Antibacterial and antibiotic-potentiation activities of the methanol extract of some cameroonian spices against Gram-negative multi-drug resistant phenotypes

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Abstract

Background: The present work was designed to evaluate the antibacterial properties of the methanol extracts of eleven selected Cameroonian spices on multi-drug resistant bacteria (MDR), and their ability to potentiate the effect of some common antibiotics used in therapy.

Results: The extract of Cinnamomum zeylanicum against Escherichia coli ATCC 8739 and AG100 strains showed the best activities, with the lowest minimal inhibitory concentration (MIC) of 64 μg/ml. The extract of Dorstenia psilurus was the most active when tested in the presence of an efflux pump inhibitor, phenylalanine Arginine-β-Naphthylamide (PAβN), a synergistic effect being observed in 56.25 % of the tested bacteria when it was combined with Erythromycin (ERY).

Conclusion: The present work evidently provides information on the role of some Cameroonian spices in the fight against multi-resistant bacteria.

Keywords: Multi-Drug Resistant bacteria, Spices, Methanol extract, Cameroon

Background

Infectious diseases are one of the leading causes of morbidity and mortality worldwide, especially in developing countries [1,3]. Following the massive use of antibiotics in human therapy, bacteria have developed several resistance mechanisms including the efflux of antibiotics [3]. Several Cameroonian spices are known to possess medicinal values [4]. In our previous report, we demonstrated that several medicinal spices inhibited the growth of MDR bacteria and were also able to improve the activity of commonly used antibiotics [5]. In our continuous search of antimicrobial drugs from medicinal plant, we designed the present work to investigate the antibacterial potential against Gram-negative MDR bacteria of some of the commonly used medicinal spices in Cameroon such as Aframomum citratum (Pereira) K. Schum. (Zingiberaceae), Aframomum melegueta (Roscoe) K. Schum. (Zingiberaceae), Scorodophloeus zenkeri Harms (Caesalpiniaeae), Tetrapleura tetraptera (Schum. & Thonn) Taub. (Mimosaceae), Fagara leprieurii (Guill and Perr) Engl. (Rutaceae), Monodora myristica (Annonaceae), Piper guineense (Schum and Thonn) (Piperaceae), Dorstenia psilurus Welwitch (Moraceae), Imperata cylin- dricium Beauv. var. koenigii Durand and Schinz (Gramineae), Pentadiplandra brazzeana Baill. (Capparaceae) and Cinnamomum zeylanicum (Linn) Cor. (Lauraceae).

Material and methods

Plant materials and extraction

The eleven edible spices used in this work were purchased from Dschang local market, West Region of Cameroon in January 2010. The collected spices material were the fruits of Aframomum citratum, Aframomum melegueta, Scorodophloeus zenkeri, Tetrapleura tetraptera,
### Table 1: Spices used in the present study and evidence of their activities

