Antibacterial activity of six medicinal Cameroonian plants against Gram-positive and Gram-negative multidrug resistant phenotypes

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Abstract

Background: Infectious diseases due to multidrug-resistant bacteria are one of the causes of treatment failures contributing to an increase in mortality and/or morbidity. In this study, we evaluated the antibacterial potential of different parts of six medicinal plants namely Alstonia boonei, Ageratum conyzoides, Croton macrostachys, Cassia obtusifolia, Catharanthus roseus and Paullinia pinnata against a panel of 36 multi-drug resistant (MDR) Gram-negative and Gram-positive bacteria.

Methods: Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of the methanol extracts from different parts of the plants were determined using broth microdilution method; standard phytochemical methods were used for phytochemical screening.

Results: Several phytochemical classes such as polyphenols, sterols, triterpenes, alkaloids, flavonoids and saponins were identified in the plant extracts. MIC values obtained ranged from 64 to 1024 μg/mL. Leaves extract of Catharanthus roseus (86.11 %), Croton macrostachys (83.33 %) and Paullinia pinnata (80.55 %) displayed the best antibacterial spectra. The lowest MIC value of 64 μg/mL was obtained with the Paullinia pinnata stems extract and Cassia obtusifolia extract against the strain of Staphylococcus aureus MRSA8. Results also showed that the tested samples generally displayed bacteriostatic effects with MBC values obtained in only 3.35 % of the cases where plant extracts were active.

Conclusion: The results obtained at the end of this study demonstrate for the first time the antibacterial activity of the studied medicinal plants against MDR bacteria. The tested plants could be a reservoir of molecules to fight against MDR bacterial infections.

Keywords: Cameroon, Gram-negative bacteria, Gram-positive bacteria, Medicinal plant, Multi-drug resistance, Antibacterial activity

Background

Infectious diseases caused by multidrug-resistant bacteria are growing steadily and are associated with a significant attributable mortality [1, 2]. The emergence of multi-drug resistant (MDR) phenotypes was first linked to nosocomial infections; but nowadays they are increasingly responsible for community infections and all pathogenic microorganisms are concerned. In Gram-negative bacteria, one of the mechanisms of resistance is the lowering of intracellular amount of antibacterial substances due to the presence of the resistance nodulation cell division (RND)-type efflux pumps. This phenomenon gives possibility to bacteria developing resistance to a wide range of antibiotics, as well as several biocides [3, 4]. Gram-positive bacteria are also a major cause of hospitalization; infections due to Staphylococcus aureus resistant to methicillin (MRSA) are a major health problem both in hospitals and community environments [5]. MRSA is responsible for
80461 severe infections and causing the death of 11,285 patients annually in the United States [6]. One of the possible ways to overcome this phenomenon of multi-resistance is the continual search for new antibacterial molecules active vis-à-vis of MDR bacteria. With regard to the broad diversity of their secondary metabolites, medicinal plants represent undeniable sources of antibacterial agents. According to WHO [7], 80 % of people in Africa have used medicinal plants for their health care; it is also estimated that among medicines sold worldwide, 30 % contain compounds derived from medicinal plants [8]. Several African medicinal plants previously investigated for biological potential showed good antibacterial activities. Some of them include *Treculia obovoidea* [9], *Albizia adianthifolia* *Laportea ovalifolia* [10], *Alchornea cordifolia*, *Pennisetum purpureum* [11]. In our continuous search of phytochemicals to combat MDR bacterial infections, we designed the present study to evaluate the antimicrobial potential of six Cameroonian medicinal plants namely *Alstonia boonei*, *Catharanthus roseus*, *Ageratum conyzoides*, *Croton macrostachys*, *Cassia obtusifolia*, and *Paullinia pinnata* vis-à-vis MDR Gram-negative and Gram-positive phenotypes.

**Methods**

**Plant materials and extraction**

Various parts of plant (Table 1) were collected from different regions in Cameroon during the month of February 2014. These include *Alstonia boonei* (leaves and bark), *Catharanthus roseus* (leaves and stem), *Ageratum conyzoides* (whole plant), *Croton macrostachys* (leaves), *Cassia obtusifolia* (whole plant), and *Paullinia pinnata* (leaves and stem). After drying, each part was powdered and soaked in methanol for 48 h at room temperature, and then filtered using Whatman filter paper N°1. The filtrate obtain were concentrated at 50 °C under reduce pressure in a vacuum to obtain each plant extract.

