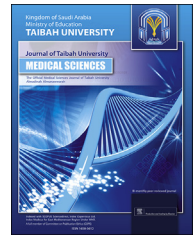




Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com



Original Article

Antibacterial activities of methanol extracts from *Alchornea cordifolia* and four other Cameroonian plants against MDR phenotypes

Flora T. Mambe, MSc^a, Igor K. Voukeng, MSc^b, Veronique P. Beng, PhD^a and Victor Kuete, PhD^{b,*}

^a Department of Biochemistry, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

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

Received 31 August 2015; revised 3 December 2015; accepted 8 December 2015; Available online 14 January 2016



المخلص

أهداف البحث: برزت الالتهابات البكتيرية المقاومة للعديد من الأدوية، خاصة التي تنجم من الأنماط سلبية الغرام، كواحدة من المخاوف الصحية الرئيسية في جميع أنحاء العالم. في هذه الدراسة؛ تم التحقق في النشاط المضاد للبكتيريا لمستخلصات المنثول لخمس من النباتات الطبية الكامرونية؛ الكورنيا كورديفوليا، واروماسناكس سيبسيوسا، ولابورتيا ايستوانس، وبينسيتوم بوربيريوم وسباتوديا كامبانولاتا ضد ١٥ بكتيريا سلبية الغرام من ضمنها الأنماط المقاومة للعديد من الأدوية.

طرق البحث: تم استخدام طريقة التخفيف الصغرى للسائل لتحديد الحد الأدنى من الكثافة المثبطة، والحد الأدنى من كثافة مبيد البكتيريا لجميع العينات. تم استخدام الطرق القياسية النباتية للفحص الأولي النباتي للمستخلصات النباتية.

النتائج: أظهر التحليل الكيميائي النباتي وجود البولي فينول، والعفص، والتربينات والستيرول في جميع المستخلصات المدروسة. تم تحديد الطبقات الكيميائية الأخرى من المركبات الثانوية بعناية. كما أظهرت النتائج أفضل الأنشطة المضادة للبكتيريا (تتراوح بين ٦٤-١٠٢٤ ميكروجرام/مل)، التي تم الحصول عليها ضد الـ ١٥ نوع من البكتيريا المختبرة، من أوراق ٩٣,٣٪، ولحاء ٨٦,٧٪ وجذور ٨٠٪. مستخلصات الكورنيا كورديفوليا، بالإضافة إلى لابورتيا ايستوانس ٨٦,٧٪ وبينسيتوم بوربيريوم ٦٦,٧٪. أقل قيمة للحد الأدنى من الكثافة المثبطة ٦٤ ميكروجرام/مل سجلت مع لحاء الكورنيا كورديفوليا ضد سودوموناس إيروجنوسا.

الاستنتاجات: تقدم هذه الدراسة نظرة عميقة إلى إمكانية استخدام النباتات المدروسة، خاصة الكورنيا كورديفوليا ولابورتيا ايستوانس في السيطرة على الالتهابات البكتيرية السلبية الغرام بما فيها الأنواع المقاومة للعديد من الأدوية.

الكلمات المفتاحية: الكورنيا كورديفوليا؛ البكتيريا سلبية الغرام؛ لابورتيا ايستوانس؛ الالتهابات البكتيرية المقاومة للعديد من الأدوية؛ النباتات الطبية

Abstract

Objectives: Multidrug-resistant (MDR) bacterial infections, especially those caused by Gram-negative phenotypes, have emerged as one of the major health concerns worldwide. In the present study, we investigated the antibacterial activity of methanol extracts from five Cameroonian medicinal plants (*Alchornea cordifolia*, *Eremomastax speciosa*, *Laportea aestuans*, *Pennisetum purpureum* and *Spathodea campanulata*) against 15 Gram-negative bacteria that included MDR phenotypes.

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

Results: Phytochemical analysis showed the presence of polyphenols, tannins, triterpenes and sterols in all of the studied extracts. Other chemical classes of secondary metabolites were selectively identified. The best antibacterial activities (MICs ranges of 64–1024 µg/mL) obtained against the 15 tested bacteria were found in extracts of leaves (93.3%), bark (86.7%) and roots (80%) of *A. cordifolia* as well as extracts of *L. aestuans* (86.7%) and *P. purpureum* (66.7%). The lowest MIC value of 64 µg/mL was recorded for the *A. cordifolia* bark extract against *Pseudomonas aeruginosa* PA01.

Conclusions: The findings of this study provide deep insights into the possible use of the studied plants, especially *A. cordifolia* and *L. aestuans*, for the control of

* Corresponding address: Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon.
E-mail: kuetevictor@yahoo.fr (V. Kuete)

Peer review under responsibility of Taibah University.



Production and hosting by Elsevier

Gram-negative bacterial infections, especially against MDR species.

Keywords: *Alchornea cordifolia*; Gram-negative bacteria; *Laportea aestuans*; MDR bacteria; Medicinal plants

© 2016 The Authors.