| Spice samples (Family) | Herbarium Voucher number | Traditional Treatment | Part used | Bioactive (or potentially active) compounds and screened activity for crude plant extract |
|------------------------|--------------------------|-----------------------|-----------|-----------------------------------------------------------------------------------------|
| Afraamomum citratum (Pereira) K. Schum. (Zingiberaceae) | 37 736/HNC | Malaria, aphrodisiac, cancer | Fruits, leaves, seeds | **Antimicrobial**: Ethylacetate extract of fruits on Ec. Pa. Sa [7] |
|                        |                          |                       |           | Cytotoxicity of fruits crude methanol extract [weak activity on leukemia CCRF-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines] [4] |
| Afraamomum melegueta (Roscoe) K. Schum. (Zingiberaceae) | 39 065/HNC | Malaria, dysentery, carminative, dysmenorrhea, fertility, rubella, leprosy, cancer [6,8] | Fruits, leaves | **Antimicrobial**: Aqueous and ethanol extract of leaves on Fo. An [9] Methanol extract of fruits (Q) on Sa. Ec. Pa. Ca. Ga [8] |
|                        |                          |                       |           | Cytotoxicity of fruits crude methanol extract [weak activity on leukemia CCRF-CEM and pancreatic MiaPaCa-2 cell lines and significant activity on CEM/ADR5000 cells with IC50 value of 7.08 μg/ml] [4] |
| Cinnamomum zeylanicum (Linn) Cor. (Lauraceae) | 22 309/SRFC | Cancer [4] | Fruits, leaves, bark | **Antimicrobial**: Cd, Cm, Lt, Fp [10,11] |
|                        |                          |                       |           | Cytotoxicity of leaves crude methanol extract [weak activity on leukemia CCRF-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines] [4] |
| Dorstenia psilurus Welwitch (Moraceae) | 44 839/HNC | Snake bite, rheumatism, head and stomach ache, hypertension, cancer [4,12,13] | Leaves, roots | **Antimicrobial**: Leaves, roots | |
| Fagara leprieurii (Guill and Perr) Engl. (Rutaceae) | 37 632/HNC | Gastritis, gingivitis, bilharzias, antidiabetic, ulcer, gonorrhea, kidney ache, sterility [4,14,15] | Bark, leaves, roots | **Antimicrobial**: Ethanol extract of the seeds on Ca. Cn. Mg. Tm. Tr. Bci. Af. Afl. Sb [15] |
|                        |                          |                       |           | Cytotoxicity of seeds crude methanol extract [weak activity on leukemia CCRF-CEM and pancreatic MiaPaCa-2 cell lines and significant activity on CEM/ADR5000 cells with IC50 value of 8.13 μg/ml] [4] |
| Imperata cylindrica Beauv. var. koenigii Durand et Schinz (ramineae) | 30 139/SRFC | Diuretic, anti-inflammatory, dysentery, urinary tract infections, cancer [4,16,17] | Leaves, roots | **Antimicrobial**: | |
|                        |                          |                       |           | Cytotoxicity of roots crude methanol extract [significant activity with IC50 values of 8.34; 7.18 and 12.11 μg/ml respectively on leukemia CCRF-CEM cells, CEM/5000 cells and pancreatic MiaPaCa-2 cell lines] [4] |
| Monodora myristica Dunal (Annonaceae) | 2 949/SRFC | Insecticidal, diuretic, constipation, anti-inflammatory, wound, worm infections, cancer [4,15,18,19] | Fruits, leaves, seeds | **Antimicrobial**: Fm. All. Af [18]; Essential oil. Af. Bs. Cgl. Ec. Ap. Sa. Sf [15]. Cytotoxicity of fruits seeds methanol extract [weak activity on leukemia CCRF-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines] [4] |
| Pentadiplandra brazzeana Baill. (Capparaceae) | 42 918/HNC | Gastric ulcer, cancer [4,20] | Fruits, leaves | **Antimicrobial**: | |
|                        |                          |                       |           | Cytotoxicity of roots crude methanol extract [weak activity on leukemia CCRF-CEM and pancreatic MiaPaCa-2 cell lines and significant activity on CEM/ADR5000 cells with IC50 value of 8.13 μg/ml] [4] |
| Piper guineense (Schum and Thonn) (Piperaceae) | 6 018/SRFC | Cough, bronchitis, rheumatism, insecticidal, anemia, carminative, stomach ache, cancer [4,8,21] | Fruits, leaves, bark | **Insecticidal**: C. [20] **Antimicrobial**: (Q); Ec. Sa. Bs. Pa. Ca. An [8,22] |
| Scorodophloeus zenkerii Harms (Caesalpiniaceae) | 44 803/HNC | Cancer [4] | Leaves, roots | **Antimicrobial**: Ethanol oil of stem bark on Ec, Sa, Bs, Cu [23] |
|                        |                          |                       |           | Cytotoxicity of fruits crude methanol extract [weak activity on leukemia CCRF-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines] [4] |
Table 1 Spices used in the present study and evidence of their activities (Continued)

