Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes

Aimé G Fankam1, Victor Kuete1*, Igor K Voukeng1, Jules R Kuiate1 and Jean-Marie Pages2

Abstract
Background: The emergence of multi-drug resistant (MDR) phenotypes is a major public health problem today in the treatment of bacterial infections. The present study was designed to evaluate the antibacterial activities of the methanol extracts of eleven Cameroonian spices on a panel of twenty nine Gram negative bacteria including MDR strains.

Methods: The phytochemical analysis of the extracts was carried out by standard tests meanwhile the liquid micro-broth dilution was used for all antimicrobial assays.

Results: Phytochemical analysis showed the presence of alkaloids, phenols and tannins in all plants extracts. The results of the antibacterial assays indicated that all tested extracts exert antibacterial activities, with the minimum inhibitory concentration (MIC) values varying from 32 to 1024 μg/ml. The extracts from Dichrostachys glomerata, Beilschmiedia cinnamomea, Aframomum citratum, Piper capense, Echinops giganteus, Fagara xanthoxyloïdes and Olax subscorpioïdea were the most active. In the presence of efflux pump inhibitor, PAßN, the activity of the extract from D. glomerata significantly increased on 69.2% of the tested MDR bacteria. At MIC/5, synergistic effects were noted with the extract of D. glomerata on 75% of the tested bacteria for chloramphenicol (CHL), tetracycline (TET) and norfloxacin (NOR). With B. cinnamomea synergy were observed on 62.5% of the studied MDR bacteria with CHL, cefepime (FEP), NOR and ciprofloxacin (CIP) and 75% with erythromycin (ERY).

Conclusion: The overall results provide information for the possible use of the studied extracts of the spices in the control of bacterial infections involving MDR phenotypes.

Background
The emergence of MDR phenotypes is a major public health problem today in the treatment of bacterial infections. The multi-drug resistance of Gram negative bacteria is a major cause of morbidity and mortality in health care services [1]. The activation of bacterial efflux pumps also plays an important role in the appearance of resistance to antibiotics [2]. The real challenge for scientists worldwide today, is to continuously find new drugs to combat resistant microorganisms, or compounds which are able to inhibit the resistance mechanisms of pathogens, therefore restoring the activity of antibiotics. Medicinal plants are rich in compounds which may be potential natural drugs and serve as alternative, less expensive and safe antimicrobials for the treatment of common ailments. Plant drugs are widely used in Africa for the treatment of many ailments and constitute the first health recourse for about 80% of the population [3]. A number of pharmaceutical products in current use worldwide are derived from plants [4]. In Cameroon, many medicinal plants including spices are used as herbal medicines. The present work was therefore designed to investigate the antibacterial potential against MDR bacteria of some of the commonly used medicinal spices in Cameroon such as Fagara xanthoxyloïdes Watern., Dichrostachys glomerata (Forsk) Chuov, Olax subscorpioïdea Oliv., Solanum melongeua L. Var ingerme D.C Hiern, Piper capense Lin.f., Xylopia aethiopica Dunal A. Rich., Aframomum citratum (Pereira). Schum, Scorodophloeus zenkeri Harms., Beilschmiedia cinnamomea (Stapf) Robyns & Wilczek, Echinops giganteus A. Rich.

* Correspondence: kuetevictor@yahoo.fr
1Department of Biochemistry, Faculty of science, University of Dschang, Cameroon
Full list of author information is available at the end of the article

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and *Mondia whitei* (Hook F). Skell. This study was also extended to the evaluation of the potencies of the above plant extracts to increase the activity of some antibiotics on MDR bacteria. The role of bacterial efflux pumps in resistance to the extracts was also studied.

**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 materials were: the fruits of *Fagara xanthoxyloides*, *Dichrostachys glomerata*, *Olax subschorpioidea*, *Solanum melongena*, *Piper capense* and *Xylopia aethiopica*, the bark of *Aframomum citratum*, *Scordophloeus zenkeri*, *Beilschmiedia cinnamomea* and the roots of *Echinops giganteus* and *Mondia whitei*. The plants were identified by Mr. Fulbert Tadjouteu of the National herbarium (Yaoundé, Cameroon) where voucher specimens were deposited under the reference numbers (Table 1).

The air dried and powdered sample (1 kg) from each spice was extracted with methanol (MeOH) for 48 h at room temperature. The extract was then concentrated under reduced pressure to give residues which constituted the crude extracts. They were then kept at 4°C until further use.

**Preliminary phytochemical investigations**

The major classes of secondary metabolites; alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes were screened according to the common phytochemical methods described by Harborne [5] with some modifications. Briefly, for alkaloids (5 mg plant extract in 10 ml methanol); a portion of 2 ml extract + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor s reagents/Wagner’s reagent/ Dragendroff reagent; creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. For tannins (5 mg plant extract in 10 ml distilled water); a portion of 2 ml + 2 ml FeCl₃; blue-black precipitate indicated the presence of tannins. For saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); a portion of 2 ml + 2 ml ether-chloroform 1:1 v/v + 4 ml NaOH 10% (w/v); red color indicated the presence of anthraquinones. For phenols (5 mg plant material in 10 ml methanol); a portion of 2 ml + 2 ml FeCl₃; violet-blue or greenish color indicated the presence of phenols.

