Activities of selected medicinal plants against multi-drug resistant Gram-negative bacteria in Cameroon

Marthe E. S. Tchana[2], Aimé G. Fankam [1], Armelle T. Mbaveng[1*], Ernestine T. Nkwengoua[2], Jackson A. Seukep[1], Francesco K. Tchouani[1], Barthélémy Nyassé[2] and Victor Kuete[1]

1. Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon.
2. Department of Organic Chemistry, Faculty of Science, University of Yaounde, Yaounde Cameroon.

Abstract

Background: Medicinal plants are used worldwide for several human ailments including bacterial infections. The present work was designed to assess the *in vitro* antibacterial activities of some Cameroonian medicinal plants including *Entada abyssinica, Entada africana*, *Pentaclethra macrophylla*, *Allexis cauliflora*, *Anthocleista leibrechtsiana*, *Carapa procera*, *Carica papaya* and *Persea americana* against Gram-negative bacteria expressing multidrug resistant (MDR) phenotypes.

Methods: The microbroth dilution was used to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the samples against eight bacterial strains belonging to four species, *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae* and *Providencia stuartii*.

Results: The extracts displayed selective antibacterial activities with the minimal inhibitory concentrations (MIC) values ranges of 64 to 1024 µg/mL. The most active extract was that from *Pentaclethra macrophylla* (TPM) that showed inhibitory activities against five of the eight (62.5%) tested bacteria. The lowest MIC value (64 µg/mL) was recorded with the crude extract of *Entada africana* against *E. coli* AG100A whilst the best MBC (256 µg/mL) value was also obtained with methanol extract of *Persea americana* against this bacterial strain.

Conclusion: The results of the present work provide baseline information on the possible use of *Pentaclethra macrophylla*, *Entada africana* and *Entada abyssinica* in the treatment of selected bacterial infections.

Keywords: Antibacterial activity; multi-drug resistant; medicinal plants.

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Introduction

The increase of bacterial resistance specifically leading to treatment failures is directly responsiblefor the current increase in morbidity and mortality associated with bacterial infections[1]. Among the known mechanisms of resistance, active e ux *via* resistance-nodulation-cell division (RND) pumps is one of the most occurring systems in Gram-negative bacteria[2]. This efflux system depends on membrane energy and efficiently expels structurally unrelated antibiotic molecules across the bacterial envelope *via* a tripartite complex (comprising an inner membrane pump, a periplasmic fusion protein, and an outer membrane channel)[3]. Today, the increase of resistance to antibiotics propels the search of new drugs to combat resistant microorganisms. Therefore, species commonly used as herbal medicine appear biologically active components isolated from plant as a good alternative, due to the variety of plants secondary metabolites and their potential to exert antimicrobial activities[4-6]. In Cameroon, several medicinal plants are used as herbal medicines to treat infectious diseases[4]. The present work was therefore designed to investigate the antibacterial potential of some commonly used medicinal plants namely *Entada abyssinica Steud.*, *Entada africana* Guill. & Perr., *Pentaclethra macrophylla* Benth. (Fabaceae), *Allexis cauliflora* (Oliv.) Pierre. (Violaceae), *Anthocleista leibrechtsiana* de Wild et Th. (Gentianaceae), *Carapa procera* DC. (Meliaceae), *Carica papaya* L. (Caricaceae) and *Persea Americana* Mill. (Lauraceae) against Gram-negative bacteria including MDR phenotypes.

