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ORIGINAL ARTICLE

Antibacterial activities of the methanol extracts of *Albizia adianthifolia*, *Alchornea laxiflora*, *Laportea ovalifolia* and three other Cameroonian plants against multi-drug resistant Gram-negative bacteria

Cedric F. Tchinda, Igor K. Voukeng, Veronique P. Beng, Victor Kuete *

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

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KEYWORDS

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Abstract In the last 10 years, resistance in Gram-negative bacteria has been increasing. The present study was designed to evaluate the *in vitro* antibacterial activities of the methanol extracts of six Cameroonian medicinal plants *Albizia adianthifolia*, *Alchornea laxiflora*, *Boerhavia diffusa*, *Combretum hispidum*, *Laportea ovalifolia* and *Scoparia dulcis* against a panel of 15 multidrug resistant Gram-negative bacterial strains. The broth microdilution was used to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the extracts. The preliminary phytochemical screening of the extracts was conducted according to the reference qualitative phytochemical methods. Results showed that all extracts contained compounds belonging to the classes of polyphenols and triterpenes, other classes of chemicals being selectively distributed. The best antibacterial activities were recorded with bark and root extracts of *A. adianthifolia* as well as with *L. ovalifolia* extract, with MIC values ranging from 64 to 1024 μg/mL on 93.3% of the fifteen tested bacteria. The lowest MIC value of 64 μg/mL was recorded with *A. laxiflora* bark extract against *Enterobacter aerogenes* EA289.

Finally, the results of this study provide evidence of the antibacterial activity of the tested plants and suggest their possible use in the control of multidrug resistant phenotypes.

1. Introduction

Bacterial infections are of particular concern globally mainly due to the development of antibiotic resistance. In the last 10 years, resistance in Gram-negative bacteria has been increasing (Pallett and Hand, 2010). Gram-negative bacteria rapidly develop drug resistance, especially in the presence of antibiotic selection pressure (Boucher et al., 2009; Peleg and

* Corresponding author at: P.O. Box 67 Dschang, Cameroon. Tel.: +237 77 35 59 27; fax: +237 22 22 60 18.
E-mail address: kuetevictor@yahoo.fr (V. Kuete).
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Hooper, 2010). In Gram-negative bacteria, efflux pumps belonging to the resistance-nodulation-cell division (RND) family of tripartite efflux pumps are largely involved in multidrug resistance (Van Bambeke et al., 2006). The spread of multidrug resistant (MDR) bacteria propels the search of novel antibacterials to combat resistant phenotypes. Botanicals constitute a good source of anti-infective compounds, in regards to the variety and diversity of their chemical structures (Cowan, 1999; Ndhlala et al., 2013; Ngameni et al., 2013). According to the World Health Organization (WHO) report, approximately 80% of the world population rely on plants or derived products for their treatment (WHO, 1993). In the past, many African plants demonstrated good antibacterial activity against Gram-negative MDR bacteria. Among the best documented plants are *Olax subscorpioïdea* (Fankam et al., 2011), *Cucurbita pepo* (Noumedem et al., 2013b), *Piper nigrum* (Noumedem et al., 2013a), *Beilschmiedia obscura* (Fankam et al., 2014), *Capsicum frutescens* (Touani et al., 2014), *Allanblackia gabonensis*, *Combretum molle*, *Gladiolus quartinianus* (Fankam et al., 2015) and *Fagara tessmannii* (Tankeo et al., 2015). In our continuos search of antibacterials from botanical source, we designed the present work to investigate *in vitro*, the antibacterial activity of the methanol extracts of six Cameroonian medicinal plants: *Albizia adianthifolia* (Schum.) (Fabaceae), *Alchornea laxiflora* (Benth.) Pax & K Hoffm. (Euphorbiaceae), *Boerhavia diffusa* Lin (Nyctaginaceae), *Combretum hispidum* Laws (Combretaceae), *Laportea ovalifolia* (Schum.) Chew (Urticaceae) and *Scoparia dulcis* Linn. (Scrophulariaceae) against MDR Gram-negative bacteria.

2. Materials and methods

2.1. Plant material and extraction

Different parts of the tested plants were collected in various parts of Cameroon in January 2014. These included the bark and roots of *A. adianthifolia*, the leaves and bark of *A. laxiflora* and *C. hispidum*, and the whole plant of *B. diffusa*, *L. ovalifolia* and *S. dulcis*. The plants were identified at the National herbarium (Yaounde, Cameroon) where voucher specimens were deposited under the reference numbers (Table 1). Each plant sample was air dried in laboratory temperature (22 ± 2 °C) and then powdered. The obtained powder (200 g) was extracted with methanol (MeOH; 1 L) for 48 h at room temperature. The extract was then concentrated under reduced pressure at about 40 °C to give residue that constituted the crude extract. All extracts were then kept at 4 °C until further use.