**Chemicals for antimicrobial assays**

Tetracycline (TET), cefepime (FEF), streptomycin (STR), ciprofloxacin (CIP), norfloxacin (NOR), chloramphenicol (CHL), cloxacin (CLX), ampicillin (AMP), erythromycin (ERY), kanamycin (KAN) (Sigma-Aldrich, St Quentin Fallavier, France) were used as reference antibiotics. *p*-Iodonitrotetrazolium chloride (INT) and phenylalanine arginine β-naphthylamide (PAßN) were used as microbial growth indicator and efflux pumps inhibitor 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* (Table 2). They were maintained on agar slant at 4°C and sub-cultured on a fresh appropriate agar plates 24 h prior to any antimicrobial test. Mueller Hinton Agar was used for the activation of bacteria. The Mueller Hinton Broth (MHB) was used for the MIC determinations.

**Bacterial susceptibility determinations**

The respective MICs of samples on the studied bacteria were determined using rapid INT colorimetric assay [6,7]. Briefly, the test samples were first dissolved in DMSO/MHB. The solution obtained was then added to MHB, and serially diluted two fold (in a 96-wells microplate). One hundred microlitres (100 l) of inoculum (1.5 x 10⁶ CFU/ml) prepared in MHB was then added. 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 h. The final concentration of DMSO was lower than 2.5% and did not affect the microbial growth. Wells containing MHB, 100 l of inoculum and DMSO at a final concentration of 2.5% served as negative control (this internal control was systematically added). The total volume in each well was 200 l. Chloramphenicol was used as reference antibiotic. The MICs of samples were detected after 18 h incubation at 37°C, following addition (40 l) of 0.2 mg/ml INT and incubation at 37°C for 30 minutes. Viable bacteria reduced the yellow dye to pink. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of microbial growth [8].

Samples were tested alone and then, in the presence of PAßN at 30 µg/ml final concentration. Two of the
Table 1 Spices used in the present study and evidence of their activities.

| Spice samples (Family) | Herbarium Voucher numbera | Part used | Bioactive (or potentially active) compoundsb and screened activity for crude plant extract |
|------------------------|---------------------------|-----------|-----------------------------------------------------------------------------------|
| Fagara xanthoxyloides  | 21793/HNC/SRF             | Fruits    | Antibacterial activity of essential oil [S: Ec, Bc, Bs, Af, Kp, Sa, Sf] [19], Cytotoxicity of fruits crude methanol extract [weak activity on leukemia CCRF-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines] [27] |
| Dichrostachys glomerata (Forsk) chuuv (Mimosaceae) | 15220/SRF-Cam | Bark, fruits | Cytotoxicity of roots crude methanol extract [weak activity on leukemia CCRF-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines] [27] |
| Aframomum citratum (Pereira). Schum (Zingiberaceae) | 37736/SRF-Cam | Leaves, fruits | Cytotoxicity of leaves crude methanol extract [weak activity on leukemia CCRF-CEM and CEM/ADR5000 cells, and pancreatic MiaPaCa-2 cell lines] [27] |
| Beilschmiedia cinnamomea (Stapf) Robyns & Wilczek (Lauraceae) | 6933/SRF-Cam | Roots | /
| Echinops giganteus A. Rich. (Asteraceae) | 23647/ SRF-Cam | Rhizomes | Antimicrobial [lupeol sitosteryl; 2, 4, 5, 7-tetrathiaoctane; 2, 4, 5, 6, 8-pentathianonane; 2, 3, 4, 6, 7-trithiapentanol; 2, 3, 4, 6, 7-trithiapentan-1-one; 2, 3, 4, 6, 7, 8-hexamethyloctane; 2, 4, 5, 7-tetrathiaoctane] [28-31], Cytotoxicity of rhizome crude methanol extract [significant activity with IC_{50} values of 6.68, 7.96 and 9.94 μg/ml respectively on leukemia CCRF-CEM cells, CEM/5000 cells and pancreatic MiaPaCa-2 cell lines] [27] |
| Mondia whitei (Hook F). Skell. (Periplocaceae) | 42920/HNC | Fruits | Reproduction system [roots water extract (400 mg/kg/day) for 55 days caused testicular lesions resulting in the cessation of spermatogenesis, degenerative changes in the seminiferous tubules and epididymides in rats] [32] |
| Olax subscorpioides Oliv. (Olacaceae) | 3528/ SRFK | Seeds | Antibacterial and cytotoxic against Artemia salina [Salibatic acid] [33,34], Cytotoxicity of leaves crude methanol extract on cancer cells [weak activity on leukemia CCRF-CEM and pancreatic MiaPaCa-2 cell lines and significant activity with IC_{50} of 10.65 μg/ml on CEM/ADR5000 cells] [27] |
| Solanum melongena L.Var inerme D.C. Hiern. (Solanaceae) | 22615/ SRFC | Fruits | Antimicrobial activity of methanol, dichloromethane and petrol ether extracts of the fruits [Q: Tr, Tr, Ca et Tb] [35] |
| Piper capense Lin.f (Piperaceae) | 7650/ SRF-Cam | Fruits | Insecticidal [N-isobutyl-ll-(3, 4-methylenedioxyphenyl)-2, 4, 5, 6, 7, 8-hexamethyloctane; N-pyrrolidyl-11-(3, 4-methylenedioxyphenyl)-2, 4, 5, 6, 7, 8-hexamethyloctane; N-isobutyl-13-(3, 4-methylene-dioxyphenyl)-2, 4, 5, 6, 7, 8-hexamethyloctane; N-isobutyl-2E, 4E-dodecatetraenamide; N-isobutyl-2E, 4E-dodecatetraenamide] [36], Cytotoxicity of fruit crude methanol extract [significant activity with IC_{50} values of 12.82 μg/ml on CEM/ADR5000 cells] [27] |
| Xylopia aethiopica (Dunal) A. Rich. (Annonaceae) | 16419/ SRF-Cam | Bark, leaves, roots, seeds | Antimicrobial [volatile oil of seeds] [19], Cytotoxicity of seeds crude methanol extract [significant activity with IC_{50} values of 3.91; 7.4 and 6.86 μg/ml respectively on leukemia CCRF-CEM cells, CEM/5000 cells and pancreatic MiaPaCa-2 cell lines] [27] |
| Scorodophloeus zenkeri Harms. (Caesalpinaceae) | 44803/ SRF-Cam | Bark | Antimicrobial [2, 4, 5, 7-Tetrahydroxane; 2, 4, 5, 6, 7-pentahydroxanone; 2, 3, 3, 7, 7-pentahydroxanone; 2, 3, 5, 7-tetrahydroxanone; 3, 3-dioxide; 2, 3, 3, 7, 7-pentahydroxanone] [37], Cytotoxicity of bark crude methanol extract on cancer cells [weak activity on leukemia CCRF-CEM and pancreatic MiaPaCa-2 cell lines and significant activity with IC_{50} of 10.65 μg/ml on CEM/ADR5000 cells] [27] |