**Preliminary phytochemical screenings**

The presence of alkaloids, triterpenes, sterols, flavonoids, polyphenols and saponins were screened according to the common phytochemical methods described by Harborne [12].

**Chemicals**

Chloramphenicol and ciprofloxacin (Sigma–Aldrich, St. Quentin Fallavier, France) were used as reference antibiotics meanwhile *p*-Iodonitrotetrazolium chloride (INT) was used as microbial growth indicator.

**Bacterial strains and culture media**

The studied microorganisms included ATCC (American Type Culture Collection) and MDR clinical strains of Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Providencia stuartii*, *Klebsiella pneumoniae* and *Enterobacter cloacae*) and Gram-positive bacteria (*Staphylococcus aureus*). Their bacterial features are summarized in Table 2; they were maintained at 4 °C on McConkey agar and Mannitol Salt Agar (MSA) for Gram negative and Gram positive bacteria respectively, and sub-cultured on Mueller Hinton Agar (MHA) for 24 h before any test. Mueller Hinton Broth (MHB) was used for MIC and MBC determinations.

**INT colorimetric assay for MIC and MBC determinations**

Minimal inhibitory concentrations (MIC) of different plant extracts were determined using broth microdilution method described by Kuete et al. [13] with some modifications [9]. Briefly, plant extracts, chloramphenicol and ciprofloxacin were dissolved in dimethylsulfoxide (DMSO)-MHB (10:90) and 100 µL each solution was added to a 96 wells microplate containing MHB, then serially diluted two-fold, followed by adding of 100 µL of inoculum prepared in MHB. The microplate was sealed and incubated for 18 h at 37 °C. The final concentration of inoculum was 1.5 ×10⁶ CFU/mL and less than 2.5 % for DMSO in each well; Wells containing DMSO 2.5 % and inoculums were used as negative control whereas chloramphenicol and ciprofloxacin consist of positive control. After 18 h incubation, 40 µL of INT (0.2 mg/mL) was added to each well and re-incubated for 30 min. MIC was defined as the lowest concentration of plant extract that inhibited bacterial growth. The determination of MBC was made by introducing 150 µL of MHB in each well of 96 well plate. Then 50 µL of the well contents which did not show any growth after incubation during MIC assays was introduced in the aforesaid plate accordingly, and incubated at 37 °C for 48 h. The MBC was defined as the lowest concentration of plant extract, which did not produce a color change after addition of INT as described previously.

**Results**

**Phytochemical composition**

The results of qualitative analysis (Table 3) showed that plant extracts contain various phytochemical classes of secondary metabolites. Polyphenols, triterpenes and saponins were present in all plant extracts except those from *Cassia obtusifolia*, *Catharanthus roseus* leaves and stem respectively.

