Original Article

The *in-vitro* antimicrobial activity of some traditionally used medicinal plants against beta-lactam-resistant bacteria

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Abstract

Background: In effort to identify novel bacterial agents, this study was initiated to evaluate the antimicrobial properties of 17 crude extracts from 12 medicinal plants against beta-lactam-resistant bacteria.

Methodology: The antimicrobial activities of plant extracts were evaluated against clinically proved beta-lactam-resistant bacteria (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Serratia marcescens*, *Acinetobacter baumannii*, *Staphylococcus aureus* and *Enterococcus* sp.) and reference strains of bacteria (*Escherichia coli* ATCC 35218, *Enterobacter aerogenes* ATCC 29751, *E. aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus hirae* ATCC 9790) by using disc-diffusion and agar-dilution assays.

Results: The crude plant extracts demonstrated broad spectrum activity against all bacteria tested with inhibition zones in the range of 8-30 mm. The minimal inhibitory concentration (MIC) values of different plant extracts against the tested bacteria were found to range from ≤ 0.3 to ≥ 10 mg ml^{-1}. The most active plant extracts were from *Dortenia picta* and *Bridelia micrantha* (MIC: 1.25-10 mg ml^{-1}) on beta-lactam-resistant Gram-negative bacilli and the extracts from *B. micrantha*, *Mallotus oppositifolius*, *Garcinia lucida*, *Garcinia. kola*, *Campylospermum densiflorum* (leaves) and *C. zenkeri* (root) on beta-lactam-resistant Gram-positive cocci (MIC: ≤ 0.3-5 mg ml^{-1}).

Conclusion: Of the 17 plant extracts studied, seven showed good antimicrobial activity against the tested bacteria. The stem bark of *B. micrantha* and the leaves of *D. picta* were most active towards beta-lactamase producing Gram-negative bacilli. This study shows that medicinal plants could be sources of compounds which can be used to fight against beta-lactam resistant bacteria.

Key words: beta-lactam-resistant bacteria, antimicrobial activity, Cameroon, Medicinal plant, beta-lactamase

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Introduction

Antimicrobial-resistant bacteria are the causes of numerous clinical problems worldwide. The development and increase of resistance among pathogens causing nosocomial and community-acquired infections are known to be associated with the widespread utilization (and sometimes overutilization) of antibiotics [1]. Infectious diseases caused by resistant microorganisms are responsible for increased health costs as well as high morbidity and mortality, particularly in developing countries.

Beta-lactams constitute one of the most important antibiotics families in worldwide use. More than fifty products were developed, exhibiting sometimes expanded spectra of action, low toxicity and in many cases, reasonable cost. Resistance to this antibiotic family can be attributed to several factors. However, the production of beta-lactamases (EC 3.5.2.6) is the major determinant of resistance [2]. These enzymes which hydrolyse the beta-lactam ring have been the subject of extensive microbiological, biochemical and genetic investigations. More than 500 beta-lactamases have been described (http:// www.lahey.org/studies/ consulted on February 20, 2008) and divided into four molecular classes: A, B, C and D [3]. The majority of these enzymes have

been described in Gram negative bacteria which are responsible for numerous infectious diseases and are generally multidrug resistant.

Previous studies in Cameroon showed high levels of resistance of commonly used antibiotics (penicillin, trimethoprim/sulfamethoxazole) in Gram negative bacilli and highlighted the emergence of multidrug-resistant bacteria which produce extended spectrum beta-lactamases [4-7].

In developing countries, due to the cost of efficient antimicrobials, a large proportion of the population utilize medicinal plants for the treatment of infectious diseases. According to the World Health Organization's estimation, traditional healing provides the primary health care needs for a large majority (80%) of the population in Africa [8]. Moreover, it is important to search for new antimicrobials to combat infectious diseases caused by multidrug-resistant bacteria including Gram negative bacilli.

In many places in Cameroon, there is a rich tradition of using herbal medicine for the treatment of various infectious diseases, inflammations, injuries, and other diseases [9]. In an ongoing programme of research and development of traditional medicine in Cameroon focused on the screening of traditionally used Cameroonian plants for antimicrobial properties, we have reported antibacterial activities on Gram positive bacteria [10], and beta-lactamase inhibitory properties of some plant extracts [11]. The present study was initiated to evaluate the antimicrobial activity of 17 crude extracts from 12 medicinal plants against beta-lactam-resistant bacteria. These plants are currently used by the population and traditional healers for the treatment of various diseases (Table 1).