a(HNC): Cameroon National Herbarium; (SRF): Société des reserves forestières; Cam: Cameroon; b(/): Not reported

*Screened activity: significant (S: CMI < 100 μg/ml), moderate (M: 100 < CMI ≤ 625 μg/ml), Weak (W: CMI > 625 μg/ml) Q: Qualitative activity based on the determination of inhibition zone [11,12]. Tm: Trichophyton mentagrophytes; Tr: Trichophyton rubrum; Tb: Trichophyton tonsurans Tb: Trichosporon beigelii; Ca: Candida albicans; Ck: Candida krusei; Af: Aspergillus flavus; Bc: Bacillus cereus; Bb: Bacillus subtilis; Ec: Escherichia coli; Kp: Klebsiella pneumoniae; Sa: Staphylococcus aureus; St: Streptococcus faecalis.

best extracts [those from D. glomerata and B. cinnamomea] were also tested in association with antibiotics at MIC/2 and MIC/5. These concentrations were selected following a preliminary assay on one of the tested MDR bacteria, P. aeruginosa PA124 (See Additional file 1, Table A1). All assays were performed in triplicate and repeated thrice. Fractional inhibitory concentration (FIC) was calculated as the ratio of MIC/Antibiotic alone and the interpretation made as follows: synergistic (<0.5), indifferent (0.5 to 4), or antagonistic (>4) [9] (The FIC values available in Additional file 1, Tables A2 and A3).

**Results**

**Phytochemical composition of the spice extracts**

The results of the phytochemical studies (Table 3) showed that all the tested extracts contain alkaloids, phenols and tannins. Anthocyanins, anthraquinones, flavonoids, saponins, sterols and triterpenes were selectively present.

**Antibacterial activity of the spice extracts**

The results of the antibacterial activity of the extract alone on a panel of Gram negative bacteria are summarized in Table 4. It appears that the extract from
D. glomerata was able to prevent the growth of all the twenty nine tested bacteria with MIC ≤ 1024 μg/ml. All other samples showed selective activity; their inhibitory activity being recorded on 28 of the 29 (96.6%) tested bacteria for B. cinnamomea, 24/29 (82.8%) for A. citratum, 19/29 (62.5%) for P. capense, 18/29 (62.1%) for E. giganteus and F. Xanthoxyloïdes, 15/29 (51.7%) for O. subscorpioïdea, 13/29 (44.8%) for X. aethiopica, 12/29 (41.4%) for M. whitei, 6/29 (20.7%) for S. melongena and 4/29 (13.79%) for S. zenkeri.