**In vitro antibacterial effect of plant extract**

The methanol extracts from different parts of plants were tested on 36 bacterial strains including 7 Gram-positive and 29 Gram-negative bacterial strains. As
| Plant family/Plant sample - Herbarium voucher number | Traditional use | Part used in this study | Potential active compounds characterized | Previously screened activity |
|----------------------------------------------------|-----------------|------------------------|----------------------------------------|-------------------------------|
| **APOCYNACEAE/Alstonia boonei De Wild – 43368/HNC** | Fever, painful micturition, insomnia, chronic diarrhea, rheumatic pains, anti-venom (snake bites), malaria, diabetes, helminths, arthritis [28, 29]. | Leaves, bark | Echitamine, echitamidine, Voaangine, akuammidine, N-α-formyllechitamidine, N-α-formyl-12-methoxyechitamidine [29]. | Antimalarial, antioxidant, analgesic, anti-inflammatory, antipyretic [30–32]. |
| **APOCYNACEAE/Catharanthus roseus L. – 5689/HNC** | Bleeding arresting, diabetes, fever, rheumatism, cancer [20, 33]. | Leaves, stem | Vincristine, vinblastine, benzoic acid, p-hydroxybenzoic acid, salicylic acid, 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, gallic acid, vanillic acid, chlorogenic acid, kaemferol trisaccharides, Quercetin trisaccharides, Syringetin glycosides [20, 34]. | Wound-healing, antimicrobial, hypoglycemic, antioxidant [18, 20, 33]. |
| **ASTERACEAE/Ageratum conyzoïdes Linn. – 19050/SFR-Cam** | Purgative, fever, ulcers, wound, mental, infectious diseases, headaches, anti-inflammatory, diarrhea [35, 36]. | Whole plant | β-caryophyllene, precocene I, friedelin, Lycopsamine, echinatine, β-sitosterol, stigmasterol, 5-methoxynobiletin, linderoflavone B, eupalestin, sabinene, α and β pinene, β-phellandrene, 1,8-cineole and limonene, eugenol [35]. | Antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antipyretic, insecticidal, antioxidant, antiplasmodial, cytotoxic [35, 37, 38]. |
| **EUPHORBIACEAE/Croton macrostachys Hochst. – 40501/HNC** | Malaria, antidiabetic, purgative mastitis, wounds, gastrointestinal Complications [39–41]. | Leaves | Neoclerodan-5,10-en-19,6β,20,12-diolide; 3α,19-Dihydroxytrachylobane; 3α,18,19-Trihydroxytrachylobane, lupeol, lupenone, betulinic acid, 28-O-acetylbetulin, betulin, lupeol acetate, zeorin, benzoic acid, methyl gallate, methyl 2,4-dihydroxy-3,6-dimethoxybenzoate, lichexanthone, β-sitosterol, and β-sitosterol palmitate, stigmasterol, botulin, crotepoxide [42, 43]. | Antibacterial, antifungal, mosquito larvicidal activity, platelet antiaggregatory, neuroprotective [25, 45–47]. |
| **FABACEAE/Cassia obtusifolia L. – 39847/HNC** | Laxative, eye infections, diarrhea, urinary tract infections, gingivitis, fever, cough [25]. | Whole plant | aloe-emodin, 1-methylauranto-obtusin-2-O-β-D-glucopyranoside, emodin, 1,2-dihydroxyanthraquinone, obtusin, chrysobutin, aurantoobutin, glucobutusin, glucaurantoobutin, 1-desmethylauranto-obtusin, 1-desmethylauranto-obtusin-2-O-β-D-glucopyranoside, 1-desmethylchryso-obtusin, 1-desmethyl-obtusin, auranto-obtusin-6-O-β-D-glucopyranoside, alaternin-1-O-β-D-glucopyranoside, chrysobutin-2-O-β-D-glucopyranoside phycion-8-O-β-D-glucopyranoside, obtusolin, O-methyl-chrysophanol, emodin-1-O-β-D-gentiobioside, chrysophanol-1-O-β-D-gentiobioside, phycion-8-O-β-D-gentiobioside, chrysophanol-1-O-β-D-glucopyranoside- (13)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranose, chrysophanic acid, phycion, questitin, 1,3-dihydroxy-8-methylanthraquinone, chrysophanol-10-O-bianthrone, torosachryson [44]. | Antiparasitic, antimicrobial, cytotoxic [24, 38, 49]. |
| **SAPINDACEAE/Paulinia pinnata L. – 10702/SRF-Cam** | Malaria, erectile dysfunction [24]. | Leaves, stem | Paullonioside A, paulinomides A and B, β-amyrin, 13β,17β-dihydroxy-28-norolean-12-ene, β-sitosterol glucopyranoside, 2-O-methyl-L-chiro-inositol, L-quebrachitols, β-sitosterol, friedelin, daucosterol, aridanin, lotoxidas. [24, 48]. | Antiparasitic, antimicrobial, cytotoxic [24, 38, 49]. |

HNC Cameroon National Herbarium, SRF-Cam Société’ des Réserve Forestières du Cameroun
shown in Table 4, extracts from leaves of Alstonia bonniei, Paullinia pinnata and Catharanthus roseus displayed wide spectra of activity in comparison to those from bark and stems of the same plants. The various plant extracts (when they were active) had MIC between 64 and 1024 μg/mL. Leaves of Catharanthus roseus showed the best spectrum of activity, inhibiting the growth of 86.11 % (31/36) of the bacteria (24/29 Gram-negative bacteria and 7/7 Gram-positive bacteria). The leaves extract of Croton macrostachys also had an interesting activity (30/36; 83.33 %), followed by extract of the leaves of P. pinnata (29/36; 80.55 %) and the whole plant extract of A. conyzoides (25/36; 69.44 %). The lowest MIC value of 64 μg/mL was obtained with the Paullinia pinnata stems extract and Cassia obtusifolia extract against the strain of Staphylococcus aureus MRSA8. In general, analysis of results shows that MBCs were obtained in 3.35 % (7/209) of cases where plant extracts were active.