Materials and Methods

Test organisms

The bacterial strains were either reference strains acquired from the American Type Culture Collection (Manassas, VA), or clinical isolates from the laboratory collection conserved at the Institute of Medical Research and Medicinal Plant Studies in Yaoundé, Cameroon.

Clinical strains were identified by conventional techniques [12] and were confirmed by API 20 E (bioMérieux, France). The isolates studied included Gram-negative bacilli (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Serratia marcescens* and *Acinetobacter baumannii*) and Gram-positive cocci (*Enterococcus* sp., *Staphylococcus aureus*, *Staphylococcus saprophyticus*). With the exception of *S. saprophyticus*, the clinical strains were resistant to at least one beta-lactam antibiotic. Reference bacterial strains were beta-lactamase producers *Escherichia coli* ATCC 35218, *Enterobacter aerogenes* ATCC 29751, *E. aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 27853, low susceptible beta-lactam strain *Enterococcus hirae* ATCC 9790, and susceptible strains *E. coli* ATCC 25922, *Staph. aureus* ATCC 25923.

Antimicrobial susceptibility of Gram-negative clinical strains was determined by E-test, according to the manufacturer's recommendation (AB Biodisk, Solna, Sweden). The antibiotics tested were amoxicillin, amoxicillin/clavulanate, piperacillin, imipenem, cephalotin, cetriaxone, cefotaxime, and ceftazidime. The double-disc (DD) synergy test [13] was used for detection of extended spectrum beta-lactamases (ESBL). A central disc of amoxicillin/clavulanate was surrounded by discs of cefotaxime, ceftriaxone, ceftazidime, and aztreonam at a distance of ca. 19 mm (center to center) on a Mueller-Hinton agar plate (Difco Laboratories, Detroit, MI) inoculated according to the standard procedures [14]. Distortion of the peripheral inhibition zones of surrounding antibiotics toward the central disc with clavulanate was indicative of an ESBL. The tests were repeated with a disc spacing of 15 mm (center to center). Each strain was routinely sub-cultured, at 37°C, on tryptic-soy agar (bioMérieux, Marcy l'Etoile, France).

Plant materials

The seeds, stem barks, leaves or roots (whichever parts of the plant are used in traditional medicine) of 12 plant species (*Picralina nitida*, *Bridelia micantha*, *Mallotus oppositifolius*, *Garcinia lucida*, *Garcinia kola*, *Dorstenia picta*, *Barteria fistulosa*, *Adenia lobata*, *Prunus africanus*, *Campylobacter excavatum*, *Campylobacter densiflorum and Campylobacter zenkeri*) were collected from various places in Cameroon from 2002 to 2006 (Table 1). Samples were identified by a botanist at the Centre for the Research on Medicinal Plants and Traditional medicine, Institute of Medical Research and Medicinal Plants Studies. A voucher specimen of each species was deposited at the National Herbarium of Cameroon.

Preparation of extracts

Each batch of plant material was air dried and powdered. Between 15 and 60 grams of each powder were then extracted with 500 ml methanol, at room temperature and with constant shaking, for 24 hours. Each extract was filtered and concentrated to dryness under reduced pressure.

Table 1. Details on the 12 medicinal plant species that were investigated.