Correspondence author:
Armelle T. Mbaveng
Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon.
P.O. Box 67; Dschang-Cameroon
armkuete@yahoo.fr

Material and methods

Plant Materials and Extraction

The plant materials used in this work were collected in different regions of Cameroon and included the leaves and roots of *Entada abyssinica* collected at Nde division, West Region in December 2012; the bark of *En-*

tada africana collected at Far Nord Region in February 2011; the bark of *Pentaclethra macrophylla* collected in November 2012 at Mfou division (Centre Region); the leaves of *Allexis cauliflora* and the bark of *Carapa procera* collected in August 2012 at Monkey mount, kribi division (South Region), *Anthocleista leibrechtsiana* collected in November 2012 in June 2012 at Pouma division (Littoral Region), the seeds of *Carica papaya* and stones of *Persea americana* collected in February 2013 at Mfoundi market (Centre Region). The botanical identification of these plants was done at the Cameroon National Herbarium in Yaounde by Mr Victor Nana, where voucher specimens were kept (Table 1). The powdered air-dried (under shade) sample from *Allexis cauliflora, Anthocleista leibrechtsiana, Carapa procera, Carica papaya* and *Persea americana* were extracted with methanol, that of *Entada africana* with the solvent mixture CH_2Cl_2/MeOH **(**1:1), those of *Entada abyssinica* with ethyl acetate and that of *Pentaclethra macrophylla* with CH_2Cl_2/MeOH (1:1) for 48 h at room temperature. The extract was then concentrated under reduced pressure under vacuum to give a residue that constituted the crude extract. They were then kept under 4^0C until use.

Table 1. Plants used in the present study and evidence of their antimicrobial activities.

Plants samples and herbarium voucher number[a]	Traditional used	part used	Known antimicrobial activities of plant extracts or compounds
Fabaceae *Entada abyssinica* 26967 SRF/ CAM	Coughs, fever, rheumatic, abdominal pains, and diarrhea, prevent miscarriage [7, 8], gonorrhea[9], Bronchite, eyes inflammation[10], snake bite[11], sleeping sickness[12].	Leaves and roots	Antimicrobial activities of methanol extracts, fractions and Compounds (5S,6R,8aR)-5-(carboxymethyl)-3,4,4a,5,6,7,8,8a-octahydro-5,6,8a-_trimethylnaphthalenecarboxylic acid, methyl 3,4,5-trihydroxybenzoate, benzene-1,2,3-triol and 2,3-dihydroxypropyltriacontanoate; **M** and **S**: Ef, Sau, Kp, St, Pm, Sf, C. gl, Cn[13]
Fabaceae *Entada africana* 8605 SFR/CAM	Abortive, stimulating agent and tonic, antidote, healing and fever-reducing beverages tonic, stomach ache, wound dressing, preventing suppuration[14].	.bark.	Ethanol extracts ¶ **Q** and W: St: St[15].
Fabaceae *Pentaclethra macrophylla* 29043 SFR/CAM	Diarrhea[16] and abortion[17].	.bark..	-
Violaceae *Allexis cauliflora* 18374 SFR/CAM	Fever, syphilis[18].	leaves	-
Loganiaceae *Anthocleista leibrechtsiana* 5843 SRF/ CAM	Infectious diseases (Personal communication)	.bark..	-
Meliaceae *Carapa procera* 26928 SFR/CAM	Wound infections[19].	bark	Antibacterial activities of ethanol extracts **Q**: *Sa , Ec, Pa*[20].
Caricaceae *Carica papaya* 33284 HNC	Typhoid fever, parasitic diseases[21], hepatic affections, dyspepsia, colic , gastric ulcer[22], toothache[23], analgesic, amebicide, antibacterial, febrifuge, hypotensive, laxative[24].	..seeds....	Antimicrobial activities of aqueous and ethanol extracts; ¶ **W** and **Q**: *Ec, Sa , St, Bs, Pv, Sd, Pm, K p, Pa*[25].
Lauraceae *Persea Americana* 57756 HNC	Diarrhea, dysentery, toothache, intestinal parasites[26], hypertension, cancer, menstrual problems, inflammation, wounds[27];	Stones	Antimicrobial activities of methanol, ethyl acetate and chloroform extracts W *and* Q*: Ec, K p, Bs, Sp, Pa, Sa, Cu, St, Ng, Ca*[28] **.**