Production and hosting by Elsevier Ltd on behalf of Taibah University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Multidrug-resistant (MDR) bacterial infections, especially those caused by Gram-negative microorganisms, have emerged as a global health concern.¹ Efflux pumps that belong to the resistance-nodulation-cell division (RND) family of tripartite efflux pumps are largely involved in the multidrug resistance of Gram-negative bacteria.² The scarcity of novel antibacterials to combat bacterial MDR phenotypes drives the search for novel drugs from natural sources. With regards to the variety and diversity of secondary metabolites, medicinal plants constitute a good source of antibacterials.³ In the past five years, studies have intensified on the search of phytochemicals from African plants to tackle MDR bacterial infections. Some of the most active plants against MDR Gram-negative bacteria include *Beilschmiedia cinnamomea*, *Fagara xanthoxyloides*, *Olex subscorpioidea*,⁴ *Solanum nigrum*,⁵ *Vernonia amygdalina*,⁶ *Peperomia fernandopoiana*,⁷ *Capsicum frutescens*,⁸ *Allanblackia gabonensis*, *Combretum molle*, *Gladiolus quartianus*,⁹ and *Fagara tessmannii*.¹⁰ In our continuous search for antibacterials from African flora, we designed the present study to investigate the *in vitro* antibacterial activity of the methanol extracts of five Cameroonian medicinal plants, that is, *Alchornea cordifolia* (Schum. & Thonn.) Müll.-Arg. (Euphorbiaceae), *Eremomastax speciosa* (Hochst.) Cufod. (Acanthaceae), *Laportea aestuans* (Linn.) Chew (Urticaceae), *Pennisetum purpureum* Schumach. (Poaceae) and *Spathodea campanulata* P. Beauv. (Bignoniaceae), against MDR Gram-negative bacteria.

Materials and Methods

Plant materials and extraction

Different parts of the selected plants were collected from various regions of Cameroon in January 2014. These included leaves, bark and roots of *A. cordifolia* and *S. campanulata* and whole plants of *E. speciosa*, *L. aestuans* and *P. purpureum*. The plants were identified at the Cameroon National Herbarium (Yaounde, Cameroon) where voucher specimens were deposited under the reference numbers shown in Table 1. Each plant sample was air-dried and powdered. Methanol is known to be powerful extraction solvent of antibacterial compounds from plants.³ Therefore, the obtained powder (200 g) was extracted with methanol (MeOH 100%; 1 L) for 48 h at room temperature. The extract was then concentrated at 68 °C

under reduced pressure to obtain the residues that constituted the crude extract. All of the extracts were kept at 4 °C until further use.

Ethics statement

No specific permits were required from the local authority for the collection of the studied plants. The medicinal plants chosen for this study are not endangered or protected by the laws of the country.

Preliminary phytochemical screening

The major phytochemical classes, such as alkaloids, triterpenes, flavonoids, anthraquinones, polyphenols, sterols, coumarins, saponins and tannins, in the plant extracts were detected according to commonly described phytochemical methods.¹¹

Antimicrobial assays

Chemicals for the antimicrobial assay

Chloramphenicol (CHL; Sigma–Aldrich, St Quentin Fallavier, France) was used as a reference antibiotic (RA). In addition, *p*-Iodonitrotetrazolium chloride (INT) was used as a microbial growth indicator.^{12,13}

Microbial strains and culture media

The studied microorganisms included sensitive and resistant strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli* and *Providencia stuartii* obtained from the American Type Culture Collection (ATCC) as well as clinical strains. Their bacterial features were previously reported.^{8,14,15} Nutrient agars were used for the activation of the tested Gram-negative bacteria, and Mueller Hinton Broth was used for the antibacterial assays.¹⁶

INT colourimetric assay for MIC and MBC determinations

The MIC determinations on the tested bacteria were conducted using the rapid *p*-Iodonitrotetrazolium chloride (INT) colourimetric assay according to the described methods¹² with certain modifications.^{17,18} The test samples and the RA were first dissolved in DMSO/Mueller Hinton Broth (MHB). The final concentration of DMSO was lower than 2.5% and did not affect microbial growth.^{19,20} The solution obtained was then added to Mueller Hinton Broth and serially diluted two-fold (in a 96-well microplate). One-hundred microlitres (100 µL) of an inoculum of 1.5×10^6 CFU/mL prepared in an appropriate broth was then added.^{17,18} The plates were covered with a sterile plate sealer and then agitated to mix the contents of the wells using a plate shaker and incubated at 37 °C for 18 h. The assay was performed in triplicate. Wells that contained adequate broth, 100 µL of the inoculum and DMSO to a final concentration of 2.5% served as the negative control. The MIC of samples was detected after 18 h of incubation at 37 °C and after the addition of 40 µL (0.2 mg/mL) of INT and were incubated at 37 °C for 30 min. Viable

Table 1: Information about the studied plants.