| Spice samples | Extraction Physical aspect Phytochemical composition | Cytotoxicity of fruits crude methanol extract | Bark, leaves, roots |
|---------------|-----------------------------------------------------|---------------------------------------------|---------------------|
| Tetrapleura tetraptera | 12 117/SRFC (Schum. & Thonn) Taub. (Mimosaceae) | Pain, arthritis, epilepsy, convulsion, gastric ulcer, cancer (4,20) | weak activity on leukaemia CCRP-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines (4) |
| Scorodophloeus zenkeri | 9.2 Creamy, brown | + | - | + | + | + | - | + | + |
| Imperata cylindricum | 10.3 Oily, brown | + | + | + | + | + | + | - | + |
| Dorstenia psilurus | 10.3 Oily, brown | + | + | + | + | + | + | - | + |
| Fagara leupriecui | 26.2 Creamy, brown | + | - | + | + | + | - | - | + |
| Monodora myristica | 23.5 Oily, brown | + | + | + | + | + | - | - | + |
| Pentadiplandra brazzeana | 4.6 Creamy, brown | + | - | - | + | + | - | - | - |
| Piper guineense | 17.5 Creamy, brown | + | + | + | + | + | - | - | - |
| Scorodophloeus zenkeri | 9.8 Creamy, dark green | + | - | - | + | + | - | - | + |
| Tetrapleura tetraptera | 29.4 brown | + | + | + | + | + | - | + | + |

(+) Present; (−) Absent; *The yield was calculated as the ratio of the obtained methanol extract according to the initial mass of the spice powder.

the seeds of Fagara leupriecui, Monodora myristica and Piper guineense, the roots of Dorstenia psilurus, Imperata cylindricum and Pentadiplandra brazzeana and the leaves of Cinnamomum zeylanicum. The plants were identified by Mr. Victor Nana of the National herbarium (Yaoundé, Cameroon) where voucher specimens were deposited under a reference number (Table 1). The extracts were obtained by methanol (MeOH) maceration as previously described [5].

Preliminary phytochemical investigations
The major secondary metabolites classes were screened according to the common phytochemical methods described by Harborne [24].

Chemicals for antimicrobial assays
Tetracycline (TET), ceftazidime (FEP), streptomycin (STR), ciprofloxacin (CIP), norfloxacin (NOR), chloramphenicol (CHL), cloxacillin (CLX), ampicillin (AMP), erythromycin (ERY), kanamycin (KAN) (Sigma-Aldrich, St Quentin Fallavier, France) were used as reference antibiotic. p-Iodonitrotetrazolium chloride (INT) and phenylalanine arginine β-naphthylamide (PAßN) were used as microbial growth indicator and efflux pumps inhibitor (EPI) respectively.

Bacterial strains and culture media
The studied microorganisms included reference (from the American Type Culture Collection) and clinical (Laboratory collection) strains of Providencia stuartii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes and Enterobacter cloacae. The bacterial strains and their features were previously reported [5]. The preliminary treatment of these organisms as well as the culture media were conducted as previously described [5].