MIC values below 100 μg/ml (Table 4) were recorded with the extract of B. cinnamomea against Enterobacter aegroens.
| Spice samples           | Extraction yield* | Physical aspect | Phytochemical composition |
|------------------------|------------------|----------------|--------------------------|
|                        |                  |                | Alkaloids | Anthocyanins | Anthraquiones | Flavonoids | Phenols | Saponins | Tannins | Sterols | Triterpenes |
| Fagara xanthoxyloides  | 12.13            | Oily brown     | +         | -          | +             | +          | +       | -        | +       | -       | -          |
| Dichrostachys glomerata| 18.29            | Brown paste    | +         | +          | +             | +          | +       | +        | +       | +       | +          |
| Aframomum citratum     | 16.32            | Brown paste    | +         | -          | -             | +          | +       | +        | +       | +       | -          |
| Beilschmiedia cinnamomea| 5.67            | Black paste    | +         | +          | +             | +          | +       | -        | +       | -       | +          |
| Echinops giganteus     | 8.87             | Oily brown     | +         | +          | +             | +          | -       | +        | +       | +       | +          |
| Mondia whitei          | 7.33             | Brown paste    | +         | +          | -             | +          | +       | +        | +       | +       | -          |
| Olax subscorpioidea    | 12.34            | Brown paste    | +         | -          | +             | +          | -       | +        | +       | -       | -          |
| Solanum melongena      | 14.30            | Black paste    | +         | +          | +             | +          | +       | +        | +       | +       | +          |
| Piper capense          | 12.87            | Brown paste    | +         | -          | -             | +          | +       | +        | +       | +       | +          |
| Xylopia aethiopica     | 26.42            | Brown paste    | +         | -          | -             | +          | +       | +        | -       | +       | +          |
| Scorodophloeus zankeri | 4.67             | Brown paste    | +         | -          | -             | +          | +       | +        | +       | -       | +          |

(+) Present; (-) Absent; *The yield was calculated as the ratio of the obtained methanol extract according to the initial mass of the spice powder
Table 4: Minimal inhibitory concentration (MIC) of the studied spice extracts and CHL on the studied bacterial species

