (32)	<0,5	<0,125 (>4)	>128
MRSA3	256	16 (16)	2	<0,125 (>16)	>128

+ : Presence of PAβN; - : Absence of PAβN ; () : AIF « Activity improvement factor »; PSA: methanol extract from the aerial parts of *Psychotria sycophylla*.

Conclusion

This work presents results that would bode well for the use of the extract of in the fight against infections caused by MDR bacteria. Most interestingly, the cellular modes of action of PSA are presented for the first time in this work. To make these results more specific in the development of a new Improved Traditional Medicine (ITM), cytotoxicity as well as acute and subacute toxicity tests could be studied.

Abbreviations

AIF : Activity improvement factor; ATCC: American Type Culture Collection; BEC: Biofilm Eradication Concentration; CFU: Colony Forming Unit; CHL: Chloramphenicol; DMSO: Dimethyl sulfoxide; DNA: Deoxyribonucleic Acid; *E. aerogenes*: *Enterobacter aerogenes*; *E. coli: Escherichia coli*; INT: p-iodonitrotetrazolium chloride ≥ 97% (INT, Sigma-Aldrich); ITM: Improved Traditional Medicine; *K. pneumoniae*: *Klebsiella pneumonia*; MBIC50: Minimum Biofilm Inhibitory Concentrations 50%; MDR: Multidrug resistant; MFS: Major Facilitator Superfamily; MHB: Mueller Hinton Broth; MIC: Minimal Inhibitory Concentration; OD: Optical Density; PAβN : Phenylalanine arginine-β- naphthylamide; *P. aeruginosa*: *Pseudomonas aeruginosa*; PBS : Phosphate Buffer Solution; PSA, methanol extract from the aerial parts of Psychotria sycophylla; *P. stuartii* : *Providencia stuartii* PolyB: Polymixin B; *P. sycophylla : Psychotria sycophylla*; RA: Reference antibiotic; RNA: Ribonucleic acid; RND: Résistance-Nodulation-Division; *S. aureus* : *Staphylococcus aureus.*

Authors' Contribution

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

Acknowledgments

We are grateful to the Alexander von Humboldt foundtaion and the Ministry of Scientific Research and Innovation for financing some consumables used in this work.

Conflict of interest

The authors declare no conflict of interest

Article history:

Received: July 17, 2022
Received in revised form: 01 September 2022
Accepted: 05 September 2022
Available online: 05 September 2022