Species (family); Voucher Number ^a	Traditional uses	Parts traditionally used	Bioactive or potentially bioactive components	Bioactivity of the crude extract
<i>Alchornea cordifolia</i> (Schum. & Thonn.) Müll.-Arg. (Euphorbiaceae); 9657/SRF/Cam	To treat rheumatic pains, fever, wounds, diarrhoea, convulsions, coughs, gonorrhoea, yaws, ulcer, rheumatic pains, bronchial troubles ^{39,40}	Leaves, seeds, bark and roots	Alchornine, alchorneinone, gentisnic acid and yohimbine ³⁹	Crude extract showed spasmolytic, ³⁹ anti-inflammatory, ⁴¹ antimicrobial, ³¹ anti-diarrhoeal ⁴² and analgesic ⁴³ activities
<i>Eremomastax speciosa</i> (Hochst.) Cufod. (Acanthaceae); 24165/SRF/Cam	Haematopoietic, antidiarrhoeal, antiulcer, treatment of female infertility, dysentery, anaemia, irregular menstruation, fracture, haemorrhoids and urinary tract infections ^{34,35}	Whole plant	Not reported	Antimicrobial effect of crude extract: (Q); <i>Ec</i> , <i>Sa</i> , <i>Ca</i> ³⁶
<i>Laportea aestuans</i> (Linn.) Chew (Urticaceae); 34812/HNC	Antihelmintic, treatment of headaches, syphilitic yaws, fever, gonorrhoea, rheumatism, menopausal disorder, antidote, asthma, hypertension, stomach-ache, diarrhoea, wounds ⁴⁴	Leaves, roots	Vanilic acid, gallic acid, ferulic acid, (6)-gingerol, capsaicin, rosemamic acid, tannic acid, p-coumaric acid, caffeic acid, scopoletin, catechin, resveratrol, genistein, apigenin, kaempferol, epicatechin, epigallocatechin, ellagic acid, myricitin acid, quercetin- 3, 7, 4-trimethyl ether, quercetin-3,7,3',4'-trimethyl ether, artemetin, kaempferol-arabinoside, quercitrin, isoquercitrin, naringin, rutin, hesperidin, limonene, alpha pinene, beta pinene, cis-ocimene, myrcene, citronellol, neryl acetate, malvidine, lycopene, carotene, lutein, hispogenin, diosgenin, neochlorogenin, hecogenin ^{45,46}	Antimicrobial effect of crude extract (Q); <i>K</i> , <i>Ec</i> , <i>Sa</i> , <i>Bs</i> , <i>St</i> , <i>Pa</i> , <i>Ca</i> ^{33,45}
<i>Pennisetum purpureum</i> Schumach. (Poaceae); 12525/SRF/Cam	Diuretic, antivenomous, Treatment of measles, wound healing ³⁷	Whole plant	Not reported	Antimicrobial effect of EO: (Q); <i>Ec</i> , <i>Pa</i> ³⁷
<i>Spathodea campanulata</i> P. Beauv. (Bignoniaceae) 22791/SRF/Cam	Treatment of mental disorders, malaria, haemorrhoids, bacterial infections, HIV, poor blood circulation, gastro-intestinal diseases, urinary tract disorders ⁴⁷	Flowers, leaves, bark	Carbohydrates, alkaloids, tannins, iridoid glucoside, phydroxy-benzoic acid, methyl p-hydroxy-benzoate ^{47,48}	Antimicrobial effect of crude extract (Q): <i>Pv</i> , <i>Ec</i> , <i>Kp</i> ³⁸

^a (HNC): Cameroon National Herbarium; (SRF/Cam): Société des Réserves Forestières du Cameroun; (Q): qualitative activity based on the inhibition zone. EO: Essential oil; *Sa*: *Staphylococcus aureus*; *Ec*: *Escherichia coli*; *Bs*: *Bacillus subtilis*; *Kp*: *Klebsiella pneumoniae*; *St*: *Salmonella typhi*; *Pa*: *Pseudomonas aeruginosa*; *Se*: *Salmonella enterica*; *Pv*: *Proteus vulgaris*; *Ca*: *Candida albicans*; Underline: Tested sample from the plant.

bacteria reduced the yellow dye to pink. The MIC was defined as the sample concentration that prevented the colour change of the medium and exhibited a complete inhibition of the microbial growth.¹² The MBC was determined by adding 50 µL aliquots of the preparations that did not show any growth after the incubation during the MIC assays in 150 µL of adequate broth. These preparations were incubated at 37 °C for 48 h. The MBC was regarded as the lowest extract concentration that did

not produce a colour change after the addition of INT, as mentioned above.^{17,18}