| Bacterial strains | F. xanthoxyloides | D. glomerata | A. citratum | B. cinnamomea | E. giganteus | M. whitei | S. melongena | O. subschorpioidea | P. capense | X. aethiopica | S. zenkeri | CHL |
|-------------------|------------------|-------------|-------------|---------------|-------------|----------|-----------|---------------|-----------|-------------|-----------|-----|
| E. coli ATCC8739  | - (1024)         | 1024 (512) | 1024 (256) | - (256)      | - (-)       | (1024)  | (128)    | - (512)       | - (512)   | - (-)       | 4 (< 2)  |
| ATCC10536        | 1024             | 512 (128)  | 1024        | 1024         | 1024        | -       | 512       | 256           | 64        | 256         | < 2 (< 2)|     |
| AG100            | 256              | 512 (256)  | 1024 (1024) | 1024 (1024)  | 1024 (1024) | 1024 (-) | 1024 (-) | 512 (-)      | - (1024)  | 1024 (1024) | - (-)    | 8 (< 2)|
| AG100A           | 1024             | 1024 (256) | 1024        | 1024         | -           | 1024     | 1024      | -             | -         | -           | < 2 (< 2)|     |
| AG100ATET        | -                | 1024 (512) | 1024        | 512          | 1024        | -       | -         | 1024          | 1024      | 1024         | - (64)   |     |
| AG102            | 1024             | 512 (256)  | 1024        | 512          | 1024        | -       | 1024      | 1024          | 1024      | 1024         | - (32)   |     |
| MC4100           | 256              | 256 (< 8)  | 512         | 256          | 512         | 1024     | 1024      | 1024          | 1024      | 1024         | - (32)   |     |
| W3110            | -                | (512)      | 256 (< 8)  | 1024 (8)     | 1024 (1024) | 1024 (512)| -         | 512 (512)     | 1024 (1024)| - (-)        | - (-)    | 4 (< 2)|
| E. aerogenes     | -                | -          | -          | -            | -           | -       | -         | -             | -         | -           | 8 (< 2)  |     |
| ATCC13048        | -                | 512 (512)  | 1024        | 512          | -           | -       | -         | -             | -         | -           | 256 (8)  |     |
| CM64             | -                | 512 (512)  | (1024)     | 1024 (1024)  | 1024 (1024) | 1024 (-) | - (-)    | - (-)         | - (-)     | - (-)        | 256 (8)  |     |
| EA3              | 1024             | -          | 1024        | 1024         | 1024        | -       | 1024      | 1024          | 1024      | 1024         | - (128)  |     |
| EA27             | 1024             | 512 (256)  | 1024        | 1024         | 1024        | -       | 512       | 1024          | 1024      | 1024         | - (256)  |     |
| EA289            | -                | 1024 (1024)| 1024        | 512          | 1024        | -       | -         | -             | -         | -           | - (64)   |     |
| EA298            | -                | 1024 (128) | -           | 1024         | -           | 1024     | -         | 256           | 512       | 1024         | 128      |     |
| EA294            | 256              | 128        | 512         | 64           | 256         | 1024     | 256       | 256           | 512       | 256         | 512      | 8   |
| E. cloacae       | -                | -          | -          | -            | -           | -       | -         | -             | -         | -           | - (8)    |     |
| ECC69            | 512 (-)          | 1024 (1024)| 1024 (1024)| 1024 (512)   | 1024 (-)    | - (-)   | - (-)    | - (-)         | - (-)     | - (-)        | - (-)    | (-)|
| BM47             | 1024 (1024)      | 1024 (256)| 1024 (1024)| 1024 (1024)  | 1024 (-)    | - (-)   | - (-)    | - (-)         | - (-)     | 512(64)     | 1024(1024)| - (-)|
| BM67             | 1024 (1024)      | 1024 (128)| 1024        | 1024         | -           | -       | -         | 1024          | 1024      | 8           | - (8)    |     |
| K. pneumoniae    | ATCC11296        | 1024 (1024)| 512 (128)  | 512 (512)    | 256 (256)   | 1024 (256)| - (1024) | - (1024)     | 1024 (512)| 1024 (256)| - (-)    | 4 (< 2)|
| KP55             | 1024             | 512 (256)  | 1024        | -            | 512         | 1024     | 1024      | -             | -         | -           | 64 (2)   |     |
| KP63             | 1024             | 512 (< 8)  | 256         | 512          | 1024        | 512      | 256       | 64            | -         | -           | 64 (2)   |     |
| K2               | 512              | 512 (32)   | 512         | 256          | 32          | 1024     | 1024      | -             | 1024      | -           | 16 (2)   |     |
| K2               | -                | 1024 (128) | -           | 1024         | -           | 1024     | 512       | -             | 1024      | -           | 32 (4)   |     |
| P. stuartii      | NEA16            | 1024       | 512 (32)   | 128          | 256         | 1024     | 1024      | 1024          | 1024      | 512         | 1024     | 32 (8)|
| ATCC29914        | 1024             | 1024 (512)| 512         | 1024         | 1024        | -       | 1024      | -             | 1024      | -           | - (16)   |     |
| PS2636           | -                | 512        | 1024        | 1024         | -           | -       | -         | -             | -         | -           | - (32)   |     |
| PS299645         | 1024 (-)         | 256 (256)  | 256         | 512          | - (-)       | - (-)   | - (-)    | - (-)         | - (-)     | - (-)        | - (-)    | (-)|
| P. aeruginosa    | PA01             | -          | 1024 (512) | 1024         | 1024        | -       | -         | -             | -         | 1024        | - (16)   |     |
| PA124            | -                | 512 (512)  | -           | 1024         | -           | -       | -         | -             | -         | -           | 32 (2)   |     |

(-): MIC not detected at up to 1024 μg/ml for the les extracts and 256 μg/ml for CHL. (): values in parenthesis are MIC of substance in the presence of PAβN at 30 μg/ml. The MIC of PAβN was 64 μg/ml on E. coli, AG100A, 512 μg/ml on ATCC11296, BM67, EA27, EA289; 1024 μg/ml on AG100Ater, ATCC13048, CM64; and > 1024 μg/ml on other bacteria. CHL: chloramphénicol.
aerogenes EA294 (64 µg/ml), E. giganteus on Klebsiella pneumoniae K24 (32 µg/ml) and X. aethiopica on Escherichia coli ATCC10536 and Klebsiella pneumoniae KP63 (64 µg/ml).

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

The various strains and MDR isolates were tested for their susceptibilities to the spice extracts, and reference antibiotic, CHL in the presence of PAßN, a well-known efflux pump inhibitor. The results presented in Table 4 showed that the activity of the extract from D. glomerata significantly increased in the presence of PAßN on 18/26 (69.2%) of the tested bacteria. The MIC values below 100 µg/ml were noted with this extract against E. coli MC4100 and W3110 (< 8 µg/ml), K. pneumoniae KP63 and K24 (< 8 µg/ml and 32 µg/ml respectively) and P. stuartii NAE16 (32 µg/ml). Apart from the extract of D. glomerata, PAßN did not induce an increased activity of other tested extract.