Discussion

Several classes of secondary metabolites such as alkaloids, triterpenes, sterols, flavonoids, polyphenols and saponins have been reported to have antibacterial properties [13–15]. Their presence in the studied plant extracts could explain the antibacterial effects of the tested samples. The need to find new molecules from medicinal plants with effective mechanisms of action against the multidrug-resistant phenotype is a necessity nowadays. All plants used in traditional medicine which have MIC values less than 8 mg/mL are considered active [16]. A plant extract has significant antibacterial

Table 2 Bacterial strains used in this study and their features

| Strains                        | Characteristics                  | References |
|-------------------------------|----------------------------------|------------|
| **Escherichia coli**          |                                  |            |
| ATCC10536                     | Reference strain                 |            |
| AG100                         | Wild-type E. coli K-12           | [50]       |
| AG100A                        | AG100 ΔacrAB::KAN6               | [50–52]    |
| AG100A_TET                    | ΔacrAB mutant AG100, over-expressing acrF gene; TETR | [53, 54] |
| AG102                         | ΔacrAB mutant AG100, over-expressing acrF gene; TETR | [53, 54] |
| MC4100                        | Wild type E. coli                | [55]       |
| W3110                         | Wild type E. coli                | [55, 56]   |
| **Enterobacter aerogenes**    |                                  |            |
| ATCC13048                     | Reference strain                 |            |
| CM64                          | CHL R resistant variant obtained from ATCC13048 over-expressing the AcrAB pump | [57] |
| EA3                           | Clinical MDR isolate; CHL R, NOR R, OFX R, SPX R, MOX R, CFT R, ATM R, FEP R | [58, 59] |
| EA27                          | Clinical MDR isolate exhibiting energy-dependent norfloxacin and chloramphenicol efflux with KAN R | [58, 59] |
| EA289                         | KAN sensitive derivative of EA27 | [60]       |
| EA294                         | EA289 acrA::KAN R                | [60]       |
| EA298                         | EA289 tolC::KAN R                | [60]       |
| **Enterobacter cloaca**       |                                  |            |
| ECC69                         | Clinical MDR isolates, CHL R     | [61]       |
| BM67                          | Clinical MDR isolates, CHL R     | [61]       |
| BM47                          | Clinical MDR isolates, CHL R     | [61]       |
| **Klebsiella pneumoniae**     |                                  |            |
| ATCC12296                     | Reference strain                 |            |
| KP55                          | Clinical MDR isolate; TET R, AMP R, ATM R, CEF R | [62] |
| KP63                          | Clinical MDR isolate; TET R, CHL R, AMP R, ATM R | [62] |
| K24                           | AcrAB-ToIC, Laboratory collection of UNR-MD1, University of Marseille, France | [61] |
| K2                            | AcrAB-ToIC, Laboratory collection of UNR-MD1, University of Marseille, France | [61] |
| **Providencia stuartii**      |                                  |            |
| NEA16                         | Clinical MDR isolate, AcrAB-ToIC | [63] |
| ATCC29916                     | Clinical MDR isolate, AcrAB-ToIC |            |
| PS2636                        | Clinical MDR isolate, AcrAB-ToIC |            |
| PS299645                      | Clinical MDR isolate, AcrAB-ToIC |            |
| **Pseudomonas aeruginosa**    |                                  |            |
| PA 01                         | Reference strain                 | [64]       |
| PA 124                        | MDR clinical isolate             |            |

Table 2 Bacterial strains used in this study and their features (Continued)