Bacterial susceptibility determinations
The respective MICs of samples on the studied bacteria were determined using rapid INT colorimetric assay [25,26] with some modifications as previously reported [5]. The inoculum concentration used was 1.5 x10⁶ CFU/ml and the samples were incubated at 37 °C for
| Bacterial strains | Tested samples and MIC in μg/ml in the absence and presence of PAßN (in parenthesis) |
|-------------------|----------------------------------------------------------------------------------|
|                   | Aframomum citratum | Aframomum melegueta | Imperata cylindricum | Cinnamomum zeylanicum | Dorstenia psilurus | Fagara leprieuri | Monodora myristica | Pentadiplandra brazzeana | Piper guineense | Scorodophloeus zenkeri | Tetrapleura tetraptera | CHL |
| E. coli ATCC8739  | 512 | 512 | 512 | 64 | - | 512 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1 |
| ATCC10536         | 1024 | 512 | 1024 | 512 | 128 | 256 | 1024 | 512 | 1024 | 512 | 1024 | 1024 | 1024 | 32 (<2) |
| AG100             | 1024 (1024) | 1024 (1024) | 1024 (256) | - (64) | - (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 4 (<2) |
| AG100A            | 512 (128) | 1024 (1024) | 1024 (128) | 512 (128) | 512 (512) | 1024 (1024) | - (-) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 512 (512) | 2 (<2) |
| AG100ATET         | 512 (512) | 1024 (1024) | 1024 (1024) | 512 (512) | 512 (128) | 1024 (1024) | - | 1024 | 512 | 1024 | 32 (<2) |
| AG102             | 1024 | - | 1024 | 1024 | 512 | 1024 | - | - | - | - | - | 16 (<2) |
| MC4100            | 512 (512) | 512 (256) | 1024 (1024) | 1024 (1024) | 512 (256) | 512 | - (-) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 512 (512) | 4 (<2) |
| W3110             | 512 (256) | 512 (512) | 512 (512) | 512 (256) | 256 | 512 | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1 (<2) |
| E. aerogenes ATCC13048 | 1024 | - | 1024 | 1024 | 1024 | 1024 | 1024 | - | - | - | - | - | 8 (<2) |
| CM64              | 1024 (1024) | 1024 (1024) | 512 (128) | 1024 (512) | 512 (256) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 512 (512) | 32 |
| EA27              | 512 (512) | 1024 (1024) | 512 (512) | 512 (512) | - (-) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 512 (512) | 64 (32) |
| EA289             | - | 1024 | - | - | 1024 | - | - | 1024 | 1024 | - | - | 1024 | 256 |
| EA298             | 1024 | 512 | - | - | - | 1024 | - | 256 | 256 | 512 | 256 | 1024 | 256 |
| EA3               | - | - | - | - | - | 1024 | - | - | - | - | - | - | - | 256 |
| E. cloacae BM47   | 512 (512) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (128) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 | 1024 | 1024 | 1024 | - (8) |
| BM67              | 512 (512) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (128) | - (-) | - (-) | - (-) | - (-) | - (-) | - (-) | - (-) | - (-) | - (32) |
| ECC169            | 512 (512) | 1024 (1024) | 1024 (1024) | 1024 (1024) | -(-) | -(-) | 1024 (1024) | -(-) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | - (32) |
| K. pneumoniae ATCC12296 | 1024 | 1024 | 1024 | 1024 | 1024 | 512 | - | - | - | - | - | 1024 | 4 |
| K2                | 1024 | - | 1024 | 1024 | 1024 | - | 1024 | - | - | - | - | - | - |
| K24               | 1024 | 1024 | 1024 | 1024 | 1024 | 512 | - | - | - | 1024 | 1024 | 32 (<2) |
Table 3 Minimal inhibitory concentration (MIC) of the studied plants extracts and chloramphenicol on the studied bacterial species

|                  | KP55 | 512 | 1024 | 256 | 512 | 1024 | 1024 | -  | -  | -  | -  | -  | -  | 1024 | 32 |
|------------------|------|-----|------|-----|-----|------|------|----|----|----|----|----|----|-----|----|
|                  | KP63 | 512 (512) | 1024 (1024) | 1024 (1024) | 512 (512) | 512 (128) | 1024 (1024) | 1024 (1024) | 512 | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 64 |
| P. stuartii      | ATCC29916 | 1024 (1024) | - (-) | - (-) | - (-) | - (-) | - (-) | - (-) | - (-) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 1024 (1024) | 8 |
|                  | NEA16 | 1024 (512) | - (-) | 1024 (1024) | 512 (512) | 512 (256) | 1024 | 1024 | - | - | 1024 | - | 64 |
|                  | PS2636 | 1024 | - | - | - | - | - | - | - | - | - | - | - |
|                  | PS299645 | 512 | 512 | 1024 | 1024 | 1024 | 1024 | - | 1024 | 1024 | 512 | 1024 | 1024 | 128 |
| P. aeruginosa    | PA01 | - | - | - | - | - | - | - | - | - | - | - | - |
|                  | PA124 | - | - | - | - | - | - | - | - | 1024 | - | - | 32 |

