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ORIGINAL ARTICLE

Antibacterial activities of the methanol extracts of *Canarium schweinfurthii* and four other Cameroonian dietary plants against multi-drug resistant Gram-negative bacteria

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Abstract Bacterial infections are among the major cause of morbidity and mortality worldwide. The present study was designed to evaluate the *in vitro* antibacterial activities of the methanol extracts of five Cameroonian edible plants namely *Colocasia esculenta*, *Triumfetta pentandra*, *Hibiscus esculentus*, *Canarium schweinfurthii* and *Annona muricata* against a panel of 19 multidrug resistant Gram-negative bacterial strains. The liquid 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 standard phytochemical methods. Results showed that all extracts contained compounds belonging to the classes of polyphenols, triterpenes and steroids, other classes of chemicals being selectively distributed. *Canarium schweinfurthii* extract showed the best activity with MIC values ranging from 64 to 1024 μg/mL against 89.5% of the 19 tested bacteria strains. MIC values below or equal to 1024 μg/mL were also recorded with *Triumfetta pentandra*, *Annona muricata*, *Colocasia esculenta* and *Hibiscus esculentus* extracts respectively against 15/19 (78.9%), 11/19 (57.9%), 10/19 (52.6%) and 10/19 (52.6%) tested bacteria. Extract from *C. schweinfurthii* displayed the lowest MIC value (64 μg/mL) against *Escherichia coli* AG100A$_{Tet}$. Finally, the results of this work provide baseline information for the use of *C. esculenta*, *T. pentandra*, *H. esculentus*, *C. schweinfurthii* and *A. muricata* in the treatment of bacterial infections including multidrug resistant phenotypes.

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1. Introduction

Bacterial infections are still among the major cause of morbidity and mortality worldwide; the situation is complicated by the appearance and emergence of multidrug resistant strains (Kuete, 2013), causing treatment failures. In

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Gram-negative bacteria, efflux pumps belong 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 bacterial MDR phenotypes propels the search of novel antibacterials to combat MDR phenotypes. Medicinal plants constitute a good source of anti-infective compounds, in regard to the variety and diversity of their secondary metabolites (Cowan, 1999; Ndhlala et al., 2013; Ngameni et al., 2013). Plants have long been used as the primary source for human treatment, and according to World Health Organization (WHO)'s report, approximately 80% of the world population rely on plants or derived products for their treatment (WHO, 1993). Fighting MDR bacterial infections with edible plants represents an attractive strategy. Several studies previously demonstrated the ability of African food plants to inhibit the growth MDR Gram-negative bacteria. Some of them include *Dichrostachys glomerata*, *Beilschmiedia cinnamomea*, *Aframomum citratum*, *Piper capense*, *Echinops giganteus*, *Fagara xanthoxyloïdes* and *Olax subscorpioïdea* (Fankam et al., 2011), *Lactuca sativa*, *Sechium edule*, *Cucurbita pepo* and *Solanum nigrum* (Noumedem et al., 2013b), *Piper nigrum* and *Vernonia amygdalina* (Noumedem et al., 2013a), *Beilschmiedia obscura*, *Pachypodanthium staudtii* and *Peperomia fernandopoiana* (Fankam et al., 2014) and *Capsicum frutescens* (Touani et al., 2014). In our continuos search for functional food plants, we designed the present work to investigate *in vitro*, the antibacterial activity of the methanol extracts of four Cameroonian food plants, *Annona muricata* Lin. (Annonaceae), *Canarium schweinfurthii* Engl. (Burseraceae), *Colocasia esculenta* (L.) Schott (Araceae), *Hibiscus esculentus* L. (Tiliaceae) and *Triumfetta pentandra* A.Rich. (Tiliaceae) against MDR Gram-negative bacteria.

2. Materials and methods

2.1. Plant material and extraction

The five edible plants used in this work were collected in Bandjoun (West Region of Cameroon) in January 2014. 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 and the 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 to give residues which 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 antibiotic (RA). *p*-iodonitrotetrazolium chloride (INT) was used as 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 was used for the activation of the tested Gram-negative bacteria while the Mueller Hinton Broth was used for antibacterial assays (Kuete et al., 2011b).

2.3.3. INT colorimetric assay for MIC and MBC determinations

The MIC determinations on the tested bacteria were conducted using rapid *p*-iodonitrotetrazolium chloride (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) or DMSO/7H9 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 Mueller Hinton Broth, 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 was 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 negative control. The MIC of samples was detected after 18 h incubation at 37 °C, following 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 and 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 addition of INT as mentioned above (Kuete et al., 2008b, 2009).