Results

In the present study, the qualitative phytochemical composition and antibacterial activity of extracts from five Cameroonian medicinal plants were determined. The results

of the phytochemical composition of the tested plants are summarized in Table 2. It appears that all of the extracts contained polyphenols, tannins, triterpenes and sterols. Apart from the leaf extracts of *L. aestuans* and *S. campanulata*, all of the other samples also contained alkaloids, whilst anthraquinone was not detected; saponins, flavonoids and coumarins were selectively distributed. The antibacterial results depicted in Table 3 showed that all of the tested extracts displayed selective activities. The best activity with MIC values that ranged from 64 to 1024 µg/mL were obtained with leaf [14/15 (93.3% of the tested bacteria)], bark [13/15 (86.7%)] and root [12/15 (80%)] extracts of *A. cordifolia*, as well as with *L. aestuans* [13/15 (86.7%)] and *P. purpureum* [10/15 (66.7%)] extracts. The lowest antibacterial spectra were obtained with leaf and bark extracts of *S. campanulata* and *E. speciosa* [6/15 (40% of the tested bacteria)] and the root extract of *S. campanulata* [3/15 (20%)]. The lowest MIC value (64 µg/mL) was recorded with the *A. cordifolia* bark extract on *P. aeruginosa* PA01. However, none of the extracts were as active as the established antibacterial compound, that is, chloramphenicol. In general, the tested extracts exerted bacteriostatic effects with a MBC/MIC ratio above 4.

Discussion

Medicinal plants constitute an important source of bioactive compounds because of the chemical diversity found in several species. In recent years, certain plants have been successfully evaluated for their antibacterial activity worldwide. The rationale of this study is based on the fact that various parts of the abovementioned medicinal plants are traditionally used to treat bacterial infections in addition to other ailments, as depicted in Table 1. The parts that are traditionally used in the treatment of bacterial infections include leaves, seeds, bark and roots of *A. cordifolia*; the whole plant of *E. speciosa* and *P. purpureum*; the leaves and roots of *L. aestuans*; as well as the flowers, leaves and bark of *S. campanulata*. Differences in the antibacterial activities were detected among the extracts. Several molecules that belong to the classes of secondary

metabolites detected in this study were found to be active against pathogenic microorganisms.^{3,21} The presence of such metabolites in the studied plant extracts can provide a preliminary explanation on their antibacterial activities. The differences in the antibacterial activities of the extracts could be due to the different chemical composition and the distinct mechanisms of action of their bioactive constituents.³ According to Kuete et al.,^{21,22} the antibacterial activity of a plant extract is considered to be significant when the MIC values are below 100 µg/mL, moderate when $100 \leq \text{MIC} \leq 625$ µg/mL and weak when $\text{MIC} > 625$ µg/mL. Consequently, the activity (MIC of 64 µg/mL) observed with *A. cordifolia* bark extract against *P. aeruginosa* PA01 can be considered to be significant. Moderate antibacterial activities ($100 \leq \text{MIC} \leq 625$ µg/mL) were obtained with the majority of the extracts (Table 2). However, the obtained MIC values are significant when considering the medicinal importance of the tested MDR bacteria.^{23–29} Both *K. pneumoniae* KP55 and KP63 were reported to be resistant to most of the commonly used antibiotics and showed high resistance to ampicillin, ceftazidime and aztreonam, with MIC values of up to 512 µg/mL.²⁴ In the present study, lower MIC values were obtained with the *A. cordifolia* bark extract (128 µg/mL) and *L. aestuans* extract (256 µg/mL) against *K. pneumoniae* KP63. In addition, MIC values of 512 µg/mL were also obtained with these extracts against *K. pneumoniae* KP63. Such activities recorded with crude extracts against MDR bacteria could be considered interesting. *P. aeruginosa* is an important nosocomial pathogen that is highly resistant to commonly used antibiotics and causes a wide spectrum of infections that might lead to substantial morbidity and mortality.³⁰ Interestingly, the bark extract of *A. cordifolia* had a MIC value of 64 µg/mL against *P. aeruginosa* PA01; this outcome clearly highlights the ability of this extract to fight MDR bacterial species. Other MDR bacteria of the Enterobacteriaceae family tested in this study, such as *E. aerogenes*, *E. coli* and *P. stuartii*, have also been classified as antimicrobial-resistant organisms of medical concern.²⁸ Interestingly, it was observed that all of those MDR strains were susceptible to the studied extracts,

Table 2: Phytochemical composition of the plant extracts.

Classes	Studied plants (% yield)* and composition								
	<i>Alchornea cordifolia</i>			<i>Eremomastax speciosa</i>	<i>Laportea aestuans</i>	<i>Pennisetum purpureum</i>	<i>Spathodea campanulata</i>		
	L (7.84%)	B (11.32%)	R (6.23%)	W (14.69%)	W (8.82%)	W (9.05%)	L (16.13%)	B (15.27%)	R (11.41%)
Alkaloids	+	+	+	+	–	+	+	+	–
Polyphenols	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	–	–	–
Anthraquinones	–	–	–	–	–	–	–	–	–
Coumarins	–	–	–	–	+	–	–	–	–
Tannins	+	+	+	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+	+	+	+
Sterols	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	–	–

(–): Absent; (+): Present; * yield calculated as the ratio of the mass of the obtained methanol extract/mass of the plant powder. The tested extracts were obtained from L: Leaves; B: bark; R: roots; W: whole plant.