(-): MIC not detected at up to 1024 μg/ml for the les extracts and 256 μg/ml for chloramphenicol. (): values in parenthesis are MIC of substance in the presence of PAßN at 20 μg/ml. The MIC of PAßN was 64 μg/ml on E. coli: AG100A, 512 μg/ml on ATCC11296. BM67. EA27; EA289; 1024 μg/ml on AG100A37, ATCC13048. CM64; and > 1024 μg/ml on other bacteria. CHL: chloramphénicol; (in bold): significant MIC value.
Table 4 Minimal inhibitory concentration (MIC) in μg/ml of antibiotics in the absence and presence sub-inhibitory concentrations of *Aframomum citratum* extract against some MDR bacteria

| Bacterial strains | Ampicillin | Cefepime | Chloramphenicol | Ciprofloxacin | Cloxacillin |
|-------------------|------------|----------|-----------------|--------------|------------|
|                   | MIC/2.5    | MIC/5    | MIC/2.5         | MIC/5        | MIC/2.5    |
| AG100Atet         | -          | -        | -               | -            | -          |
| AG102             | -          | -        | 128 (8)         | -            | -          |
| CM64              | -          | 256 (1)  | -               | -            | -          |
| XP63              | -          | 32 (8)   | -               | -            | -          |
| PA124             | 128 (6) 4 | 64 (2)   | -               | -            | -          |

MIC/2.5: concentration of plant extract added equal to 204.8 μg/ml for AG100Atet, KP63; and to 409.6 μg/ml for PA124. CM64. AG102.

MIC/5: concentration of plant extract added equal to 102.4 μg/ml for AG100Atet, KP63; and to 204.8 μg/ml for PA124. CM64. AG102.

18 h [5]. The final concentration of DMSO was lower than 2.5 % and this concentration also served as negative control [5]. Chloramphenicol was used as reference antibiotic. The MICs of samples were detected after 18 h incubation at 37 °C, following addition (40 μl) of 0.2 mg/ml INT and incubation at 37 °C for 30 minutes [5]. MIC was defined as the lowest sample concentration that prevented the color change of the medium and exhibited complete inhibition of microbial growth [27].

Samples were tested alone and then, in the presence of PAßN at 20 mg/L final concentration as previously reported [5]. Four of the best extracts, those from *A. citratum*, *C. zeylanicum*, *D. psilurus* and *T. tetrapeta* were also tested in association [5] at the concentrations selected following a preliminary assay on *P. aeruginosa* PA124 (See Additional file 1: Table S1). All assays were performed in triplicate and repeated thrice. Fractional inhibitory concentration (FIC) [5] were calculated and the interpretations were made as follows: synergistic (<0.5), indifferent (0.5 to 4), or antagonistic (>4) [28] (The FIC values available in Additional file 1: Table S2 and S3).

Table 5 Minimal inhibitory concentration (MIC) of antibiotics in absence and presence of *Cinnamomum zeylanicum* extract (μg/ml)

| Bacterial strains | Amoxicillin | Cefepime | Chloramphenicol | Ciprofloxacin | Cloxacillin |
|-------------------|------------|----------|-----------------|--------------|------------|
|                   | MIC/2.5    | MIC/5    | MIC/2.5         | MIC/5        | MIC/2.5    |
| AG100Atet         | -          | -        | -               | -            | -          |
| AG102             | -          | -        | 128 (8)         | -            | -          |
| CM64              | -          | 256 (1)  | -               | -            | -          |
| KP63              | -          | 32 (8)   | -               | -            | -          |
| PA124             | 128 (6) 4 | 64 (2)   | -               | -            | -          |

MIC/2.5: concentration of plant extract added equal to 204.8 μg/ml for AG100Atet, KP63; and to 409.6 μg/ml for PA124. CM64. AG102.

MIC/5: concentration of plant extract added equal to 102.4 μg/ml for AG100Atet, KP63; and to 204.8 μg/ml for PA124. CM64. AG102.

*: Folds decreasing of MIC. S: synergy. I: indifference. nt: not tested; (−): MIC > 256 μg/ml.
Table 6 Minimal inhibitory concentration (MIC) of antibiotics in absence and presence extracts *Dorstenia psilurus* (μg/ml)

| Bacterial strains | Antibiotics and MIC in absence | Antibiotics and MIC in presence of *Dorstenia psilurus* extract |
|-------------------|-----------------------------|-----------------------------|
|                   | Alone MIC/2.5 MIC/5          | Alone MIC/2.5 MIC/5          |
| AG100A tet        | -                           | -                           |
| AG102             | -                           | -                           |
| CM64              | -                           | -                           |
| KP63              | -                           | -                           |
| PA124             | -                           | -                           |

MIC/2.5: concentration of plant extract added equal to 102.4 μg/ml for AG100A tet, CM64, KP63, AG102 and to 204.8 μg/ml for PA124.