3. Results and discussion

The results of qualitative phytochemical analysis showed that all the tested plant extracts contained polyphenols, triterpenes and sterols. Except the extract from *C. esculenta*, all the crude extracts contained alkaloids; other secondary metabolite classes being selectively distributed (Table 2). The results summarized in Table 3 showed that the tested extracts displayed

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
Annona muricata Lin. (Annonaceae); 18681/SRF/Cam	Treatment of wounds and insomnia; antiparasitical insecticidal (Rajeswari et al., 2012)	Leaves, seeds, bark and roots	Epomuricenins-A and B, montecristin, cohibins-A and B, muridienins-1 and 2, muridienins-3 and 4, muricadienin and chatenaytrienins-1, 2 and 3 and sabadelin, murihexol, donhexocin, annonacin A and annonacin B (Rajeswari et al., 2012)	Aqueous and ethanolic extracts: (Q); *Sa*, *Vc*, *Ec*, *Se*, *Lv* and *On* (Vieira et al., 2010) and *Pv*, *Sp*, *Bs*, *St*, *Kp*, *Ea* (Rajeswari et al., 2012); hexane, ethyl acetate and methanol extract on: *Lb* and *Lp*; crude extract tested against *Hv* (Rajeswari et al., 2012)
Canarium schweinfurthii Engl. (Burseraceae); 16929/SRF/Cam	Treatment of malaria, constipation, diarrhea rheumatism and sexually transmitted diseases (Koudou et al., 2005)	Fruits, leaves and rhizomes	Saponins, cardiac glycosides, tannins, flavonoids and steroids (Ngbede et al., 2008)	EO: *Bc*, *Ef*, *Ec*, *Li*, *Se*, *Sd*, *Sa*, *Pm*, *Sc* and *Ca* (Obame et al., 2007)
Colocasia esculenta (L.) Schott (Araceae); 42352/HNC	Scorpion and snake bite (Nakade et al., 2013)	Leaves and tubers	Quinones, alkaloids, saponins, tannins, phenols, terpenoids, glycosides and steroids (Nakade et al., 2013)	Ethyl acetate extract: (Q) *St*, *Kp*, *Pa*, *Sp*, *Bs*, *Pv*, *Ec* (Nakade et al., 2013) aqueous and methanolic extracts: (Q) *Vspp* (Lee et al., 2010)
Hibiscus esculentus L. (Tiliaceae); 8537/SRF/Cam	Inflammation, anti-ulcer, analgesic hypoglycemic, anti-cancer, hypoglycemic (Daly, 1997; Uraku et al., 2010)	Fruits and leaves	/	Crude extract on *Salmonella*, *Shigella* and *Enterobacter* (Nwaiwu et al., 2012)
Triumfetta pentandra A.Rich. (Tiliaceae); 9014/SRF/Cam	Induce fertility and implantation of the fetus (Ngondi et al., 2005; Okoli et al., 2007)	Leaves, stems and roots	Triumfettamide, triumfettoside, heptadecanoic acid, β-sitosterol glucopyranoside, friedeline, lupeol, betuline, maslinic acid, 2-hydroxyoleanolicacid and the mixture of stigmasterol and β-sitosterol (Sandjo et al., 2008; Sandjo and Kuete, 2013)	/

* (HNC): Cameroon National Herbarium; (SRF/Cam): Société des Réserves Forestières du Cameroun; (Q): qualitative activity based on the inhibition zone./: not reported; EO: Essential Oil; *Sa*: *Streptococcus aureus; Vc: Vibriocholerae; Ec: Escherichia coli; Lv: Litopenaeusvannmaei; On: Oreochromisnicoticus; Hv: Herpes virus; Lb: Leishmania braziliensis; Lp: Lieshmaniapanamensis; Pv: Proteusvulgaris; Sp: Streptococcus pyogenes; Bs: Bacillus subtilis; Kp: Klebsiellapneumoniae; St: Salmonella typhi; Sd: Shigelladysenteriae; Pa: Pseudomonas aeruginosa; Vspp: Vibriospecies; Bc: Bacillus cereus; Ef: enterococcusfaecalis; Li: Listeria innocua; Se: Salmonella enterica; Pm: Proteus mirabilis; Sc: Staphylococcus camorum; Ca: Candida albicans.*

Table 2 Phytochemical composition of the plant extracts.

Classes	Studies plants, extraction yield* (%) and composition				
	C. esculenta (Leaves; 6.25%)	*T. pentandra* (Leaves; 5.50%)	*H. esculentus* (Fruits; 2.98%)	*C. schweinfurthii* (Fruits; 0.87%)	*A. muricata* (Leaves; 4.50%)
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.

Table 3 Minimal inhibitory concentration (MIC) in μg/mL of methanol extracts from the studied plants and chloramphenicol.