References

1. Astagneau P, Ancelle T. 2011. Surveillance épidémiologique : ´ principes, méthodes et applications en santé publique. *Medecine Sciences Publications* p360. Lavoisier : Paris, France.
2. Lemaoui C, Layaida H, Badi A, Foudi N. 2017. Stratégies actuelles de lutte contre la résistance aux antibiotiques. *J Anti-infect.* 19(1):12-19.
3. World Health Organization (WHO). 2017. Antimicrobial Resistance, World Health Organization, Geneva, Switzerland, http://www.who.int/mediacentre/news/releases/2017/bacteriaantibiotics-needed/en/
4. CDC (Centres for Disease Control and Prevention). (2013). *Antibiotic resistance threats in the United States, 2013*.U.S. Department of Health and Human Services, p113.
5. E. Cerceo E, Deitelzweig SB, Sherman BM, Amin AN. 2016. Multidrug-resistant gram-negative bacterial infections in the hospital setting: overview, implications for clinical practice, and emerging treatment options. *Microb Drug Resist.* 22(5): 412–431.
6. Lomovskaya OO, Bostian KA. 2006. Practical applications and feasibility of efflux Pump inhibitors in the clinic–a vision for applied use. *Biochem Pharmacol.* 71:910–918.
7. Blanco P, Hernando-Amado S, Reales-Calderon JA, Corona F, Lira F, Alcalde-Rico M, Bernardini A, Sanchez MB, Martinez JL. 2016. Bacterial Multidrug Efflux Pumps: Much More Than Antibiotic Resistance Determinants. *Microorganisms*. 4: 14
8. Tchinda CF, Voukeng IK, Beng VP. Kuete V. 2016. Antibacterial activities of the methanol extracts of *Albizia adianthifolia, Alchornea laxiflora, Laportea ovalifolia* and three other Cameroonian plants against multidrug resistant gram-negative bacteria. *Saudi J Biol Sci.* 24:950-955.
9. Mbaveng AT, Sandjo LP, Tankeo SB, Ndifor AR, Pantaleon A, Nagdjui BT, Kuete V. 2015. Antibacterial activity of nineteen selected natural products against multidrug resistant Gram-negative phenotypes. *Springerplus.* 4, 823.
10. 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*. 16:388
11. Komguem J, Meli AL, Manfouo RN, Lontsi D, Ngounou FN, Kuete V, Kamdem HW, Tane P, Ngadjui BT, Sondengam BL, Tane P, Connolly, J. D. 2005. Xanthones from *Garcinia smeathmannii* (Oliver) and their antimicrobial activity. *Phytochemistry.* 66: 1713-1717.
12. Mbaveng AT, Ndontsa BL, Kuete V, Nguekeu YMM, Celik I, Mbouangouere R, Tane P, Efferth T. 2018. A naturally occuring triterpene saponin ardisiacrispin B displayed cytotoxic effects in multi-factorial drug resistant cancer cells via ferroptotic and apoptotic cell death. *Phytomedicine.* 43:78-85
13. Kuete V, Sandjo LP, Djeussi DE, Zeino M, Kwamou GM, Ngadjui B, Efferth T. 2014. Cytotoxic flavonoids and isoflavonoids from *Erythrina sigmoidea* towards multifactorial drug resistant cancer cells. *Invest New Drugs* .32:1053–1062
14. 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.
15. Dzotam J.K, Kuete V. 2017. Antibacterial and antibiotic-modifying activity of methanol extracts from six cameroonian food plants against multidrug-resistant enteric. *BioMed Res Int*. 2017: 1583510.
16. 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
17. Manekeng HT, Mbaveng AT, Nguenang GS, Seukep JA, Wamba BEN, Nayim P, Yinkfu NR, Kuete V. 2018. Anti-staphylococcal and antibiotic-potentiating activities of seven Cameroonian edible plants against resistant phenotypes. *Invest Med Chem Pharmacol.* 1(1):7.
18. 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.
19. Ngongang FCM, Fankam AG, Mbaveng AT, Wamba BEN, Nayim P, Beng VP and Kuete V. 2020. Methanol Extracts from Manilkara zapota with moderate antibacterial activity displayed strong antibiotic-modulating effects against multidrug-resistant phenotypes. *Invest Med Chem Pharmacol.* 3(1):37
20. Tchinda, C.F., Voukeng, I.K., Veronique, P. Beng V.P., Kuete, V. 2020. Mechanisms of action of roots crude extract and adianthifolioside GS1 from Albizia adianthifolia (Fabaceae) against MDR Gram negative enteric bacteria. *Invest Med Chem Pharmacol.* 3(2):46
21. 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.
22. Youmbi LM, Atontsa BCK, Tankeo SB, Wamba BEN, Nayim P, Nganou BK, Bitchagno GTM, Simo IK, Mpetga JDS, Penlap VB and Kuete V. 2020. Antibacterial potential and mechanism of action of botanicals and phytochemicals from Stachytarpheta cayennensis (Verbenaceae) against Gram negative multidrugresistant phenotypes expressing efflux pumps. *Invest Med Chem Pharmacol.* 3(1):35
23. 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.
24. Guefack MGF, Tankeo SB, Ngaffo CMN, Bonsou IN, Nayim P, Wamba BEN, Kuete V, Mbaveng AT. 2022. Antibacterial and antibiotic-modulating activities of Rhinella jimi and three other animal extracts against multidrug-resistant Gram-negative phenotypes. *Invest Med Chem Pharmacol.* 5(1):61
25. Demgne OMF, Tchinda CF, Mbaveng AT, Beng VP, Kuete V. 2022. Antibacterial and antibiotic-potentiating activities of nine Cameroonian medicinal plants against multidrug-resistant bacteria expressing active efflux pumps. *Invest Med Chem Pharmacol.* 5(1):58.
26. Mambe FT, Tchinda CF, Wamba BEN, Nayim P, Ashu F, Manekeng HT, Beng VP, Kuete V. 2022. Modes of action of the methanol extract and 3-Oβ-galactopyranosyl-