[a](HNC): Cameroon National Herbarium; (SRF): Société des reserves forestière du Cameroun; [b](-): Not reported. Screened activity: Significant (S:MIC < 100 µg/mL), moderate (M:100 < MIC≤ 625 µg/mL), Weak (W:MIC > 625 µg/mL)[28], Q:qualitative activity based on the determination of the inhibition zone. Ca:*Candida albicans;* St: *Salmonella typhi;* An*:Aspergilus nicfer;* Bs*:Bacillus subtilis;* EC*:Escherichia coli;* Kp:*Klebsiella pneumoniae;* Pa:*Pseudomonas aeruginosa;* Pvt:*Proteus vulgaris;* Sau: *Staphylococcus aureus; Cn: Cryptococcus neoformans,* Cu: *Candida utilis,* Sc: *Saccharomyces cereviciae;*Sm:*Streptococcus mutans;* Sa:*Streptococcus aeoginosa;* La :*Lactobacillus acidophillus;* ML:*Micrococcus luteus;* San:*Streptococcus anginosus;* Ea:*Enterobacter aerogenes;* Rs:*Rhizoctonia solanic;* Bp:*Bacillus punilus;* Sb:*Shigella boydii;* Ss:*Shigella sonnei;* Sd:*Shigella dysenteria;* Vc*:Vibrio cholerae;* Csp:*Citrobacter sp;* Pm:*Proteus mirabilis;Eag: Enterobacter agglomerans Ecl: Enterobacter cloacae* Sal*:Staphylococcus albus;* Sma: *Serratia marcescens;* Sp: *Schizosaccharomyces pombe;* Ha:*Hansenula anomala;* Scl:*Sclerotinia libertiana;* PC:*Penicillim crustasum;* Mm:*Mucor mucedo;* Rc:*Rhizopus chinensis;*Ecl:*Enterobacter cloacae*; PS*:Providencia stuartii;* Pl*:Paenibacillus larvae***;** Vhs: *Herpes simplex virus*;Vp: Virus of poliomyelitis;VI: *Influenza virus*; Mm: *Morganella morganii Sd: Shigella dysenteriae; Pv: Proteus vulgaris, Cf: Citrobacter freundii,*

Bacterial strains and culture media

The studied microorganisms included references (from the American Type Culture Collection) and clinical (Laboratory collection) strains of *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae,* and *Providencia stuartii* (Table 2). They were maintained on agar slant at 4°C and sub-cultured on a fresh appropriate agar plates 24 hrs prior to any antimicrobial test. Mueller Hinton Agar (MHA) was used for the activation of bacteria for 24 h prior to use and the Mueller Hinton Broth (MHB) was used for the MIC determinations.

Table 2. Bacterial strains and features

Bacteria	Features	References
Escherichia coli		
ATCC8739	Reference strain	
AG100A	AG100⊿*acrAB*::KAN^R	29, 30
Enterobacter aerugenes		
ATCC13048	Reference strain	
CM64	CHLR resistant variant obtained from ATCC13048 over-expressing the *AcrAB* pump	31
Klebsiella pneumoniae		
ATCC11296	Reference strain	
Kp55	Clinical MDR isolate, TET^R ,AMP^R ,ATM^R , and CEF^R	32
Providencia stuartii		
ATCC29916	Reference strain	
NAE16	Clinical MDR isolate, *AcrAB-TolC*	33

AMP^R, ATM^R, CEF^R, CFT^R, CHL^R, FEP^R, KAN^R, MOX^R, NAL^R, NOR^R STR^R, and TET^R Resistance to ampicillin, aztreonam, cephalothin, cefadroxil, chloramphenicol, cefepime, kanamycin, moxalactam, streptomycin, and tetracycline; MDR: multidrug resistant., OMPF and OMPC: Outer Membrane Protein F and C respectively. AcrAB-TolC : efflux pump AcrAB associate to TolC porine.