Table 3: MICs and MBCs in µg/mL of methanol extracts from the studied plants and chloramphenicol.

Bacterial strains	Tested samples MIC and MBC (in bracket) values (µg/mL)										
	<i>Alchornea cordifolia</i>			<i>Eremomastax speciosa</i>	<i>Laportea aestuans</i>	<i>Pennisetum purpureum</i>	<i>Spathodea campanulata</i>			CHL	
	L	B	R	W	W	W	L	B	R		
<i>Escherichia coli</i>											
ATCC8739	512 (–)	256 (–)	1024 (–)	1024 (–)	1024 (–)	512 (–)	512 (–)	512 (–)	–	–	2 (64)
ATCC10536	512 (–)	256 (–)	1024 (–)	1024 (–)	1024 (–)	1024 (–)	1024 (–)	–	–	–	2 (32)
AG100ATet	1024 (–)	1024 (–)	1024 (–)	–	256 (–)	1024 (–)	1024 (–)	–	512 (–)	–	32 (256)
AG102	512 (–)	1024 (–)	1024 (–)	–	512 (–)	512 (–)	512 (–)	512 (–)	512 (–)	–	32 (256)
<i>Enterobacter aerogenes</i>											
ATCC13048	512 (–)	1024 (–)	1024 (–)	1024 (–)	512 (–)	512 (–)	512 (–)	–	1024 (–)	–	16 (128)
CM64	256 (–)	512 (–)	512 (–)	–	512 (–)	512 (–)	512 (–)	512 (–)	1024 (–)	–	256 (–)
EA 27	512 (–)	–	1024 (–)	–	512 (–)	–	–	–	–	–	32 (256)
EA 289	128 (–)	512 (–)	128 (–)	1024 (–)	256 (–)	128 (512)	128 (512)	1024 (–)	1024 (–)	512 (–)	32 (256)
<i>Klebsiella pneumoniae</i>											
ATCC11296	512 (–)	128 (–)	1024 (–)	–	–	512 (–)	512 (–)	1024 (–)	1024 (–)	1024 (–)	32 (256)
KP55	1024 (–)	512 (–)	1024 (–)	–	512 (–)	–	–	–	–	–	64 (256)
KP63	512 (–)	128 (–)	–	–	256 (–)	512 (–)	512 (–)	–	–	–	32 (256)
<i>Providencia stuartii</i>											
ATCC29916	512 (–)	512 (–)	512 (–)	1024 (–)	256 (–)	–	–	–	–	–	64 (256)
NEA 16	512 (–)	256 (–)	512 (–)	–	512 (–)	–	–	512 (–)	–	1024 (–)	64 (256)
<i>Pseudomonas aeruginosa</i>											
PA01	512 (–)	64 (–)	–	1024 (–)	1024 (–)	512 (–)	512 (–)	–	–	–	64 (–)
PA124	–	–	–	–	–	–	–	–	–	–	256 (–)

–: > 1024 (MIC) or not determined: The tested extracts were obtained from L: Leaves; B: bark; R: roots; W: whole plant; CHL: chloramphenicol.

especially those from *A. cordifolia* and *L. aestuans*. Therefore, the overall antibacterial activity of *A. cordifolia* and *L. aestuans* could be considered to be significant. Likewise, it has been demonstrated that the 50% aqueous-ethanol extract of the leaves of *A. cordifolia* exhibits antibacterial activities against a panel of bacterial and fungal strains.³¹ The reported MIC values varied from 10 to 20 mg/mL against various strains and isolates of Gram-negative bacteria, such as *E. coli*, *P. aeruginosa*, *Enterobacter clacae*, *Citrobacter freundii*, *K. pneumoniae*, *Shigella flexneri*, *Salmonella paratyphi A* and *Acinetobacter baumannii*.³¹ However, the extract was more active against Gram-positive bacteria and had MIC values below 2.5 µg/mL against different strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* as well as against anaerobic bacteria, such as *Peptostreptococcus spp.* and *Staphylococcus assacharolyticus*.³¹ This result is in accordance with the present study because we also found that this plant showed activity against MDR bacterial strains. Nonetheless, it should be observed that better activities were obtained in the present work against Gram-negative bacteria, which suggests that methanol could be a better extraction solvent for antibacterial compounds of *A. cordifolia* than a 50% water–ethanol mixture. This hypothesis is consolidated by the fact that a water extract and ethanol extract of *A. cordifolia* separately showed low or no antibacterial activity against Gram-negative bacteria. Indeed, the reported MIC values of the ethanol extracts were above 11.88 mg/mL against *Helicobacter pylori*, *Salmonella typhi*, *Salmonella enteritidis*, *S. flexneri* and *E. coli*, whilst the water extract displayed MIC values above 18.75 mg/mL, with no activity at up to 300 mg/mL against *S. enteritidis* and *E. coli*.³² Although, the results

obtained in the present study with *A. cordifolia* and *L. aestuans* are mostly moderate, they are similar to previously obtained data with several other Cameroonian plants, such as *O. subscorpioidea*,⁴ *S. nigrum*,⁵ *V. amygdalina*,⁶ *P. fernandopoiana*,⁷ *C. frutescens*,⁸ *A. gabonensis*, *C. molle*, *G. quartianus*,⁹ and *F. tessmannii*.¹⁰