S. aureus

| Strains                        | Characteristics                  | References |
|-------------------------------|----------------------------------|------------|
| ATCC 25923                    | Reference strain                 |            |
| MRSA 3                        | Clinical MDR isolate OFX R, KAN R, TET R, ERM R | [65] |
| MRSA 4                        | Clinical MDR isolate OFX R, KAN R, CHL R, CIP R |            |
| MRSA 6                        | Clinical MDR isolate OFX R, FLX R, KAN R, TET R, CIP R, IM/CS R | |
| MRSA 8                        | Clinical MDR isolate OFX R, FLX R, KAN R, ERM R, CIP R, IM/CS R |            |
| MRSA 11                       | Clinical MDR isolate OFX R, KAN R, ERM R, CIP R, IM/CS R |            |
| MRSA 12                       | Clinical MDR isolate OFX R, FLX R, KAN R, ERM R, IM/CS R |            |

**AMPS**: AMP R, ATM R, CEF R, CFT R, CHL R, CIP R, ERM R, FEP R, FLX R, IM/CS R, KAN R, MOX R, OFX R, STR R, TET R, Resistance to ampicillin, aztreonam, cefazolin, cefadroxil, chloramphenicol, Ciprofloxacin, Erythromycin, cepaflox, Flocloxef, Imipenem/ Cilastatin sodium, kanamycin, mosalactam, streptomycin, and tetracycline; MDR multidrug resistant

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activity if MIC is 100 μg/mL, moderate if its MIC is between 100 and 625 μg/mL and low when MIC is above 625 μg/mL [17]. Based on the above criteria, it can be deduced that all tested plants had antibacterial activity as MIC values below 8 mg/mL were obtained with each extract on at least one bacterial strain. MIC values above 625 μg/mL were obtained with extract from A. boonei bark against 2/36 (5.5 %) tested bacteria as well as with C. roseus stem extract against 6/36 (16.7 %) microorganisms tested, indicating that they rather displayed low antibacterial effects. Nonetheless, the activity obtained with the Paullinia pinnata stems extract and Cassia obtusifolia extract against the strain of Staphylococcus aureus MRSA8 (MIC value of 64 μg/mL) could be considered important. Moderate activity was obtained in many cases. In fact, MIC values ranged from 128 to 512 μg/mL were obtained with extract from A. conyzoides (whole plant) against 12/36 (33.3 %) tested bacteria, A. boonei leaves against 19/36 (52.8 %), C. obtusifolia (whole plant) against 17/36 (47.2 %), C. roseus leaves against 18/36 (50 %), C. macrostachys (leaves) against 25/36 (69.4 %), and P. pinnata stem and leaves against 13/36 (36.1 %) and 19/36 (52.8 %) respectively.

Though the antibacterial activities of some of the tested plants have already been reported, their effects against MDR phenotypes are being documented for the first time. The extract from the leaves of C. roseus had a broad antibacterial activity (31/36; 86.11 %); Nayak and Pereira [18] and Kamaraj et al. [19] reported the antibacterial activity of this plant extract on some sensitive bacteria. Several alkaloids were isolated from this plant [20, 21] and these compounds could also be responsible for the antibacterial activity of this plant [22]. MIC values obtained with extract of leaves of C. macrostachys are between 128 and 1024 μg/mL; Antibacterial compounds previously isolated from this plant include the triterpenoid, lupeol [23]. The extract of P. pinnata possessed a good activity (MIC of 64 μg/mL) against S. aureus MRSA8 while the extract from the leaves was active against 80.55 % (29/36) of the studied microorganisms. Lunga et al. [24] demonstrated the activity of this plant on strains of Salmonella sp. with a bacteriostatic effect, corroborating our findings. The extract of C. obtusifolia significantly inhibited the growth of S. aureus MRSA8 with MIC of 64 μg/mL, and was active on 22 of the 36 tested microorganisms. The activity obtained in this study is much better than that mentioned by Doughari et al. [25]. In fact, they obtained MIC of 2000 μg/mL and 1000 μg/mL on clinical isolate of S. aureus and P. aeruginosa respectively. This could be due to the difference of phytochemical composition as the environmental conditions influence the availability as well as the amounts of some secondary metabolites in the plant. One of the best suited secondary metabolite from this plant is emodin (anthraquinone) which possesses a good antibacterial activity against S. aureus [26]; this could explain the interesting activity observed vis-à-vis of MRSA in this study. The extract of A. conyzoides had a relatively low activity on all studied microorganisms. Nevertheless, MIC of 256 μg/mL vis-a-vis E. aerogenes EA-CM64 and EA27, P. stuartii PS2636, S. aureus MRSA 4 which are multi-drug resistant clinical strains were obtained; this could explain the use of this plant in traditional medicine. Leaves and bark extracts of A. boonei had a moderate activity against Gram-negative bacteria whilst bark extract was not active against Gram-positive species; this is explained by the fact that some antimicrobial compounds have specific activity spectrum (narrow) and therefore will not be active on certain categories or certain species of microorganisms [27]. Though the overall activity of the tested plants can be considered moderate, the results of this study are interesting taking in account the fact that most of the tested bacterial strains were MDR phenotypes.
|                      | A. conyzaïdes (whole plant) | A. boonei | C. obtusifolia (whole plant) | C. roseus | C. macrostachys (leaves) | P. pinnata | Reference drugs |
|----------------------|-----------------------------|-----------|-------------------------------|-----------|-------------------------|-----------|-----------------|
|                      |                            | Leaves    | Bark                          | Leaves    | Stem                    | Leaves    | (Stem) Chloramphenicol |