Bacterial susceptibility determinations

The respective MICs of samples on the studied bacteria were determined by using rapid *p*-Iodonitrotetrazolium chloride (INT, Sigma-Aldrich, St. Quentin Fallavier, France) colorimetric assay[34]. Briefly, the test samples were first dissolved in dimethylsulfoxide (DMSO)/ MHB. The solution obtained was then added to MHB, and serially diluted two fold (in a 96-well microplate). One hundred microlitres (100 µL) of inoculum (1.5 × 10^6 CFU/mL) prepared in MHB was then added. The turbidity of the microbial suspension was adjusted with a densitometer to a McFarland standard of 0.5 that is equivalent to 1-5 x 10^8 CFU/mL. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a shaker and incubated at 37○C for 18 hrs. The final concentration of DMSO was set at 2.5% (a concentration at which DMSO does not affect the microbial growth). Wells containing MHB and 100 µl of inoculums served as a negative control. Chloramphenicol (CHL) was used as reference antibiotic. The MICs of samples were detected after 18 h of incubation at 37○C, following addition (40 µL) of 0.2 mg/mL INT and incubation at 37○C for 30 min[5]. Viable bacteria reduced the yellow dye to pink. MIC was defined as the lowest sample concentration that exhibited complete inhibition of microbial growth and then prevented this change MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth.

For the determination of MBC, the microplates were filled by 150 µL of MHB without extract of plant; for wells not having received a INT (during the reading of the MIC), 50 µL of the contents of the wells corresponding to the concentrations higher or equal to the MIC was taken and introduced into these microplates. These were then incubated during 48 h à 37^{0}C, followed by revelation with the INT. All the concentrations among which we did not observe pink coloring were taken as bactericidal and the lowest was noted as MBC.

Results

The data summarized in Table 3 shows the antibacterial activities of the tested strains. All extracts were active on at least one of the eight tested bacteria with the MIC values ranging from 64 to 1024 µg/mL. The most active extracts were those of *P. macrophylla* (TPM), *E. africana* (TM2), bark of young from plant *E. abyssinica* (TM1') and bark of old plant from *E. abyssinica* (TM1) with the respective inhibitory activities recorded against 62.5 %, 50%, 37.5% and 37.5%. The lowest MIC value (64 µg/mL) was obtained with *E. africana* (TM2) extract against *E. coli* AG100A. This strain was the most sensitive amongst the tested bacteria towards all the plant extracts whilst no activity was recorded against *E. aerugenes* CM64 at the tested concentrations. The MIC of chloramphenicol was lower compared to those of the tested extract on all bacteria used in this study. However they were still high and varied from 8 to above 256 µg/mL. This confirms the high level of resistance of studied bacterial strains. The extracts of *P. americana* (AV), *E. africana* (TM2) and *A. cauliflora* (ACT) showed MBC values of 128; 256 and 512µg/mL respectively against *Echerichia coli* AG100A.

Table 3 Minimal inhibitory concentration (MIC) and minimal inhibitory bactericidal concentration (MBC) of test plant extracts and chloramphenicol (µg/mL).

Bacterial species	Tested samples, MIC and MBC (in bracket)											
	ACT	ALD	AV	CPE	PAY	TM2	TM1' (Bark)	TM1' (leaves)	TM1 (bark)	TM1 (roots)	TPM	CHL
Escherichia coli												
ATCC 8739	-	-	-	-	-	-	-	128(512)	-	-	-	8(128)
AG100A	512 (512)		128 (128)		-	**64** (256)	-	-	-	-	1024(-)	16(128)
Klebsiella												
	-	-	-	-		-	1024(-)	1024(-)	1024(-)	512(-)	1024(-)	16(256-)
KP55	-	-	-	-		-	512(1024)	-	128(512)	-		32(1024)
	-	-	-	-	-	-	512(-)	-	1024(-)	256(-)		8(128)
CM64	-	-	-	-	-	-	-	-	-	-	-	>256(-)
	-	1024 (-)	-	-	-		1024(-)		-	-	1024(-)	32(1024)
NAE16		-	-	-	512(-)		-		-	-	-	32(1024)