Effects of the association of some spice extracts with antibiotics

To evaluate the possible synergistic effects of the extracts with antibiotics, four of the most active samples (F. xanthoxyloïdes, D. glomerata, B. cinnamomea and O. subscorpioïdea) were selected. A preliminary study using P. aeruginosa PA124, one of the MDR bacteria used in this work, 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. The results (see Additional file 1, Table A1) allow the selection of MIC/2 and MIC/5 as the sub-inhibitory concentrations of the extracts from D. glomerata and B. cinnamomea, which were then tested on eight MDR bacteria, E. coli AG100, AG100TET, K. pneumoniae KP55, E. aerogenes EA3, EA27, EA289, CM64 in addition to P. aeruginosa PA124. The results are summarized in Tables 5 and 6. Synergistic effects were observed with the association between D. glomerata (Table 5, Additional file 1, Table A2) and B. cinnamomea (Table 6, Additional file 1, Table A3) and most of the antibiotics on the studied MDR bacteria. At MIC/2, synergistic effects were noted with the extract of D. glomerata on 25% (2/8) of the tested bacteria for CLX and AMP, 50% (4/8) for KAN, 62.5% (5/8) for CHL, FEP, STR, CIP, 75% (6/8) for ERY and 87.5% (7/8) for NOR and TET. Increase in MIC values of > 8 fold were recorded at MIC/2 with CHL, TET, STR, CIP, NOR (Table 5). At MIC/5, synergistic effects were noted on 50% of the eight tested MDR bacteria in the case of STR and CIP, 62.5% in the case of ERY and 75% in the case of CHL, TET and NOR.

The extract of B. cinnamomea at MIC/2 (Table 6) also induced significant increase of the activity of several antibiotics, the synergistic effects being noted on 25% of the tested bacteria in the case of CLX and AMP, 50% in the case of KAN, 62.5% in the case of FEP and STR, 75% in the case of CHL, TET and CIP, 87.5% in the case of ERY and 100% for NOR. With this extract, synergistic effects were also observed at MIC/5 on 25% of the studied MDR bacteria in the case of CLX and AMP, 37.5% in the case of STR and KAN, 50% in the case of TET, 62.5% in the case of CHL, FEP, NOR and CIP and 75% in the case of ERY.

Discussion

Phytochemical composition of the spice extracts

Phytochemical screening revealed the presence of several classes of secondary metabolites. Though the detection of such metabolites does not automatically predict the antimicrobial activity of a plant extract, it has clearly been demonstrated that several compounds belonging to the investigated classes of metabolites showed antibacterial activities [4,10-12].

Antibacterial activity of the spice extract

Phytochemicals are routinely classified as antimicrobials on the basis of susceptibility tests that produce MIC in the range of 100 to 1000 µg/ml [13]. Activity is considered to be significant if MIC values are below 100 µg/ml for crude extract and moderate when 100 < MIC < 625 µg/ml [11]. Therefore, the activity recorded with B. cinnamomea and E. giganteus respectively on E. aerogenes EA294 and K. pneumoniae K24, and X. aethiopica on E. coli ATCC10536 and K. pneumoniae KP63 can be considered significant. Alternative criteria have been described by Fabry et al. [14], which consider extracts having MIC values below 8000 µg/ml to have noteworthy antimicrobial activity. Under these less stringent criteria, and considering the fact that the spices tested are used as food ingredients with limited toxicity, the overall activity recorded with several extracts, most notably those of D. glomerata, B. cinnamomea, A. citratum, P. capense, E. giganteus, F. xanthoxyloïdes and O. subscorpioïdea, could be considered important. Besides, some of the tested samples were more active than CHL used as reference antibiotic on some of the MDR bacteria such as E. cloacaee ECCI69 and BM47, E. aerogenes EA27 and EA289, highlighting the importance of the results reported herein. It can be noted that all the investigated phytochemical classes were detected in the extracts of D. glomerata, S. melongena and M. withei. Contrary to D. glomerata extract that exhibited a good spectrum of activity, the inhibition potential of S. melongena and M. withei was lower and seems not in correlation with their chemical composition. This clearly confirms the fact that the presence of secondary metabolites does not automatically predict the antimicrobial activity of a plant.
Table 5 Minimal inhibitory concentration (MIC) in μg/ml of antibiotics in the absence and presence of the sub-inhibitory concentrations of *D. glomerata* extracts against MDR bacteria.