2.2. Preliminary phytochemical screening

The major phytochemical classes such as alkaloids, triterpenes, flavonoids, anthraquinones, polyphenols, sterols, coumarins, saponins and tannins (Table 2) were investigated according to the common described phytochemical methods (Harbone, 1973; Ngameni et al., 2013; Poumale et al., 2013; Wansi et al., 2013).

2.3. Antimicrobial assays

2.3.1. Chemicals for antimicrobial assay

Chloramphenicol (CHL) (Sigma–Aldrich, St. Quentin Fallavier, France) was used as reference antibiotics (RA). *p*-Iodonitrotetrazolium chloride (INT) was used as the microbial growth indicator (Eloff, 1998; Mativandlela et al., 2006).

2.3.2. 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 (Lacmata et al., 2012; Seukep et al., 2013; Touani et al., 2014). Nutrient agar (Sigma–Aldrich) was used for the activation of the tested Gram-negative bacteria while the Mueller Hinton Broth (Sigma–Aldrich) was used for antibacterial assays (Kuete et al., 2011b).

2.3.3. INT colorimetric assay for minimal inhibitory concentration and minimal bactericidal concentration determinations

The MIC determination on the tested bacteria were conducted using rapid INT colorimetric assay according to described methods (Eloff, 1998) with some modifications (Kuete et al., 2008b, 2009). The test samples and RA were first of all dissolved in DMSO/Mueller Hinton Broth (MHB) broth. The final concentration of DMSO was lower than 2.5% and does not affect the microbial growth (Kuete et al., 2007, 2008a). The solution obtained was then added to MHB, and serially diluted two fold (in a 96-wells microplate). One hundred microliters (100 µL) of inoculum 1.5×10^6 CFU/mL prepared in appropriate broth were then added (Kuete et al., 2008b, 2009). The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a plate shaker and incubated at 37 °C for 18 h. The assay was repeated thrice. Wells containing adequate broth, 100 µL of inoculum and DMSO to a final concentration of 2.5% served as the negative control. The MIC of samples was detected after 18 h incubation at 37 °C, following the addition (40 µL) of 0.2 mg/mL of INT and incubation at 37 °C for 30 min. Viable bacteria reduced the yellow dye to pink. MIC was defined as the sample concentration that prevented the color change of the medium thus exhibited complete inhibition of microbial growth (Eloff, 1998). The MBC was determined by adding 50 µL aliquots of the preparations, which did not show any growth after incubation during MIC assays, to 150 µL of adequate broth. These preparations were incubated at 37 °C for 48 h. The MBC was regarded as the lowest concentration of extract, which did not produce a color change after the addition of INT as mentioned above (Kuete et al., 2008b, 2009).

3. Results and discussion

The results of Table 2 reporting the qualitative phytochemical analysis indicated that all the tested plant extracts contained polyphenols and triterpenes. Except the extract from *L. ovalifolia*, all other crude extracts contained alkaloids, other secondary metabolite classes being selectively distributed (Table 2). The antibacterial data compiled in Table 3 showed that all the tested extracts displayed selective antibacterial activities. The best activity was recorded with bark and root extracts of *A. adianthifolia* as well as with *L. ovalifolia* extract, with MIC values ranging from 64 to 1024 µg/mL against 14/15 (93.3%) tested bacteria. The antibacterial activity with MIC

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Table 1 Information on the studied plants.