(1→4)-β-D-galactopyranosyl.-oleanolic acid from Acacia polyacantha against multiresistant Gram-negative bacteria. *Invest Med Chem Pharmacol.* 5(1):60.
27. 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..
28. Kofogoue AC, Tchinda CF, Kuete V. (2022). Antibacterial and antibiotic-potentiating activities of Desmodium uncinatum, Neoboutonia glabrescens, Ternstroemia cameroonensis and eight other Cameroonian medicinal plants against multidrug resistant bacteria expressing active efflux pumps. *Invest Med Chem Pharmacol.* 5(1):62.
29. Wamba NEB, Nayim P, Mbaveng TA, Voukeng KI, Dzotam KJ, Ngalani OJT, Kuete V. 2018. Syzygium jambos displayed antibacterial and antibiotic-modulating activities against resistant phenotypes. *Evid Based Complement Alternat Med*. 2018: 5124735.
30. Yang H, Zhang H, Yang C, Chen Y (2016) Chemical constituents of plants from the *genus Psychotria*. *Chem Biodivers* 13(7):807–820.
31. Demgne OMF, MbougniaJFT, Seukep AJ, Mbaveng AT, Tene M, Nayim P, · Wamba BEN, Guefack MGF, Beng VP, Tane P, Kuete V. 2021. Antibacterial phytocomplexes and compounds from *Psychotria sycophylla* (Rubiaceae) against drug-resistant bacteria. *Advances in Traditional Medicine* https://doi.org/10.1007/s13596-021-00608-0
32. Kuete V, Wabo FG, Ngameni B, Mbaveng TA, Metuno R, Etoa F-X, Lall N. 2007. Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* (Ixonanthaceae). *J Ethnopharmacol.* 114(1):54–60
33. Newton SM, Lau CG, Gurcha SS, Besra GS, Wright CW. 2002. The evaluation of forty-three plant species for *in vitro* antimycobacterial activities, isolation of active constituents from *psoraleac corylifolia* and *sanguinaria cadensis*. *J Ethnopharmacol.* 79: 57-63.
34. Leejae S, Taylor PW, Voravuthikunchai, SP. 2013. Antibacterial mechanisms of rhodomyrtone against important hospital-acquired antibiotic-resistant pathogenic bacteria. *J Medl Microbiol*. 62: 78–85.
35. Chaib K, Kouidhi B, Jraf H, Mahdouani K, Bakhrouf A. 2011. Antibacterial activity of thymoquinone, an active principle of Nigella sativa and its potency to prevent bacterial biofilm formation. *BMC Complement Altern Med.* 11:29- 34
36. Tegos PG, Haynes M, Strouse, JJ, Khan TMM, Bologa GC, Oprea, IT, Sklar AL. 2011. Microbial efflux pump inhibition: tactics and strategies. *Curr Pharmaceut Des.* 17(13):1291-1302.
37. Van Bambeke F, Pagès JM, Lee VJ. 2010. Inhbitor of bacterial efflux pumps as adjuvants in antibacterial therapy and diagnostic tools for detection of resistance by efflux. *Front Anti-infect Drug Discov.* 1: 138-175.
38. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Albert-Franco S, Ngadjui BT, Pagès J. M. 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.
39. Laudy AE, EwaKulin´ska E, Tyski S. 2017. The Impact of Efflux Pump Inhibitors on the Activity of Selected Non-Antibiotic Medicinal Products against Gram-Negative Bacteria. *Molecules.* 22: 114.
40. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. (2020). Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J Pharm Anal.* 10(4):277-290.
41. Sampathkumar B, Khachatourians GG, Korber D R. 2003**.** High pH during trisodium phosphate treatment causes membrane damage and destruction of *Salmonella enterica* serovar Enteritidis. *Appl Environ Microbiol.* 69: 122–129.
42. Zhang J, Ye KP, Zhang X, Pan DD, Sun YY, Cao JX. 2017. Antibacterial Activity and Mechanism of Action of Black Pepper Essential Oil on Meat-Borne *Escherichia coli*. *Front Microbiol.* 7: 1-10.
43. Kohanski MA, Dwyer DJ, Collins JJ. 2010. How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology*, 8: 423-435.
44. Ahmed A, Khan AK, Anwar A, Ali SA, Shah MR. 2016. Biofilm inhibitory effect of chlorhexidine conjugated gold nanoparticles against *Klebsiella pneumoniae*. *Microbial Pathology*, 98: 50-56.
45. Murugan K, Sangeetha S, Kalyanasundaram VB, Saleh AS. 2013. *In vitro* and in silico screening for *Andrographis paniculata* quorum sensing mimics: New therapeutic leads for cystic fibrosis *Pseudomonas aeruginosa* biofilms. *Plant Omics*, 6(5):340-346.
46. Onsare JG, Arora DS. 2015. Antibiofilm potential of flavonoids extracted from *Moringa oleifera* seed coat against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*. *J Appl Microbiol.* 118(2):313-325.