Family	Botanical name	Voucher number	Site of collection	Part used	Uses in traditional medicine
Apocynaceae	*Picralima nitida* (Stapf.) T. & H.Durand	1941/SRFK	Mbalmayo (Centre)	Seeds Leaves Roots	Hypertension, fever, malaria, anti-inflammatory, antimicrobial
Euphorbiaceae	*Bridelia micantha* (Hochst.) Baill.	19699/SRFCam	Yaoundé (Centre)	Stem bark	Cough, antimicrobial, diarrhoea, gastric ulcer, intestinal worms, eye diseases
	Mallotus oppositifolius (Geiseler) Müll.Arg.	4964/SRFK		Leaves	Dysentery, intestinal worms, diarrhoea,
Clusiaseae	*Garcinia lucida* Vesque	17974/ SRFCam	Lolodorf (Sud)	Seed Stem bark	Gastric ulcer, fermentation of palm wine, gynecological infections, anti-poison, gastro-intestinal infections, snake bites
	G. kola Heckel	9815/ SRFCam	Ngok Mapubi (Centre)	Stem bark	
Moraceae	*Dorstenia picta* Bur.	32453/ SRFCam	Tombel (South west)	Leaves	Diarrhoea, infected wounds, anti-inflammatory, antimicrobial, eye diseases, snake bites
Passifloraceae	*Barteria fistulosa* Mast.	19809/ SRFCam	Kaya (Centre)	Leaves	Infected wounds, fever, rheumatism
	Adenia lobata (Schum. & Thonn.)	43292/HNC	Baham (West)	Stem	Cough, colic
Rosaceae	P*runus africanus* (Hook. F.) Kalkman	35610/ HNC	Balembo (West)	Leaves	Dermatological infection, prostatis, abdominal pain, purgative
Ochnaceae	*Campylobacter excavatum* (Tiegh.) Farron	41530/ HNC	Kribi (Sud)	Leaves	Chest and gastric pains
	C. densiflorum (De Wild. & T.Durand) Farron	30055/ HNC		Roots Leaves	
	C. zenkeri (Engl. ex Tiegh.) Farron	41982/ HNC		Leaves Roots	

Antimicrobial assays

The antimicrobial activity of each crude extract was measured *in vitro* against 18 microbial cultures representing 5 Gram-positive cocci and 13 Gram-negative bacilli. The antimicrobial properties were investigated by disc diffusion and agar dilution methods, as recommended by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) [14,15].

Disc diffusion

Each dried extract was dissolved in 50% methanol to give 200 mg ml^{-1}, and sterilized by filtration through a 0.22-µm-pore filter (Millipore, Billeria, MA). The antimicrobial activities of each extract

Table 2. Susceptibility of Gram-negative bacilli to beta-lactam antibiotics.

Strains	Minimum inhibitory concentration (µg/ml) of*							
	AC	XL	PP	CE	TX	CT	TZ	IP
K. pneumonia HGY19	≥ 256	12	≥ 256	32	0.047	0.047	0.50	0.19
K. pneumonia HGY6	≥ 256	12	≥ 256	≥ 256	≥ 256	≥ 256	6	0.19
K. oxytoca U103	≥ 256	8	≥ 256	128	1.5	1.5	12	0.125
E. cloacae HGY18	≥ 256	16	≥ 256	≥ 256	48	48	≥ 256	0.19
S. mercescens HGY10	≥ 256	16	≥ 256	≥ 256	≥ 256	≥ 256	2	0.25
S. marcescens HGY4	≥ 256	32	≥ 256	≥ 256	≥ 256	≥ 256	3	0.38
A. baumannii HGY12	32	16	≥ 256	96	12	8	2	0.125
A. baumannii HGY13	≥ 256	12	≥ 256	≥ 256	8	8	3	0.19

* AC: amoxicillin; XL: amoxicillin/clavulanic acid; PP: piperacillin; CE: cephalothin; TX: ceftriaxone; CT: cefotaxime; TZ: ceftazidime; IP: imipenem

were then investigated by disc diffusion, using for each plate 100 µl saline containing 10^7 colony-forming units (cfu) [on Mueller-Hinton agar (bioMérieux)]. Filter paper discs (6 mm diameter) were placed on the pre-inoculated agar surface and impregnated with 30 µl (6 mg dried extract/disc) of diluted plant extract. Negative controls were prepared with the solvent used to prepare the plant extracts (50% methanol) while gentamicin (1 mg ml^{-1}) was used as the positive control.

The plates were then incubated a 37°C for 24 hours before the diameters of inhibition zones around each disc were measured. All tests were performed twice and the antimicrobial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts.

Determination of minimal inhibitory concentrations

The minimal inhibitory concentrations (MIC) of each active extract (extract which inhibited growth of the majority of the tested bacteria) were then determined using an agar-dilution method. Each target species of microorganism was cultured overnight on tryptic-soy agar and then suspended in sterile saline to give 10^4 cfu ml^{-1}. Each dried plant extract was dissolved in 50% methanol to yield 200 mg ml^{-1}, before sterilization, by filtration through a 0.22-mm-pore filter. Each extract was then serially diluted with sterile distilled water before being mixed with sterile molten Mueller–Hinton agar to give final concentrations between 0.03 and 10 mg dried extract/ml. Each agar solution was vortexed and then immediately poured into a Petri dish. A suspension of one of the test organisms (at 10^4 cfu ml^{-1}) was spotted on the cold agar with a micropipette. The inoculated plates were incubated at 37°C for 24 hours. At the end of the incubation period, the plates were evaluated for the presence or absence of microbial growth. The lowest concentration of an extract at which there was no visible growth was taken as the MIC for the extract–microbe combination under consideration. As controls, the MIC of gentamicin against each bacterial species was similarly determined. Gentamicin was serially diluted with sterile distilled water to give final concentrations between 0.125 and 32 µg ml^{-1}.