**Escherichia coli**

|                      | ATCC8739                   | 512 (−)  | −                             | 512 (−)  | −                       | 1024 (−) | 2 (128)         |
|                      | ATCC10536                  | 256 (−)  | −                             | 512 (−)  | 1024 (−)                | 256 (−)  | 128 (−)         |
|                      | AG100                      | 1024 (−) | −                             | 256 (1024)| 1024 (−)                | 256 (−)  | 128 (−)         |<2 (64) |
|                      | AG100A                     | 1024 (−) | −                             | 512 (−)  | 128 (−)                 | 256 (−)  | <2 (128)        |
|                      | AG100ATET                  | 1024 (−) | 512 (−)                       | 1024 (−) | 256 (−)                 | 1024 (−) | 16 (−)          |
|                      | ATCC13048                  | 1024 (−) | 512 (−)                       | 256 (−)  | 256 (−)                 | 256 (−)  | 128 (−)         |

**Pseudomonas aeruginosa**

|                      | PA 01                      | −         | −                             | 256 (−)  | 512 (−)                 | 256 (−)  | 256 (−)         | 1024 (−) | 32 (−)         |
|                      | PA 124                     | −         | −                             | −        | −                       | −        | −               | 128 (−)  |               |

**Enterobacter aerogenes**

|                      | ATCC13048                  | 1024 (−) | 512 (−)                       | 512 (−)  | −                       | 128 (−)  | 4 (32)          |
|                      | EA-CM64                    | 256 (−)  | −                             | 512 (−)  | −                       | 256 (−)  | 512 (−)         | 1024 (−) | 256 (−)         |
|                      | EA3                        | −         | −                             | 256 (−)  | 1024 (−)                | 128 (−)  | −               | 256 (−)  |               |
|                      | EA27                       | 256 (−)  | 512 (−)                       | 512 (−)  | 1024 (−)                | 512 (−)  | 512 (−)         | 32 (−)   |
|                      | EA289                      | 512 (−)  | 1024 (−)                      | 256 (−)  | 512 (−)                 | 1024 (−) | 512 (−)         | 64 (−)   |
|                      | EA298                      | 1024 (−) | −                             | 1024 (−) | 512 (−)                 | 1024 (−) | 512 (−)         | 128 (−)  |

**Providencia stuartii**

|                      | NEA16                      | 1024 (−) | 512 (−)                       | 1024 (−) | 1024 (−)                | 512 (−)  | 128 (−)         | 1024 (−) | 32 (256)        |
|                      | ATCC29916                  | 512 (−)  | 512 (−)                       | −        | −                       | 256 (−)  | 1024 (−)        | 16 (256) |
|                      | PS2636                     | 256 (−)  | −                             | −        | 256 (−)                 | 256 (−)  | −               | 16 (256) |
|                      | PS299645                   | 1024 (−) | 512 (−)                       | −        | 256 (−)                 | 512 (−)  | 512 (−)         | 64 (−)   |

**Klebsiella pneumoniae**

|                      | ATCC11296                  | 512 (−)  | 512 (−)                       | 1024 (−) | 1024 (−)                | 512 (−)  | 1024 (−)        | 8 (256)  |
|                      | KP55                       | 512 (−)  | 512 (−)                       | 256 (−)  | 512 (−)                 | 256 (−)  | 256 (−)         | 32 (256) |
|                      | KP63                       | 1024 (−) | 1024 (−)                      | 512 (−)  | −                       | 1024 (−) | 256 (−)        | 32 (−)   |
|                      | K2                         | 1024 (−) | 256 (−)                       | 1024 (−) | 512 (−)                 | 512 (−)  | −               | 64 (256) |