Using an agar diffusion assay, Oloyede and Ayanbadejo have observed that the methanol extract of *L. aestuans* had a moderate antimicrobial activity at 200 mg/mL against a panel of Gram-negative and Gram-positive bacteria as well as fungi, with inhibition zone diameters that varied from 10 mm to 20 mm. In contrast, the values for gentamicin at 10 mg/mL or 70% tioconazole (used as positive controls) varied from 24 to 28 mm.³³ Our data are in accordance with that study because we also observed that the methanol extract of this plant had a moderate activity on the majority of tested Gram-negative bacteria, with MIC values that generally ranged from 256 to 1024 µg/mL (Table 3). Traditionally, *E. speciosa* has been used to heal health conditions related to bacterial infections, such as diarrhoea, ulcers, dysentery and urinary tract infections.^{34,35} However, the antibacterial activity observed in the present study was rather low (MIC values of 1024 µg/mL) or null. This outcome may be due to the fact most of the studied bacteria are MDR phenotypes or to the poor antibacterial potential of this plant. Indeed, the low antibacterial activity of the 95% ethanol extract was also reported by Okokon et al. against *E. coli* and *S. aureus*.³⁶ Similarly, *P. purpureum* and *S. campanulata* used in folk medicine to manage microbial infections also displayed a null or moderate antibacterial activity against the studied bacteria. The reported data are in conformity

with previously documented data because Njoku et al. have demonstrated that *P. purpureum* had a low antibacterial activity against *E. coli* and *P. aeruginosa*,³⁷ whereas Rajesh et al. have reported the low inhibitory effects of *S. campanulata* against *Proteus vulgaris*, *E. coli* and *K. pneumoniae*.³⁸

Conclusion

The results of the present investigation suggest that the extracts of the studied plants, in particular those from *A. cordifolia* and *L. aestuans*, can be used as potential leads to discover new antibacterials to control certain bacterial infections, especially those that involve MDR bacterial species.

Competing interest

The authors declare that there are no conflicts of interest.

Authors' contributions

FTM and IKV performed the study; VK and VPB supervised the work; VK designed the experiments, wrote the manuscript, supervised the work and provided the bacterial strains; all of the authors read and approved the final version of the manuscript.

Acknowledgements

The authors are thankful to the Cameroon National Herbarium for the identification of plants.