Results

All the Gram-negative clinical strains tested are resistant to aminopenicillin (amoxicillin), ureidopenicillin (piperacillin) and first-generation cephalosporins (cephalothin) (Table 2). Characteristics of clavulanate-induced distortions of inhibition zones indicative of extended spectrum beta-lactamase (ESBL) production were found in three (*K. pneumonia* HGY6, *Kl. oxytoca* U103, *E. cloacae* HGY18) of these strains around the disc containing third-generation cephalosporin and/or aztreonam. Examination of the disc susceptibility tests of clinical strains (results not shown) indicates that the *S. marcescens* strains are high-level cephalosporinase-producers and Gram-positive clinical strains (*S. aureus* U127 and *Enterococcus* sp. P054) are resistant to penicillin.

The 12 plant species investigated are reported in Table 1, with the parts used and traditional medicinal uses. The antimicrobial activity of crude extracts from these plants against bacteria (Gram-negative bacilli and Gram-positive cocci) resistant to beta-lactam antibiotics examined in the present study was assessed qualitatively by measuring the inhibition zone diameters and quantitatively by determining minimal inhibitory concentrations.

The results for the 17 studied plant extracts are summarized in Tables 3 (disc diffusion) and 4 (MICs) for Gram-negative bacilli and in Table 5 for Gram-positive cocci. In the disc-diffusion assays, each of the tested plant extracts inhibited the growth of at least one of the species of beta-lactam-resistant-Gram-negative bacilli and *E. coli* ATCC 25922 (beta-lactam susceptible strain). The extracts of *P. nitida*

(seeds), *B. micrantha*, *M. oppositifolius*, *G. lucida*, *D. picta*, *P. africanus* and *C. zenkeri* (leaves) demonstrated broad spectrum activity against all tested Gram-negative bacilli with inhibition zones in the range of 9-23 mm (Table 3). However, the remaining plant extracts, *P. nitida* (leaves, root), *G. kola*, *A. lobata*, *C. excavatum*, *C. densiflorum*, *C. zenkeri* (root) showed selective activity (inhibition zone range of 8-17 mm) against tested bacteria, with no activity on one of the ESBL producing strains, *K. oxytoca* U103 (Table 3). The MIC values of different plant extracts against Gram-negative bacteria were found in the range of 1.25 - ≥ 10 mg ml^{-1} (Table 4). The most active plant extract was *D. picta* (MIC values: 1.25-5 mg ml^{-1}) followed by the extract of *B. micrantha* (MIC values: 1.25-10). *Garcinia lucida* and *G. kola* showed good activity on non-fermenter Gram-negative bacilli (*Ps. aeruginosa* and *Ac. baumannii*) (MIC, 2.5-10 mg ml^{-1}) (Table 4). The extract of *P. africanus* which exhibited good activity on all test organisms by diffusion test (inhibition zone: 16-23 mm) demonstrated good activity only on *A. baumannii* (2.5-5 mg ml^{-1}) and *E. coli* (5 mg ml^{-1}) strains.

Table 5 gives the antimicrobial activity of different plant extracts against Gram-positive bacteria. With the exception of the extracts of *P. nitida* (leaves) and *B. fistulosa*, which did not show any activity on one (*S. aureus* ATCC 25923) and two isolates (*S. aureus* ATCC 25923 and *S. aureus* U127) respectively, the remaining plant extracts demonstrated good activity on all tested isolates. However, the highest activity was observed on the beta-lactam-susceptible *S. saprophyticus* isolate with wide inhibition zone diameters (16-26 mm) and low MIC values (≤ 0.3-5 mg ml^{-1}). Compared to the activities on beta-lactam-resistant Gram-negative bacilli, the extracts of *B. micrantha*, *M. oppositifolius*, *G.lucida*, *G. kola*, *C. densiflorum* (leaves) and *C. zenkeri* (root) demonstrated high potency on beta-lactam-resistant Gram-positive cocci (*Enterococcus* sp P054 and *S. aureus* U127) (MIC : ≤ 0.3-5 mg ml^{-1}) (Table 5).