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Table 4 MIC and MBC (in bracket) of plant extracts and reference drugs (Continued)

| Enterobacter cloacae     |        |        |        |        |        |        |        |        |
|--------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| ECCI69                   | -      | 1024 (-)| 1024 (-)| 1024 (-)| 1024 (-)| 1024 (-)| 1024 (-)| 1024 (-)| 1024 (-)| -      |
| BM47                     | -      | -      | -      | 1024 (-)| -      | 512 (-)| 1024 (-)| 1024 (-)| 256 (-)| -      |
| BM67                     | 512 (-)| 512 (-)| 1024 (-)| 512 (-)| 256 (-)| -      | 256 (-)| 1024 (-)| -      | -      |
| BM94                     | 1024 (-)| 512 (-)| 1024 (-)| 512 (-)| 512 (-)| -      | 512 (-)| 1024 (-)| -      | 128 (-)|

| Staphylococcus aureus    |        |        |        |        |        |        |        |
|--------------------------|--------|--------|--------|--------|--------|--------|--------|
| ATCC25923                | 512 (-)| 256 (-)| -      | 256 (1024)| 512 (-)| 1024 (-)| 256 (-)| 256 (-)| 128 (1024)| 2 (8) |
| MRSA 3                   | -      | -      | -      | -      | -      | 1024 (-)| -      | -      | -      | -      | 32 (128) |
| MRSA 4                   | 256 (-)| 256 (-)| -      | 128 (1024)| 512 (-)| -      | 256 (-)| 256 (-)| 128 (512)| 64 (128) |
| MRSA 6                   | -      | 128 (-)| -      | 256 (-)| 1024 (-)| 512 (-)| 512 (-)| 256 (-)| 256 (-)| 64 (128) |
| MRSA 8                   | -      | 128 (-)| -      | 64 (512)| 128 (-)| 1024 (-)| 512 (-)| 256 (-)| 64 (512)| 16 (64) |
| MRSA 11                  | 1024 (-)| -      | -      | 512 (-)| 1024 (-)| 1024 (-)| 1024 (-)| 512 (-)| 128 (512)| 32 (32) |
| MRSA 12                  | -      | 128 (-)| -      | 256 (-)| 1024 (-)| 1024 (-)| 1024 (-)| 512 (-)| 256 (-)| 256 (-)|

(●): MIC or MBC not detected up to 1024 μg/mL for plant extracts and 256 μg/mL for reference drugs.
Conclusion
The present study demonstrates that plants studied and mostly C. macrostachys, C. roseus and P. pinnata contain phytochemicals with valuable antibacterial activities vis-à-vis multi-drug resistant phenotypes. They could be used in the management of bacterial infections including MDR phenotypes.

Abbreviations
A. coryzae: Agaratum coryzae; Alstonia boonei; Alstonia boonei; ATCC: American type culture collection; C. macrostachys: Croton macrostachys; C. roseus: Catharanthus roseus; C. abutilosifolia: Canna abutilosifolia; CFU: Colony forming unit; DMSO: Dimethyl sulfoxide; E. aerogenes: Enterobacter aerogenes; E. cloacae: Enterobacter cloacae; E. coli: Escherichia coli; INT: p-lodotonintrateilum chloride; K. pneumoniae: Klebsiella pneumoniae; MBC: Minimal bactericidal concentration; MDR: Multi-drug resistant; MHA: Mueller Hinton Agar; MHB: Mueller Hinton Broth; MIC: Minimum inhibitory concentration; MRSA: Methicillin resistant Staphylococcus aureus; MSA: Mannitol Salt Agar; P. aeruginosa: Pseudomonas aeruginosa; P. pinnata: Paulinia pinnata; P. stuartii: PROVIDENCIA stuartii; RND: Resistance nodulation division; S. aureus: Staphylococcus aureus

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Availability of data and materials
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Authors’ contributions
IKV carried out the study; IKV and VK designed the experiments and wrote the manuscript; VB and TP supervised the work; VK provided the bacterial strains; all authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
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Ethics approval and consent to participate
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