References

- Xu ZQ, Flavin MT, Flavin J. Combating multidrug-resistant gram-negative bacterial infections. **Expert Opin Investig Drugs** 2014; 23(2): 163–182.
- Van Bambeke F, Pages JM, Lee VJ. Inhibitors of bacterial efflux pumps as adjuvants in antibiotic treatments and diagnostic tools for detection of resistance by efflux. **Recent Pat Antiinfect Drug Discov** 2006; 1(2): 157–175.
- Cowan MM. Plant products as antimicrobial agents. **Clin Microbiol Rev** 1999; 12(4): 564–582.
- Fankam AG, Kuete V, Voukeng IK, Kuiate JR, Pages JM. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. **BMC Complement Altern Med** 2011; 11: 104.
- Noumedem JA, Mihasan M, Lacmata ST, Stefan M, Kuiate JR, Kuete V. Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria. **BMC Complement Altern Med** 2013; 13: 26.
- Noumedem JA, Mihasan M, Kuiate JR, Stefan M, Cojocar D, Dzoyem JP, Kuete V. *In vitro* antibacterial and antibiotic-potential activities of four edible plants against multidrug-resistant Gram-negative species. **BMC Complement Altern Med** 2013; 13: 190.
- Fankam AG, Kuiate JR, Kuete V. Antibacterial activities of *Beilschmiedia obscura* and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes. **BMC Complement Altern Med** 2014; 14: 241.
- Touani FK, Seukep AJ, Djeussi DE, Fankam AG, Noumedem JA, Kuete V. Antibiotic-potential activities of four Cameroonian dietary plants against multidrug-resistant Gram-negative bacteria expressing efflux pumps. **BMC Complement Altern Med** 2014; 14: 258.
- Fankam AG, Kuiate JR, Kuete V. Antibacterial and antibiotic resistance modifying activity of the extracts from *Allanblackia gabonensis*, *Combretum molle* and *Gladiolus quartianus* against Gram-negative bacteria including multi-drug resistant phenotypes. **BMC Complement Altern Med** 2015; 15: 206.
- Tankeo S, Damen F, Awouafack M, Mpetga J, Tane P, Eloff J, Kuete V. Antibacterial activities of the methanol extracts, fractions and compounds from *Fagara tessmannii*. **J Ethnopharmacol** 2015; 169: 275–279.
- Harbone J, editor. *Phytochemical methods: a guide to modern techniques of plant analysis*. London: Chapman & Hall; 1973.
- Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. **Planta Med** 1998; 64(8): 711–713.
- Mativandela SPN, Lall N, Meyer JJM. Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. **S Afr J Bot** 2006; 72(2): 232–237.
- Lacmata ST, Kuete V, Dzoyem JP, Tankeo SB, Teke GN, Kuiate JR, Pages JM. Antibacterial activities of selected Cameroonian plants and their synergistic effects with antibiotics against bacteria expressing MDR phenotypes. **Evid Based Complement Altern Med** 2012; 2012: 623723.
- Seukep JA, Fankam AG, Djeussi DE, Voukeng IK, Tankeo SB, Noumedem JA, Kuete AH, Kuete V. Antibacterial activities of the methanol extracts of seven Cameroonian dietary plants against bacteria expressing MDR phenotypes. **Springerplus** 2013; 2: 363.
- Kuete V, Kamga J, Sandjo LP, Ngameni B, Poumale HM, Ambassa P, Ngadjui BT. Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita* Vahl. (Moraceae). **BMC Complement Altern Med** 2011; 11: 6.
- Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. Antimicrobial activity of the crude extract, fractions and compounds from stem bark of *Ficus ovata* (Moraceae). **J Ethnopharmacol** 2009; 124(3): 556–561.
- Kuete V, Wansi JD, Mbaveng AT, Kana Sop MM, Tadjong AT, Beng VP, Etoa FX, Wandji J, Meyer JJM, Lall N. Antimicrobial activity of the methanolic extract and compounds from *Teclea afzelii* (Rutaceae). **S Afr J Bot** 2008; 74(4): 572–576.
- Kuete V, Ngameni B, Simo CC, Tankeu RK, Ngadjui BT, Meyer JJ, Lall N, Kuiate JR. Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa* and *Ficus cordata* (Moraceae). **J Ethnopharmacol** 2008; 120(1): 17–24.
- Kuete V, Wabo GF, Ngameni B, Mbaveng AT, Metuno R, Etoa FX, Ngadjui BT, Beng VP, Meyer JJ, Lall N. Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* (Ixonanthaceae). **J Ethnopharmacol** 2007; 114(1): 54–60.
- Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. **Planta Med** 2010; 76(14): 1479–1491.
- Kuete V, Efferth T. Cameroonian medicinal plants: pharmacology and derived natural products. **Front Pharmacol** 2010; 1: 123.
- Mallea M, Chevalier J, Bornet C, Eyraud A, Davin-Regli A, Bollet C, Pages JM. Porin alteration and active efflux: two *in vivo* drug resistance strategies used by *Enterobacter aerogenes*. **Microbiology** 1998; 144(Pt 11): 3003–3009.
- Chevalier J, Pages JM, Eyraud A, Mallea M. Membrane permeability modifications are involved in antibiotic resistance in *Klebsiella pneumoniae*. **Biochem Biophys Res Commun** 2000; 274(2): 496–499.
- Pradel E, Pages JM. The AcrAB-TolC efflux pump contributes to multidrug resistance in the nosocomial pathogen *Enterobacter aerogenes*. **Antimicrob Agents Chemother** 2002; 46(8): 2640–2643.