| Bacterial strains | Antibiotics and MIC in absence and presence D. glomerata extracts at MIC/2 and MIC/5 |
|-------------------|--------------------------------------------------------------------------------------------|
|                   | Chlamphenicol | Cloxacillin | Ampicillin | Erythromycin | Kanamycin |
| PA124             | 32           | 32 (1)      | 16 (2)     | -            | 64        |
|                   | MIC/2        | 16 (2)      | -          | -            | 64        |
|                   | MIC/5        | -           | -          | -            | 64        |
|                   | CM64         | 256 (1)     | -          | -            | -         |
|                   | MIC/2        | 256         | -          | -            | 64        |
|                   | MIC/5        | 256         | 256       | -            | 64        |
|                   | EA3          | -           | 32 (> 16) | 32 (> 16)    | -         |
|                   | MIC/2        | -           | 256       | 256          | -         |
|                   | MIC/5        | -           | 64        | 32 (2)       | 64 (1)    |
|                   | EA27         | 256         | 32 (8)    | 64 (4)       | 64 (4)    |
|                   | MIC/2        | 256         | 64 (4)    | 64 (4)       | 64 (1)    |
|                   | MIC/5        | -           | -         | 64 (1)       | 32        |
|                   | EA289        | -           | -         | -            | -         |
|                   | MIC/2        | -           | -         | -            | 32 (1)    |
|                   | MIC/5        | -           | -         | 32 (1)       | 16        |
|                   | KP55         | 64          | 8 (8)     | 16 (4)       | -         |
|                   | MIC/2        | -           | -         | -            | -         |
|                   | MIC/5        | 256         | 128 (2)   | 64 (1)       | 32        |
|                   | AG100        | 8           | < 2 (> 4) | 256          | 64 (4)    |
|                   | MIC/2        | < 2 (> 4)   | 64 (4)    | 64 (1)       | 32 (1)    |
|                   | MIC/5        | 256         | 64 (1)    | 32 (1)       | 16 (1)    |
|                   | AG100TET     | 64          | 8 (8)     | 16 (4)       | -         |
|                   | MIC/2        | 16 (4)      | 32 (1)    | 16 (1)       | -         |
|                   | MIC/5        | -           | 256       | 128 (2)      | 32 (2)    |
|                   | AG100TET     | 8           | 1 (8)     | 2 (4)        | -         |
|                   | MIC/2        | 8           | 32 (16)   | 16 (1)       | -         |
|                   | MIC/5        | 256         | 32 (16)   | 16 (1)       | 32 (2)    |
|                   | Tetracycline | -           | -         | -            | -         |
|                   | Cefepime     | -           | -         | -            | -         |
|                   | Streptomycin | -           | -         | -            | -         |
|                   | Ciprofloxacin| -           | -         | -            | -         |
|                   | Norfloxacin  | -           | -         | -            | -         |

MIC/2: concentration of plant extract added equal to 256 μg/ml for PA124, CM64, EA3, EA27, KP55, AG100; and to 512 μg/ml for EA289 and AG100TET.

MIC/5: concentration of plant extract added equal to 102.4 μg/ml for PA124, CM64, EA3, EA27, KP55, AG100; and to 204.8 μg/ml for EA289 and AG100TET.

(): Values in bracket are folds increase of activity. S: synergy, I: indifference; (>): > 512 μg/ml
Table 6 Minimal inhibitory concentration (MIC) of antibiotics in the absence and presence of the sub-inhibitory concentrations of *B. cinnamomea* extract (μg/ml) against some MDR bacteria.

| Bacterial strains | Chloramphenicol | Cloxacillin | Ampicillin | Erythromycin | Kanamycin |
|------------------|-----------------|-------------|------------|--------------|-----------|
|                  | Alone MIC/2      | MIC/5       | Alone MIC/2 | MIC/5        | Alone MIC/2 | MIC/5 |
| **PA124**        | 32 < 0.5 (32)    | -           | -          | -            | 64 8 (8)   | 32 (2) |
| **CM64**         | 256 -            | -           | -          | -            | 6 < 0.5 (32) | > 32 |
| **EA3**          | 16 (> 32) 5      | 32 -        | 256 -      | -            | 64 8 (8)   | 32 (2) |
| **EA27**         | 256 8 (32) s     | 16 (16) s   | 256 < 0.5 (512) s | 16 (16) s | 64 64 (1) f | 64 (1) f |
| **EA289**        | 256 -            | -           | -          | -            | 256 32 (8) | 64 (4) f |
| **KP55**         | 64 8 (8) s       | 8 (8) s     | -          | -            | 256 128 (2) | 128 (2) |
| **AG100**        | 16 (2) s         | 32 (2) s    | -          | 128 (> 4) s  | 128 (> 4) s | 32 (1) f |
| **AG100**        | 8 < 2 (> 4) s    | 4 (2) s     | 256 256 (1) | 256 (1)      | 64 < 2 (> 32) | 4 (8) |
| **Bacterial strains** | **Tetracycline** | **Cefepime** | **Ampicillin** | **Erythromycin** | **Kanamycin** |
| **PA124**        | 4 2 (2) s        | 4 (1) s     | -          | 64 (> 8) s   | -          | 16 (1) |
| **CM64**         | 32 8 (4) s       | 8 (4) s     | 256 32 (8) | 32 (8) s     | 8 < 2 (> 4) s | < 2 (> 4) s |
| **EA3**          | 2 1 (2) s        | 2 (1) s     | -          | 16 4 (4) s   | 4 (4) s    | 64 4 (16) s |
| **EA27**         | 16 1 (16) s      | 4 (4) s     | 256 16 (16) s | 64 (4) s | 8 < 0.5 (> 16) s | 2 (4) s |
| **EA289**        | 8 4 (2) s        | 4 (2) s     | -          | 256 (> 2) s  | 256 (> 2) s | 64 16 (4) s |
| **KP55**         | 4 1 (1) s        | 4 (1) s     | -          | 64 (> 8) s   | 16 (8) s   | 64 (16) s |
| **AG100**        | 4 2 (2) s        | 2 (2) s     | -          | 64 (> 4) s   | 64 (4) s   | 64 4 (16) s |
| **AG100**        | < 2 < 2          | < 2 256 64 (4) s | 256 256 (1) | 256 (1)      | 4 < 2 (> 2) s | < 2 (> 2) s |