With respect to the tested part of plant, it was observed that the seeds of *P. nitida* seem to be more active on Gram-negative bacteria than leaves and roots (*P. nidita*), whereas there is no significant difference in activity on Gram-positive bacteria. For all tested bacteria there is no activity difference according to the tested parts of *G. lucida*, *C. zenkeri* and *C. densiflorum* (Tables 3, 4, 5).

Discussion

The aim of this study was to evaluate the antimicrobial properties of 17 plant extracts against beta-lactam-resistant bacteria. Resistance to beta-lactam is due to numerous contributing factors among which the production of beta-lactamase is the most important [2]. All beta-lactam-resistant Gram-negative bacteria used in this study are beta-lactamase-producing isolates and some are ESBL producers. Further studies indicated that the produced ESBLs are the class A SHV-12 (*K. oxytoca* U103; *E. cloacae* HGY18) and CTX-M-1 (*K. pneumonia* HGY6) enzymes [16]. ESBLs confer resistance to practically all beta-lactams including third-generation cephalosporins and monobactam. This phenotype is generally combined with resistance to non-beta-lactam antibiotics [17]. For the best management of infectious diseases caused by beta-lactam-resistant bacteria in developing countries where the efficient antibiotics are not affordable for the majority of the population, it is important to find alternative agents from medicinal plants.

All beta-lactam-resistant Gram-negative bacilli demonstrated varying levels of susceptibility in terms of inhibition zone diameters, which ranged from 8 to 23 mm. However, only two plant extracts (*B. micrantha* and *D. picta*) showed high potency (MIC values: 1.25-10 mg ml^{-1}) on all tested isolates. Moreover, the extract of *P. africanus* which demonstrated high activity on the basis of inhibition zone diameters (16-23 mm) showed low potency at the MIC level (MIC ≥ 10 for eight of 13 tested isolates). This observation indicated that the relationship between inhibition zone diameters and the MIC values was far from evident. This could be explained by the fact that in crude plant extracts some constituents may influence the diffusion properties of the active compound as already observed by others [18,19]. Previous studies revealed the high activity of *M. oppositifolius* extracts on *P. aeruginosa* NCTC (MIC: 32.5µg ml^{-1}) and *S. aureus* (MIC 25 µg ml^{-1}) [20]. Our study showed reduced activity on these bacteria.

In general, the activity of plant extracts is high on Gram-positive cocci when compared to Gram-negative bacilli. This finding was already reported [10,21] and could be explained by the different cell wall structures of these bacteria. Gram-negative bacteria possess an outer phospholipidic membrane with structural lipopolysaccharide components which is not found in Gram-positive bacteria. This

Table 3. Antimicrobial activities of the medicinal-plant extracts as measured against Gram-negative bacilli in disc-diffusion assays.

Plant species	Methanol extract	Diameter (mm) of zone of inhibition*												
		E. coli 25922	*E. coli* 35218	*E. aerogenes* 29751	*E. aerogenes* 13048	*E. cloacae* HGY18	*K. pneumoniae* HGY19	*K. pneumoniae* HGY6	*K. oxytoca* U103	*S. marcescens* HGY4	*S. marcescens* HYG10	*P. aeruginosa* 27853	*A. baumannii* HGY12	*A. baumannii* HGY13
P. nitida	Leaves	17	17	16	16	17	-**	-	-	13	15	16	-	15
	Roots	-	-	-	9	10	10	-	-	-	-	11	-	15
	Seeds	17	16	15	14	15	17	15	17	18	18	18	16	17
B. micrantha	Stem bark	15	14	14	14	16	15	16	16	16	16	16	15	17
M. oppositifolius	Leaves	16	16	19	14	19	18	14	15	15	13	16	17	18
G. lucida	Seeds	15	13	11	12	13	11	9	10	13	11	14	17	17
	Stem bark	15	10	11	15	13	10	13	8	13	14	13	15	15
G. kola	Stem bark	14	12	13	13	12	13	-	-	11	12	13	13	12
D. picta	Leaves	18	21	18	17	20	17	17	16	19	23	20	21	16
B. fistulosa	Leaves	-	-	-	13	14	8	-	-	12	10	17	16	13
A. lobata	Stem	-	-	8	12	10	8	-	8	11	10	-	16	16
P. africanus	Stem bark	22	20	20	15	17	18	18	19	16	18	19	23	19
C. excavatum	Leaves	14	-	8	9	9	11	-	-	9	8	10	14	14
C. densiflorum	Roots	15	13	10	13	16	12	-	-	12	12	15	-	16
	Leaves	16	15	14	12	15	14	15	-	15	14	15	-	14
C. zenkeri	Leaves	13	12	13	13	13	14	14	15	13	14	13	13	13
	Roots	12	13	11	11	11	10	13	-	10	10	12	9	12
Gentamicin		40	27	29	31	33	-	-	16	-	-	29	31	12