MIC/5: concentration of plant extract added equal to 204.8 μg/ml for AG100A tet, CM64, KP63, AG102 and to 409.6 μg/ml for PA124.

Table 7 Minimal inhibitory concentration (MIC) of antibiotics in absence and presence extracts *Tetrapleura tetraptera* (μg/ml)

| Bacterial strains | Antibiotics and MIC in absence | Antibiotics and MIC in presence of *Tetrapleura tetraptera* extract |
|-------------------|-----------------------------|-----------------------------|
|                   | Alone MIC/2.5 MIC/5          | Alone MIC/2.5 MIC/5          |
| AG100A tet        | -                           | -                           |
| AG102             | -                           | -                           |
| CM64              | -                           | -                           |
| KP63              | -                           | -                           |
| PA124             | -                           | -                           |

Results

Phytochemical composition of the spice extracts

The results of qualitative analysis showed that each plant contains various phytochemical compounds such as alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, steroids, tannins and triterpenes as shown in Table 2.

Antibacterial activity of the spice extracts

The results summarized in Table 3 summarize the MIC of the extract tested alone or in combination with PAβN on the tested microorganisms. Its shows that all the studied extracts were active on at least one microbial strain. *A. citratum* showed the best activity, it inhibitory effect being recorded on 85% (24/28) of the tested bacteria. Other
samples were less active, their inhibitory potencies being observed on 75% of tested bacteria (21/28) for *I. cymindricum* and *C. zeylanicum*, 67.9% (19/28) for *A. melegueta*, *D. psilurus*, *F. leprieurii* and *T. tetraperta*; 64.3% (18/28) for *M. myristica* and *S. zenkeri*; 50% (14/28) for *P. guineense* and 42.9% (12/28) for *P. brazzeana*.

**Role of efflux pumps in susceptibility of gram negative bacteria to the tested spice extracts**

Potentiating effect of EPI was not observed on tested bacteria when associated with *M. myristica*, *P. brazzeana*, *T. tetraperta* and *S. zenkeri*. PA$_\beta$N weakly increased the activity of *A. citratum*, *A. melegueta*, *F. leprieurii*, *I. cymindricum*, *C. zeylanicum* and *P. guineense*. The activity of *D. psilurus* in the presence of EPI significantly increased on most of the tested bacteria (except against *P. stuartii* ATCC29916, *E. coli* ECC169 and *E. aerogenes* EA27) (see Table 3).

**Effects of the association of some spice extracts with antibiotics**

*A. citratum*, *C. zeylanicum*, *D. psilurus* and *T. tetraperta* (Tables 4, 5, 6 and 7) were associated to antibiotics in view of evaluating the possible synergistic effect of these associations. A preliminary study using *P. aeruginosa* PA124 was carried out with ten antibiotics (CLX, AMP, ERY, KAN, CHL, TET, FEP, STR, CIP and NOR) to select the appropriate sub-inhibitory concentrations to be used. MIC/2.5 and MIC/5 were then selected as the sub-inhibitory concentrations (see Additional file 1: Table S1). All of these four extracts were then tested in association with antibiotics previously listed on strains of *E. coli* AG100A$_\text{TET}$ and AG102, *E. aerogenes* CM64, *K. pneumoniae* KP63 and *P. aeruginosa* PA124. No antagonistic effect (FIC $>4$) was observed between extracts and antibiotics meanwhile indifference was observe between *T. tetraperta* and antibiotics in most of the case (see Tables 5, 6, and 7, Additional file 1: S2, S3, S4 and S5). Significant increase of the activity was observed with the association of the extracts of *A. citratum* and *D. psilurus* on *E. aerogenes* CM64 and *K. pneumoniae* KP63, and with *C. zeylanicum* against *K. pneumoniae* KP63. A significant decrease (synergy effect) of MIC values was also observed when ERY was associated with various extracts, and when extracts of *A. citratum* and *C. zeylanicum* were each combined with aminoglycosides (KAN, STR), the best activity being noted against *E. aerogenes* CM64.