MIC/2: concentration of plant extract added equal to 512 μg/ml for PA124, CM64, EA3, EA27, AG100; and to 256 μg/ml for EA289, KP55 and AG100A<sub>TET</sub>

MIC/5: concentration of plant extract added equal to 204.8 μg/ml for PA124, CM64, EA3, EA27, KP55, AG100; and to 102.4 μg/ml for EA289 and AG100A<sub>TET</sub>

(): Values in bracket are folds increase of activity. S: synergy, I: indifference; (>): > 512 μg/ml
extract though it is a good indication of its possible pharmacological potential.

To the best of our knowledge, the antibacterial activity of B. cinnamomea and P. capense is being reported for the first time. Moreover, the present work reports for the first time the activity of the tested spices on MDR bacteria. Nevertheless, the antimicrobial potential of some of the plants or related genus were demonstrated on sensitive strains. Banso and Adeyemo [15] reported the presence of antibacterial tannins in the genus Dichrostachyys. Chouna et al. [16] also demonstrated that Beilschmiedia anacardioïdes was significantly active against Bacillus subtilis, Micrococcus luteus and Streptococcus faecalis. Plants of the genus Echinops such as E. ellenbeckii and E. longisetus were found active on Staphylococcus aureus [17] meanwhile the antibacterial activity of the essential oils and alkaloids from F. xanthoxyloïdes was also documented [18,19]. The aqueous and ethanol extracts from O. subscorpioïdea were found active on both bacteria and fungi [20]. The results obtained in the present work therefore provide additional information on the studied plants and are in consistence with some of the previous reports.

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

Tripartite efflux systems, mainly those clinically described as AcrAB-ToLC in Enterobacteriaceae or MexAB-OprM in P. aeruginosa, are associated with a major human health problem as they play a central role in multidrug resistance of pathogenic Gram negative bacteria [21-23]. PAßN has been reported as a potent inhibitor of the RND efflux system and is especially active on AcrAB-ToLC and MexAB-OprM [22,24,25]. To determine the role of efflux pumps in this work, the concentration of PAßN used (30 μg/ml) had no intrinsic effect on the bacteria as previously determined [26]. In contrast, with these conditions significant increase of the antibacterial activity of D. glomerata extract was noted, showing that one or more active compounds from this plant could be substrate(s) of efflux pumps acting in resistant strains of E. coli, K. pneumoniae and P. stuartii. These data suggest that possible association of the extract of D. glomerata and efflux pump inhibitor can be envisaged to improve the fight against MDR phenotypes.

Effects of the association of extracts from D. glomerata and B. cinnamomea with antibiotics

The association of natural products such as plant extracts and antibiotics constitutes an alternative in the fight against MDR bacteria. Significant synergistic effects were noted with both D. glomerata and B. cinnamomea extracts when they were associated with several antibiotics. Such effects might be due either to the action of the active compounds or possible inhibition of the efflux pumps by other compounds of the extracts. The lowest synergistic effects were observed with β-lactamines (CLX and AMP), obviously due to the fact their target are localized in the bacterial cell coat. However, the synergistic effects observed indicate that active compounds of the extract could also present different mode(s) of action from those of the studied antibiotics.

Conclusion

The overall results of the present work provide baseline information for the possible use of the studied spice extracts in the treatment of bacterial infections involving MDR phenotypes. In addition to these antibacterial activities, the data reported herein indicated that possible combinations of the extract of D. glomerata with an efflux pump inhibitor, and also the association of extract of this plant as well as that from B. cinnamomea with several antibiotics could be used in the control of bacterial infections involving MDR phenotypes.

Additional material

Additional file 1: Table S1. Activities of antibiotics in combination with the sub-inhibitory concentrations of some plants extracts on Pseudomonas aeruginosa PA124. Table S 2. Fractional Inhibitory Concentrations (FIC) of the association between antibiotics and extract of D. glomerata at MIC/2 and MIC/5 (μg/ml) against MDR bacteria. Table S 3. Fractional Inhibitory Concentrations (FIC) of association between antibiotics and extract of B. cinnamomea at MIC/2 and MIC/5 (μg/ml).

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Author details

1Department of Biochemistry, Faculty of science, University of Dschang, Cameroon. 2Transporteurs Membranaires, Chimiorésistance et Drug Design, UMR-MD1, IFR 88, UFRs de Médecine et de Pharmacie, Marseille, France.

Authors’ contributions

PAF carried out the study; VK designed the experiments and wrote the manuscript; VK, IKV, JRK and JMP supervised the work; VK and JMP provided the bacterial strains; All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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