* *E. coli* 25922: *E. coli* ATCC 25922; *E. coli* 35218: *E. coli* ATCC 35218; *E. aerogenes* 29751: *E. aerogenes* ATCC 29751; *E. aerogenes* 13048: *E. aerogenes* ATCC 13048 ; *P. aeruginosa* 27853: *P. aeruginosa* ATCC 27853.

** No activity

Table 4. Minimal inhibitory concentrations of the active plant extracts on Gram-negative bacilli, as determined in the agar-dilution assays.

Plant species	Methanol Extract	Minimal inhibitory concentration (mg/ml)*												
		E. coli 25922	*E. coli* 35218	*E. aerogenes* 29751	*E. aerogenes* 13048	*E. cloacae* HGY18	*K. pneumoniae* HGY19	*K. pneumoniae* HGY6	*K. oxytoca* U103	*S. marcescens* HGY4	*S. marcescens* HYG10	*P. aeruginosa* 27853	*A. baumannii* HGY12	*A. baumannii* HGY13
P. nitida	Leaves	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10
	Seeds	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10
B. micrantha	Stem bark	1.25	10	10	10	2.5	10	10	10	5	5	1.25	1.25	2.5
M. oppositifolius	Leaves	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10
G. lucida	Seeds	5	≥ 10	10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	10	10	10	2.5	5
	Stem bark	5	≥ 10	≥ 10	≥ 10	10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	5	2.5	5
G. kola	Stem bark	10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	NT	NT	≥ 10	≥ 10	10	5	5
D. picta	Leaves	2.5	2.5	2.5	5	2.5	5	5	5	2.5	2.5	2.5	2.5	2.5
B. fistulosa	Leaves	NT	NT	NT	≥ 10	≥ 10	≥ 10	NT	NT	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10
P. africanus	Stem bark	5	5	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	2.5	5
C. densiflorum	Roots	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10
	Leaves	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10
C. zenkeri	Leaves	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	NT	≥ 10	≥ 10	≥ 10	NT	≥ 10
	Roots	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10	NT	≥ 10	≥ 10	≥ 10	≥ 10	≥ 10
Gentamicin **		0.5	0.5	1	0.5	2	≥ 32	≥ 32	≥ 32	≥ 32	≥ 32	1	1	≥ 32

* *E. coli* 25922: *E. coli* ATCC 25922; *E. coli* 35218: *E. coli* ATCC 35218; *E. aerogenes* 29751: *E. aerogenes* ATCC 29751; *E. aerogenes* 13048: *E. aerogenes* ATCC 13048 ; *P. aeruginosa* 27853: *P. aeruginosa* ATCC 27853.

**Minimal inhibitory concentration of gentamicin is given in µg/ml

Table 5. Antimicrobial activities of the medicinal-plant extracts, against Gram-positive bacilli in disc diffusion and agar dilution assays.