Bacterial strains	Tested samples, MIC and MBC values (μg/mL) and MBC/MIC ratio																	
	Colocasia esculenta			*Triumfetta pentandra*			*Hibiscus esculentus*			*Canarium schweinfurthii*			*Annona muricata*			*Chloramphenicol*		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Escherichia coli																		
ATTC8739	1024	–	–	–	–	–	1024	–	–	512	1024	–	–	–	–	4	–	–
AG100	1024	–	–	256	1024	4	1024	–	–	128	–	256	256	–	–	4	256	64
AG100A	256	1024	4	1024	–	–	1024	–	–	512	1024	1024	1024	–	–	2	64	32
AG102	–	–	–	1024	–	–	1024	–	–	512	1024	1024	1024	–	–	8	–	–
AG100ATet	1024	–	–	512	–	–	512	–	–	64	1024	512	512	–	–	64	256	4
W3110	1024	–	–	1024	–	–	1024	–	–	512	1024	1024	1024	–	–	8	16	2
Enterobacter aerogenes																		
ATCC13048	–	–	–	512	–	–	–	–	–	512	–	–	–	–	–	8	128	16
EA289	1024	–	–	512	–	–	–	–	–	256	–	–	–	–	–	64	512	8
EA27	–	–	–	1024	–	–	1024	–	–	128	–	1024	1024	–	–	64	512	8
EA298	–	–	–	–	–	–	–	–	–	1024	–	–	–	–	–	32	256	8
CM64	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	256	256	1
Klebsiella pneumoniae																		
ATCC11296	256	–	–	256	–	–	512	–	–	256	–	512	512	–	–	4	512	128
KP55	1024	–	–	512	–	–	–	–	–	512	–	512	512	–	–	64	128	2
KP63	512	–	–	1024	–	–	1024	–	–	256	–	256	256	–	–	256	256	1
K24	–	–	–	1024	–	–	–	–	–	1024	–	–	–	–	–	64	256	4
Pseudomona aeruginosa																		
PA01	–	–	–	1024	–	–	1024	–	–	512	–	1024	1024	–	–	16	256	16
PA124	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	64	512	8
Providencia stuartii																		
ATCC29916	–	–	–	1024	–	–	–	–	–	512	–	–	–	–	–	8	32	4
NEA16	1024	–	–	512	–	–	–	–	–	256	1024	1024	1024	–	–	32	256	8

R: MBC/MIC; -: > 1024 (MIC) or not determined.

selective antibacterial activities. The best activity was recorded with *Canarium schweinfurthii* extract, with MIC values ranging from 64 to 1024 μg/mL against 17/19 (89.5%) tested bacteria. MIC values below or equal to 1024 μg/mL were also recorded with *Triumfetta pentandra*, *Annona muricata*, *Colocasia esculenta* and *Hibiscus esculentus* extracts respectively against 15/19(78.9%), 11/19 (57.9%), 10/19 (52.6%) and 10/19 (52.6%) tested bacteria. The lowest MIC value (64 μg/mL) was recorded with the extract from *C. schweinfurthii* against *E. coli* AG100ATet. Extract from *C. schweinfurthii* also displayed the best spectrum of bactericidal effect with a ratio MBC/MIC $\leqslant$4 obtained on five tested bacterial strains.

Differences in antibacterial activities were noted between the extracts. 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 $100 \leqslant \text{MIC} \leqslant 625$ μg/mL and weak when MIC > 625 μg/mL. Consequently, the activity (MIC of 64 μg/mL) observed with *C. schweinfurthii* extract against *E. coli*AG100ATet can be considered important. The antimicrobial properties of compounds from *C. schweinfurthii* have been reported (Longanga Otshudi et al., 2000) and the present study provides additional data on the ability of this plant to fight MDR bacteria. Moderate or weak antibacterial activities ($625 \leqslant \text{MIC} \leqslant 1024$ μg/mL) were obtained with the majority of the extracts. However, the obtained MIC values are very important when considering that extracts are from edible plant parts and also when considering the medicinal importance of the tested MDR bacteria (Chevalier et al., 2000; Mallea et al., 1998, 2003; Pradel and Pages, 2002; Kuete et al., 2010, 2011a; Tran et al., 2010). It has been demonstrated that the ethyl acetate extract from leaves of *C. esculenta* exhibits antibacterial activity against the strains of *S. typhii*, *K. pneumoniae*, *P. aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris* and *E. coli* (Nakade et al., 2013). This is in accordance with the present study, as we also found that this plant showed activity against *E. coli*, *K. pneumoniae* and *P. aeruginosa* strains.

4. Conclusion

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

Competing interest

The authors declare that there are no conflicts of interest.

Authors' contributions

JKD and FTK carried out the study; 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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