**Discussion**

**Phytochemical composition of the spice extracts**

The phytochemical studies revealed the presence of secondary metabolite such as alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, sterols, tannins and triterpenes; several molecules belonging to these classes of secondary metabolites were found active on pathogenic microorganisms [29].

**Antibacterial activity of the spice extract**

Although this is the first time that plants used in this work are studied for their activities vis-à-vis multi-resistant bacteria, plants belonging to some of the genus studied herein, like the *Aframomum* genus are well documented for their antimicrobial activity [6]. Some antibacterial compounds, such as acridone and chelerythrine have previously been isolated from the fruits of *F. leprieurii* [14,30]. The antimicrobial activity of *P. brazzeana* and *S. zenkeri* is mainly due to some sulfur compounds. In fact, sulfur compounds with antimicrobial properties have been previously isolated from the two plants [7,31]. Several alkaloids of the genus *Piper* proved to be responsible for the activity of *P. guineense* [32]. The detection of this class of secondary metabolites in the extract studied herein can explain the observed activities. According to Krishnaiah et al. [16], the antimicrobial activity of *I. cymindricum* can be due to the presence of tannins in this plant. However, tannins were not detected in the extract of *I. cymindricum* as found in the present work (Table 2), suggesting that other classes of secondary metabolites might be responsible for the antibacterial activity of this plant.

**Role of efflux pumps in susceptibility of gram negative bacteria to the tested spice extracts**

The significant increase of the activity of the extract of *D. psilurus* in the presence of EPI, indicates that bioactive constituents of this plant extract are substrate of efflux pumps. Efflux through AcrAB-TolC pumps was reported as essential mode of resistance of several Gram-negative MDR bacteria to a number of flavonoids pumps. Efflux through AcrAB-TolC pumps was reported as essential mode of resistance of several Gram-negative MDR bacteria to a number of flavonoids isolated from the plants of the genus Dorstenia, such as isobavachalcone, kanzonol C, stipulin, etc. [4,15,33-35]. This suggests that possible combination of the extract of *D. psilurus* with EPI can be envisaged to overcome MDR bacteria.

**Effects of the association of extracts with antibiotics**

The results obtained by combining the antibiotic with the extracts of *A. citratum*, *C. zeylanicum*, *D. psilurus* and *T. tetraperta* indicate that these extracts contain chemical compounds that can modulate the activity of antibiotics against bacteria expressing MDR phenotypes. The methanol extracts of *A. citratum*, *C. zeylanicum* and *D. psilurus* showed a synergistic effect with antibiotics inhibiting bacterial cell wall synthesis (AMP and CEF) on *K. pneumoniae* KP63. The intrinsic mode of action of the active extracts is to be investigated.

**Conclusion**

The present work evidently provides information in the role of some Cameroonian spices in the fight against multi-resistant bacteria. The study also highlights the potential of *D. psilurus* as a strong antibacterial agent.
when the extract is combined with efflux pump inhibitor and several antibiotics.

Additional file

Additional file 1: Table S1. Activities of antibiotics in combination with the sub-inhibitory concentrations of some plants extracts on Pseudomonas aeruginosa PA124. S2. Fractional inhibitory Concentrations of the association between antibiotics and extracts of Actinomadura citratum at MIC/2.5 and MIC/5 (μg/ml) against MDR bacteria. S3. Fractional inhibitory Concentrations of the association between antibiotics and extracts of Cinnamomum zeylanicum at MIC/2.5 and MIC/5 (μg/ml) against MDR bacteria. S4. Fractional inhibitory Concentrations of the association between antibiotics and extracts of Dorstenia subinnotatus at MIC/2.5 and MIC/5 (μg/ml) against MDR bacteria. S5. Fractional inhibitory Concentrations of the association between antibiotics and extracts of Tetrapleura tetraptera at MIC/2.5 and MIC/5 (μg/ml) against MDR bacteria.

Competing interest

The authors declare that they have no competing interest.

Authors’ contributions

IKV carried out the study, VK designed the experiments and wrote the manuscript, VK, GAF, JAKN, JPD, JRK and JMP supervised the work; VK and JIMP provided the bacterial strains; all authors read and approved the final manuscript.

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