Plant species	Extract	Antimicrobial activities*									
		Inhibition zone (mm)					Minimal inhibitory concentration (mg/ml)				
		E. hirae 9790	*Enterococcus sp* P054	*S. aureus* 25923	*S. aureus* U127	*S. saprophyticus*	*E. hirae* 9790	*Enterococus sp* P054	*S. aureus* 25923	*S. aureus* U127	S. *saprophyticus*
P. nitida	Leaves	17	14	-***	12	17	1.25	≥ 10	NT****	≥ 10	1.25
	Roots	18	17	13	21	23	≥ 10	≥ 10	≥ 10	≥ 10	1.25
	Seeds	19	20	22	22	20	2.5	≥ 10	≥ 10	≥ 10	≤ 0.3
B. micrantha	Stem bark	27	15	16	25	17	5	1.25	1.25	1.25	2.5
M. oppositifolius	Leaves	17	20	26	26	26	1.25	2.5	2.5	2.5	≤ 0.3
	Seeds	18	20	22	20	25	5	1.25	≤ 0.3	1.25	≤ 0.3
G. lucida	Stem bark	20	18	19	19	25	2.5	1.25	≤ 0.3	1.25	≤ 0.3
G. kola	Stem bark	15	19	20	18	23	≤ 0.3	0.625	0.625	0.625	≤ 0.3
D. picta	leaves	20	20	21	20	22	0.625	10	5	5	≤ 0.3
B. fistulosa	Leaves	20	17	-	-	17	5	≥ 10	NT	NT	5
A. lobata	Stem	16	21	19	19	22	≥ 10	≥ 10	≥ 10	≥ 10	10
P. africanus	Stem bark	20	22	23	30	29	0.625	≥ 10	5	5	≤ 0.3
C. excavatum	leaves	19	15	12	15	16	≥ 10	≥ 10	≥ 10	≥ 10	2.5
C. densiflorum	roots	17	18	12	12	20	5	10	10	≥ 10	2.5
	leaves	28	16	17	16	22	1.25	10	5	5	1.25
C. zenkeri	leaves	20	15	11	18	16	≥ 10	≥ 10	10	≥ 10	5
	roots	17	17	18	18	20	≤ 0.3	2.5	2.5	5	≤ 0.3
Gentamicin **		32	23	14	38	37	8	2	16	≤ 0.125	≤ 0.125

*Ehi 9790: *E. hirae* ATCC 9790 ; Ensp P054 *Enterococcus* sp P 054 ; Sau 25923: *S. aureus* ATCC 25923 ; Sau U127 : *S. aureus* U 127. Ssa: *S. saprohyticus.*
**Minimal inhibitory concentration of gentamicin is given in µg/ml.
*** -: No activity
**** NT: Not tested

composition makes the cell wall impermeable to lipophilic solutes, and the porins in the cell wall do not allow the penetration of high molecular mass hydrophilic solutes, with an exclusion limit of about 600 Da .

To our knowledge, there was no previous report on the antimicrobial activities on beta-lactam-resistant bacteria or the chemical natures of the potentially antimicrobial compounds of the 12 plant species investigated in the present study. However, some studies revealed the presence of bergapten, coumarin, beta-sitosterol, beta-sitosterol glucopyranoside, oleanolic, naringenic acid, and prorepensin in *D. picta* [22]; flavonoids and xanthones in *G. kola* [23, 24], cycloartane derivatives, anthocyane, flavonoids, saponins triterpenes and alkaloids in *G. lucida* [25-27], friedelin, taraxerone, epifriedelinol, taraxerol in *B. micrantha* [28, 29], sterols in *P. africanus* [30]. It is probable that some of these compounds, alone or in combination, are responsible for the observed antimicrobial properties as previously shown. In addition, previous studies showed that most of these plant extracts are beta-lactamase inhibitors [11]; therefore, active plant extracts had not only antimicrobial properties, but also anti- beta-lactamase activities. Moreover, some of the plant extracts showed good antifungal activities on yeast (*D. picta*), filamentous fungi (*G. kola*, *G. lucida*, *B. micrantha*) and all fungi (*P. africana*) (results not shown). This study supports the traditional antimicrobial use of the tested plant species in various infectious diseases. This study also confirms the antimicrobial activity of *B. micrantha* [31-33].

Concerning the safety of the plants studied, *G. lucida* has little toxicity to Vero cells and the host cells [25], whereas the extract of *B. micrantha* is cytotoxic and possess acute systemic toxicity [34].

Nothing is known of the human toxicities of the other plants.

In conclusion, medicinal plants could be sources of compounds which might be useful in managing beta-lactam resistant bacteria and extended spectrum beta-lactamase-producing entrerobacteria as previously demonstrated [35,36]. However, further studies about the absence of toxicity of plant extracts and the isolation of active compounds are important to propose these plants as alternative approaches to resistance management.

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