26. Mallea M, Mahamoud A, Chevalier J, Alibert-Franco S, Brouant P, Barbe J, Pages JM. Alkylaminoquinolines inhibit the bacterial antibiotic efflux pump in multidrug-resistant clinical isolates. **Biochem J** 2003; 376(Pt 3): 801–805.
27. Tran QT, Mahendran KR, Hajjar E, Ceccarelli M, Davin-Regli A, Winterhalter M, Weingart H, Pages JM. Implication of porins in beta-lactam resistance of *Providencia stuartii*. **J Biol Chem** 2010; 285(42): 32273–32281.
28. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Alibert-Franco S, Ngadjui BT, Pages JM. Efflux pumps are involved in the defense of Gram-negative bacteria against the natural products isobavachalcone and diospyrone. **Antimicrob Agents Chemother** 2010; 54(5): 1749–1752.
29. Kuete V, Alibert-Franco S, Eyong KO, Tangmouo JG, Bolla JM, Alibert-Franco S, Ngadjui BT, Pages JM. Antibacterial activity of some natural products against bacteria expressing a multidrug-resistant phenotype. **Int J Antimicrob Agents** 2011; 37(2): 156–161.
30. Cardoso O, Alves AF, Leitao R. Surveillance of antimicrobial susceptibility of *Pseudomonas aeruginosa* clinical isolates from a central hospital in Portugal. **J Antimicrob Chemother** 2007; 60(2): 452–454.
31. Okeke IN, Ogundaini AO, Ogungbamila FO, Lamikanra A. Antimicrobial spectrum of *Alchornea cordifolia* leaf extract. **Phytother Res** 1999; 13(1): 67–69.
32. Adeleye Adeyemi I, Omonigbehin AE, Stella S, Oluwatosin O, Sobande Jumoke S. Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull.Arg., *Boerhavia diffusa* (L) and *Bridellia micrantha* (Hoscht) Baill. used in traditional medicine in Nigeria on *Helicobacter pylori* and four diarrhoeagenic bacterial pathogens. **Afr J Biotechnol** 2008; 7: 3761–3764.
33. Oloyede GK, Ayanbadejo OE. Phytochemical, toxicity, antimicrobial and antioxidant screening of extracts obtained from *Laportea aestuans* (Gaud). **J Med Sci** 2014; 14: 51–59.
34. Oben J, Assi S, Agbor G, Musoro D. Effect of *Eremomastax speciosa* on experimental diarrhoea. **Afr J Trad Complement Altern Med** 2006; 3(1): 95–100.
35. Kuete V, Voukeng IK, Tsobou R, Mbaveng AT, Wiench B, Beng VP, Efferth T. Cytotoxicity of *Elaeophorbium drupifera* and other Cameroonian medicinal plants against drug sensitive and multidrug resistant cancer cells. **BMC Complement Altern Med** 2013; 13: 250.
36. Okokon J, Antia B, Udoh A, Akpan M. Antianaemic and antimicrobial activity of *Eremomastax speciosa*. **J Pharmacol Toxicol** 2007; 2: 196–199.
37. Njoku O, Okorie I, EC O Okafor J. Investigation on the phytochemical and antimicrobial properties of *Pennisetum purpureum*. **J Med Arom Plant Sci** 2004; 26: 311–314.
38. Rajesh K, Harsha R, Mohammed G, Hareesh A, Thammanna G, Dinesha R, Satish K, Irfan A. Antimicrobial activity of ethanol extract of leaf and flower of *Spathodea campanulata* P. Beauv **Res J Pharm Biol Chem Sci** 2010; 3: 691–698.
39. Ogungbamila FO, Samuelsson G. Smooth muscle relaxing flavonoids from *Alchornea cordifolia*. **Acta Pharm Nord** 1990; 2(6): 421–422.
40. Adeneye AA, Oreagba AI, Ishola IO, Kalejaiye HA. Evaluation of the anti-arthritis activity of the hydroethanolic leaf extract of *Alchornea cordifolia* in rats. **Afr J Tradit Complement Altern Med** 2014; 11(2): 402–410.
41. Manga HM, Brkic D, Marie DE, Quetin-Leclercq J. *In vivo* anti-inflammatory activity of *Alchornea cordifolia* (Schumacher & Thonn.) Mull. Arg. (Euphorbiaceae). **J Ethnopharmacol** 2004; 92(2–3): 209–214.
42. Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, Cimanga K, De Bruyne T, Apers S, Totte J. Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. **Phytomedicine** 2000; 7(1): 31–38.
43. Osadebe PO, Okoye FB. Anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. **J Ethnopharmacol** 2003; 89(1): 19–24.
44. Essiet U, Edet N, Bala D. Phytochemical and physicochemical analysis of the leaves of *Laportea aestuans* (Linn) Chew and *Laportea aestuans* (Schumacher) Chew (male and female). **Asian J Plant Sci Res** 2011; 1: 35–42.
45. Oloyede G, Oyelola M. Chrysen-2-ol derivative from west indian wood nettle *Laportea aestuans* (L.) chew inhibits oxidation and microbial growth *in vitro*. **EXCLI J** 2013; 12: 894–906.
46. Okereke S, Elekwa I, Nmaju A. Gas chromatographic, hypoglycemic and hypolipidemic effects of leaves of *Laportea aestuans* in alloxan induced diabetes in male albino rats. **IOSR J Environ Sci Toxicol Food Technol** 2014; 42: 42–46.
47. Zahid Z, Aniruddha P, Sagar D, Subur K, Rana Z. Comparative phytochemical screening of flowers and bark of *Spathodea campanulata*. **Int J Appl Biol Pharm Technol** 2011; 2: 233–235.
48. Sowjanya P, Hapsana P, Kiran B, Vagdevi G, Srinivasa B. Pharmacognostical and physicochemical standardization of leaves of *Spathodea campanulata* P. Beauv **J Pharmacogn Phytochem** 2013; 2: 189–192.

How to cite this article: Mambe FT, Voukeng IK, Beng VP, Kuete V. Antibacterial activities of methanol extracts from *Alchornea cordifolia* and four other Cameroonian plants against MDR phenotypes. *Journal of Taibah University Medical Sciences* 2016;11:121–127.