Species (family); voucher Number*	Traditional uses	Parts used traditionally	Bioactive or potentially bioactive components	Bioactivity of crude extract
Albizia adianthifolia (Schum.) (Fabaceae); 24729/SRF/Cam	Treatment skin diseases, bronchitis, inflamed eyes, tapeworm, headaches and sinusitis (Van-Wyk and Gerick, 2000; Watt and Breyer-Brandwyk, 1962)	Leaves, bark and roots	Adianthifoliosides A, B, D (Haddad et al., 2004, 2003), lupeol and aurantiamide acetate (Tamokou Jde et al., 2012), prosapogenins (Haddad et al., 2002)	*Ethylaceate fraction extracts*: antimicrobial on Ec, Ef, Pa, Pm, Kp, Sa, Sf, St, Ca, Ct, Ck, Cg, Cl, Cn (Tamokou Jde et al., 2012); *Aqueous extract*: antioxidant (Beppe et al., 2014; Tamokou Jde et al., 2012)
Alchornea laxiflora (Benth.) Pax & K Hoffm. (Euphorbiaceae); 9661/SRF/Cam	Treatment inflammatory and infectious diseases, poliomyelitis and measles. (Ogundipe et al., 2001; Oladunmoye and Kehinde, 2011)	Leaves, bark and roots	Quercetin-7,4′-disulphate, quercetin, quercetin-3′,4′-disulphate, quercetin-3,4′-diacetate, rutin and quercetrin (Ogundipe et al., 2001)	*Methanol fraction of leave extracts*: antimicrobial on Ba, Bc, Ec, Kp, Pa, Pf, Sa, Ag, Af, As, Ca, Cp (Akinpelu et al., 2015); *Crude extract*: antioxidant (Farombi et al., 2003)
Boerhavia diffusa Lin (Nyctaginaceae); 15247/SRF/Cam	Treatment of diabetes, asthma, Bronchial infection (Kouakou et al., 2009)	Whole plant	Boeravinones G, H (Ahmed-Belkacem et al., 2007)	*Crude extract of leaves*: Antioxidant and hepatoprotective properties (Olaleye et al., 2010), *antimicrobial activity*: Pa, Ec, St, Sf (Wagh and Vidhale, 2010)
Combretum hispidum Laws (Combretaceae); 48289/HNC	Treatment of stomach aches, diarrhea, gastro-intestinal disorders, liver complaints, skin infections, urinary tract infections (Adjanohoun et al., 1996; Burkill, 1985; Jiofack et al., 2009)	Leaves, roots	Not reported	*Crude extract of bark*: anti-hepatotoxic, anti-inflammatory, antiparasitic, mollucidal effect (Schmelzer and Gurib-Fakim, 2013)
Laportea ovalifolia (Schum.) Chew (Urticaceae) 44306/HNC	Treatment of headache, internal ulcers, diabetes, bronchitis and filariasi (Focho et al., 2009; Momo et al., 2006)	Leaves and roots	Laportoside A and Laportomide A (Tazoo et al., 2007)	*Crude extract of leaves*: antidiabetic and hypolipidemic effects (Momo et al., 2006)
Scoparia dulcis Linn. (Scrophulariaceae) 53478/HNC	Treatment of anemia, burns, headaches, bronchitis, gastric disorders, hemorrhoids, insect bites, skin wounds, hypertension. (Freire et al., 1996)	Whole plant	Scoparinol (Ahmed et al., 2001); scoparic acid, scopadulcic acid, scopadulciol and scopadulin (Zulfiker et al., 2011)	*Crude extracts*: anti-diabetic, anti-inflammatory properties and antioxidant capacity *in vivo* (Adaikpoh et al., 2007; Freire et al., 1996)

* (HNC): Cameroon National Herbarium; (SRF/Cam): *Société des Réserves Forestières du Cameroun*; *As*: *Aspergillus niger*; *Ag*: *Aspergillus glaucus*; *Af*: *Aspergillus flavus*; Ba: Bacillus anthracis; Bc: Bacillus cereus; *Ca*: *Candida albicans*; *Cg*: *Candida glabrata*; *CK*: *Candida krusei*; *Cl*: *Candida lusitaniae*; *Cn*: *Cryptococcus neoformans*; *Cp*: *Candida pseudotropicalis*; *Ct*: *Candida tropicalis*; *Ec*: *Escherichia coli*; *Ef*: *Enterococcus faecalis*; *Kp*: *Klebsiella pneumoniae*; *Pa*: *Pseudomonas aeruginosa*; *Pf*: *Pseudomonas fluorescens*; *Pm*: *Proteus mirabilis*; *Sa*: *Staphylococcus aureus*; *Sf*: *Shigella flexneri*; *St*: *Salmonella typhi*.

Table 2 Qualitative phytochemical composition of the plant extracts.

Classes	Studies plants and composition								
	Albizia adianthifolia		*Alchornea laxiflora*		*Boerhavia diffusa*	*Combretum hispidum*		*Laportea ovalifolia*	*Scoparia dulcis*
	B	R	L	B	W	L	B	W	W
Alkaloids	+	+	+	+	+	+	+	–	+
Polyphenols	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	–	+
Anthraquinones	+	+	–	–	+	+	+	–	–
Coumarins	+	+	–	–	–	–	–	+	–
Tannins	+	–	+	–	+	+	+	+	+
Triterpenes	+	+	+	+	+	+	+	+	+
Sterols	+	+	+	+	–	+	+	–	+
Saponins	+	+	+	–	+	+	+	+	+

(–): Absent; (+): Present; the tested extracts were obtained from (L: Leaves; B: bark; R: roots; W: whole plant).