(-):> 1024 µg/mL for the extracts; (in braket): MBC in µg/mL; AV: *P. Americana,* TM2: *E. Africana;* T M1': Young *E. abyssinica,* TM1: Old *E. abyssinica*, TPM:*P. macrophylla*, ACT: *Al. cauliflora;* ALD: *A. leibrechtsiana*, CPE: *Carapa procera* PAY: *C. papaya,* CHL: Chloramphenicol. (in bold): significant activity

Discussion

Plants constitute a good source of anti-infective agents and were found to be effective in the fight against microbial infections[35]. A number of secondary metabolites derived from plants such alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes have previously showed antibacterial activities[4,5,36]. The extracts are considered to possess significant activity when they have MIC below 100 µg/mL, moderate activity when their MICs vary between 100 and 625 µg/mL or weak activity their display MICs above 625 µg/mL[4]. Consequently, the activity of *E. Africana* (TM2) against *E. coli* AG100A (64 µg/mL) could be considered important. Nevertheless, the overall activity of the studied plants could be considered as selective and rather moderate or weak. To the best of our knowledge, the *in vitro* antibacterial activity of *P. macrophylla* is being reported for the first time. Nevertheless, the aqueous and ethanol leaf extracts of this plant were previously tested for their anti-diarrheal activity using experimental animal models. Diarrheal infections are also caused by pathogenic bacteria such as *E. coli* and other enterobacteriaceae. Besides, it was demonstrated several tannins, alkaloids, saponins, flavonoids, steroids and or terpenoids have antibacterial activities[37].

Further detection of this class of chemical in this extracts will therefore provide better understanding on its antibacterial potential. The antimicrobial activities of plants of the genus *Entada* have also been demonstrated[13,15]. Teke and al.[13] demonstrated that the methanol extract, fractions and compounds from the stem bark of *E. abyssinica* have moderate activities against bacteria and fungi. The weak activity observed in this work is therefore consistent with their studies. The weak antibacterial activities of the methanol extract of *E. abyssinica* stem bark have also been reported[38], validating the low inhibitory potential of the plant as documented herein. The presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides have been reported in *E. abyssinica* and *E. africana*[13,39].

The activities recorded in this study may be due to the presence of such chemical classes chemicals in the tested extracts. Although the activity recorded with the methanol extract of *P. americana* was found weak, this plant is known to possess antimicrobial activities against sensitive bacteria and fungi[28]. The result of antibacterial activity obtained with the extract of the seeds of *C. papaya* is in accordance with those obtain by Ogunjobi and Ogunjobi[25] who previously demonstrated the antibacterial activity of ethanol and aqueous extract of the seeds of *C. papaya* on the various bacteria stains. Ogunjobi and Ogunjobi[25] also revealed showed that the seeds of this plant contain reducing sugars, phenols, alkaloids and tannins which could be responsible for the inhibitory activities of this plant as observed against *K. pneumoniae* ATCC11296 and *P. stuarti* NAE16. The antibacterial activity of the ethanol extract of *C. procera* has also been demonstrated against *S. aureus, E. coli*, and *P. aeruginosa* strains[20]. The present study therefore brings additional information on the antibacterial activities of this plant against multi-resistant bacteria. To the best of our knowledge, the antibacterial activities of *A. leibrechtsiana* and *A. cauliflora* extracts are being reported here for the first time. The weak antibacterial activities of most of the studied plants could be due to the resistance features of the studied bacterial strains. However, their effects on at least one bacterial species could justify their use in African traditional medicine in the treatment of microbial infections as reported in Table 1.

Conclusions.

The present work provides a supportive information of the antibacterial activities of the tested medicinal plants and the possibility to use the extracts from *Pentaclethra macrophylla*, *Entada africana*, *Entada abyssinica* in the control of selected bacterial infections.

Competing interests

The authors declare that they have no competing interest.

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