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

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Received 10 July 2015; revised 16 January 2016; accepted 19 January 2016
Available online 25 January 2016

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
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; Nd hlala et al., 2013; Ng ameni 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 subscorpioides (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), Allu nhlackia gabonensis, Combretum molle, Gladiolus quartinianus (Fankam et al., 2015) and Fagara tessmannii (Tan keo et al., 2015). In our continuous 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; Ng ameni et al., 2013; Pou mal et al., 2013; Wansì 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 micro- bial growth indicator (Eloff, 1998; Mativandel a 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 × 10^8 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
### 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-Brandwijk, 1962) | Leaves, bark and roots | Adiantifoliolides A, B, D (Haddad et al., 2004, 2003), lupeol and aurantiumide acetate (Tamokou Jde et al., 2012), prosapogenins (Haddad et al., 2002) | *Ethylacetate 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; Oladammye and Kehinde, 2011) | Leaves, bark and roots | Quercitin-7,4′-disulphate, quercetin, quercetin-3′,4′-disulphate, quercetin-3′-diturate, rutin and quercetin (Ogundipe et al., 2001) | *Methanol fraction of leaf extracts*: antimicrobial on Ba, Be, Ec, Kp, Pa, Pf, Sa, Ag, Af, As, Ca, Cg, Ck, Cg, Cm, Cl, Cj, Cn, Cr, C0, Cu, Bc,  |
| *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*: antioxidant (Farombi et al., 2003) |
| *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; Burkhill, 1985; Jofack et al., 2009) | Leaves, roots | Not reported | *Crude extract of bark*: anti-hepatoxictic, 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 filariasis (Foch 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, scopadulcic and scopadulin (Zulfikar 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 glaues; Af: Aspergillus flavus; Ba: Bacillus anthracis; Bc: Bacillus cereus; Ca: Candida albicans; Cg: Candida glabrata; Ck: Candida kruzei; Cj: Candida lusitaniae; Cn: Cryptococcus neoforms; Cp: Candida pseudotropicalis; Ct: Candida tropicalis; Ec: Escherichia coli; Ef: Enterococcus faecalis; Kp: Klebsiella pneumoniae; Pf: Pseudomonas aeruginosa; Pm: Pseudomonas fluorescence; Pr: Proteus mirabilis; Sa: Staphylococcus aureus; Sf: Shigella flexneri; St: Salmonella typhii.

### 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).
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.

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.

Acknowledgements

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

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