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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)									
	Albizia adianthifolia		*Alchornea laxiflora*		*Boerhavia diffusa*	*Combretum hispidum*		*Laportea ovalifolia*	*Scoparia dulcis*	CHL
	B	R	L	B	W	L	B	W	W	
Escherichia coli										
ATCC8739	128 (–)	128 (1024)	256 (–)	1024 (–)	1024 (–)	-	–	512 (–)	–	2 (64)
ATCC10536	512 (–)	256 (–)	128 (–)	512 (–)	1024 (–)	–	–	1024 (–)	–	2 (32)
AG100ATet	256 (–)	256 (–)	–	–	1024 (–)	–	–	512 (–)	1024 (–)	32 (256)
AG102	1024 (–)	1024 (–)	256 (–)	512 (–)	512 (–)	1024 (–)	512 (–)	512 (–)	1024 (–)	32 (256)
Enterobacter aerogenes										
ATCC13048	256 (–)	128 (1024)	512 (–)	512 (–)	1024 (–)	–	–	256 (–)	–	16 (128)
CM64	256 (–)	128 (512)	512 (–)	512 (–)	1024 (–)	512 (–)	512 (–)	512 (–)	1024 (–)	256 (–)
EA 27	256 (–)	256 (–)	128 (1024)	–	1024 (–)	512 (–)	–	512 (–)	–	32 (256)
EA 289	128 (–)	128 (–)	128 (1024)	64 (1024)	512 (–)	256 (–)	1024 (–)	256 (–)	256 (–)	32 (256)
Klebsiella pneumoniae										
ATCC11296	128 (–)	128 (–)	256 (–)	256 (–)	–	–	–	1024 (–)	–	32 (256)
KP55	256 (–)	256 (–)	512 (–)	512 (–)	–	1024 (–)	–	512 (–)	–	64 (256)
KP63	128 (–)	128 (–)	512 (–)	–	1024 (–)	–	–	256 (–)	–	32 (256)
Providencia stuartii										
ATCC29916	512 (–)	512 (–)	–	512 (–)	–	1024 (–)	–	256 (–)	1024 (–)	64 (256)
NEA 16	512 (–)	1024 (–)	128 (–)	–	512 (–)	512 (–)	–	128 (–)	512 (–)	64 (256)
Pseudomonas aeruginosa										
PA01	256 (–)	128 (–)	512 (–)	512 (–)	–	512 (–)	–	256 (–)	–	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.

values ranged from 64 to 1024 μg/mL for leaves [12/15 (80%) of the tested bacteria] and bark [10/15 (66.7%)] of *A. laxiflora*, [10/15 (66.7%)] for *B. diffusa*, for leaves [8/15 (53.3%)] and bark [3/15 (20%)] of *C. hispidum* and [6/15 (40%)] for *S. dulcis* extracts was obtained. The lowest MIC value (64 μg/mL) was recorded with *A. laxiflora* bark extract against *E. aerogenes* EA289. In almost all cases, the tested extract exerted bacteriostatic effects with a ratio MBC/MIC above 4.

Several molecules belonging to the detected classes of secondary metabolites were found active on pathogenic microorganisms (Awouafack et al., 2013; Cowan, 1999; Ndhlala et al., 2013; Tsopmo et al., 2013). The presence of such metabolites in the studied plant extracts can provide a preliminary explanation on their antibacterial activities. Differences were observed in the antibacterial activities of the extracts. These could be due to the differences in their chemical composition as well as in the mechanism of action of their bioactive constituents (Cowan, 1999). According to Kuete (2010), Kuete and Efferth (2010), the antibacterial activity of a plant extract is considered significant when MIC values are below 100 μg/mL, moderate when 100 ⩽ MIC ⩽ 625 μg/mL and weak when MIC > 625 μg/mL. Consequently, the activity (MIC of 64 μg/mL) observed with *A. laxiflora* bark extract against *E. aerogenes* EA289 can be considered important. Moderate antibacterial activities (100 ⩽ MIC ⩽ 625 μg/mL) were obtained with the majority of the extracts. However, the obtained MIC values are very important when considering the medicinal importance of the tested MDR bacteria (Chevalier et al., 2000; Kuete et al., 2010, 2011a; Mallea et al., 1998, 2003; Pradel and Pages, 2002; Tran et al., 2010). The antibacterial activities of *A. adianthifolia*, *A. laxiflora* and *B. diffusa* was reported on many sensitive species (Akinpelu et al., 2015; Tamokou Jde et al., 2012; Wagh and Vidhale, 2010). The present work therefore provides additional data on their ability to combat MDR phenotypes.

4. Conclusion

The results of the present investigation suggest that the extracts of the studied plants can be used as potential leads to discover new drugs to control some bacterial infections, especially those involving MDR bacterial species.

Authors' contributions

CTF and IKV carried out the study; VK and VPB supervised the work; VK designed the experiments, wrote the manuscript, supervised the work and provided the bacterial strains; all authors read and approved the final manuscript.

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