Antibiotic-potentiation activities of four Cameroonian dietary plants against multidrug-resistant Gram-negative bacteria expressing efflux pumps

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Abstract

Background: The continuous spread of multidrug-resistant (MDR) bacteria, partially due to efflux pumps drastically reduced the efficacy of the antibiotic armory, increasing the frequency of therapeutic failure. The search for new compounds to potentiate the efficacy of commonly used antibiotics is therefore important. The present study was designed to evaluate the ability of the methanol extracts of four Cameroonian dietary plants (Capsicum frutescens L. var. facilulatum, Brassica oleacea L. var. italica, Brassica oleacea L. var. butyris and Basilicum polystachyon (L.) Moench) to improve the activity of commonly used antibiotics against MDR Gram-negative bacteria expressing active efflux pumps.

Methods: The qualitative phytochemical screening of the plant extracts was performed using standard methods whilst the antibacterial activity was performed by broth micro-dilution method.

Results: All the studied plant extracts revealed the presence of alkaloids, phenols, flavonoids, triterpenes and sterols. The minimal inhibitory concentrations (MIC) of the studied extracts ranged from 256-1024 μg/mL. Capsicum frutescens var. facilulatum extract displayed the largest spectrum of activity (73%) against the tested bacterial strains whilst the lower MIC value (256 μg/mL) was recorded with Basilicum polystachyon against E. aerogenes ATCC 13048 and P. stuartii ATCC 29916. In the presence of PAβN, the spectrum of activity of Brassica oleacea var. italica extract against bacteria strains increased (75%). The extracts from Brassica oleacea var. butyris, Brassica oleacea var. italica, Capsicum frutescens var. facilulatum and Basilicum polystachyon showed synergistic effects (FIC ≤ 0.5) against the studied bacteria, with an average of 75.3% of the tested antibiotics.

Conclusion: These results provide promising information for the potential use of the tested plants alone or in combination with some commonly used antibiotics in the fight against MDR Gram-negative bacteria.

Keywords: Cameroonian dietary plants, Potentiation, Gram-negative bacteria, Multidrug resistant, Efflux pumps

Background

The spread of multidrug-resistant bacteria, partially due to the inappropriate use of common antibiotics, drastically reduced the efficacy of the antibiotic armory, increasing the frequency of therapeutic failure. The over-expression of efflux pumps is the main resistance mechanism observed in many bacteria [1]. In Gram-negative bacteria, many of these efflux pumps belong to the resistance-nodulation-cell division (RND), family of tripartite efflux pumps [2]. In the fight against microbial infections including those due to MDR bacteria, investigations are being carried out to discover new effective, none or less-toxic and available antibacterial drugs. Many scientist are also investigating synergistic compounds to potentiate the activity of the commonly used antibiotics [3]. The present work was designed to evaluate the in vitro ability of some edible plants namely Capsicum frutescens L. var. facilulatum (Solanaceae) or ‘chili pepper’, Brassica oleacea L. var. italica commonly known as ‘Broccoli’ and Brassica
oleacera L. var. butyris (Brassicaceae) or ‘Cauliflower’; and Basilicum polystachyon (L.) Moench. (Lamiaceae) or ‘Musk Basil’ to potentiate the effect of common antibiotics against Gram-negative MDR phenotypes.

Methods

Plant material and extraction

The plants used in this study were collected in Douala (Littoral Region of Cameroon) in January 2013. The plants were further identified at the National Herbarium (Yaoundé, Cameroon) where voucher specimens were deposited under a reference number (Table 1). Air dried and powdered sample (0.1 g) of each plant was extracted by maceration with methanol (0.3 L) for 48 h at room temperature (25°C). After filtration using Whatman No. 1 filter paper, the filtrate of each plant was concentrated under reduced pressure in a rotary evaporator, and dried at room temperature to give the crude extract. The extraction yield was calculated (Table 2). These extracts were then stored at 4°C until further use.

Preliminary phytochemical screenings

The secondary metabolite classes such as alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes were screened according to the standard phytochemical methods described by Harbone [16].

Bacteria strains and culture media

The studied microorganisms included both reference (from the American Type Culture Collection, ATCC) and clinical (Laboratory collection) strains of Escherichia coli, Enterobacter aerogenes, Providencia stuartii, Pseudomonas aeruginosa and Klebsiella pneumoniae (Table 3). They were maintained at 4°C and sub-cultured on a fresh appropriate Mueller Hinton Agar (MHA) for 24 h before any antibacterial test. The Mueller Hinton Broth (MHB) was used for all antibacterial assays.

Chemicals for antibacterial assays

Nine commonly used antibiotics including tetracycline (TET), cefepime (CEP), streptomycin (STR), ciprofloxacin (CIP), norfloxacin (NOR), chloramphenicol (CHL), amoxicillin (AMP), erythromycin (ERY), kanamycin (KAN) (Sigma-Aldrich, St Quentin Fallavier, France) were used for potentiation assay.

Table 1 Information on plants used in this study

| Plants samples and herbarium voucher number | Parts used | Popular names | Traditional used | Known antimicrobial activities of plants |
|--------------------------------------------|------------|---------------|------------------|-----------------------------------------|
| Capsicum frutescens L. var. faciulatum (Solanaceae) | Fruits | Green pepper | Antimutagenic [4], allergy, cancer and viral infection [5] | Antibacterial activities of aqueous and methanolic extracts against Sa, St, Vc [6,7], antifungal activities of lectin against Af, [8]; antifungal activities of saponin CAY-1 against Ca, Aspergillus Spp and dermatophytes Tm, Tr et Mc [9]. |
| Brassica oleacera L. var. italicla (Brassicaceae) | Leaves | Broccoli | Oxidative stress, cytotoxic [10] | Antibacterial activities of ethanolic extracts against Sa, Bc, Pa [11]. Antifungal activities against Sc, Te, Hm, Pm [12]. |
| Brassica oleacera L. var. butyris (Brassicaceae) | Leaves | Flower cabbage | Cytotoxic effect, antiproliferative, Oxidative stress [13] | Antibacterial activities of sulfur compounds MMTSO, AITC, MMTSO, against Pp, Lm, Lp, Lb Lm Sa, Ea, Ec, Bs and antifungal against strains Sc, Te, Hm, Pm [12]. |
| Basilicum polystachyon (L.) Moench. (Lamiaceae) | Leaves | Cotimandjo (Cameroon) | Infectious diseases, gastroenteritis [14] | Strong activities of acidic extracts against Gram (+), but less activities against Gram (-). Strong antibacterial activities of ethanolic and methanol extracts against An [15]. |

Af, Am, Ca, Cr, Tr, Mt, Mc, Sa, Bc, Ec, Pa, Sc, Te Hm, Pr, Pp, Lp, Lb, Lm, Bs, Ea, St, Te, Hm, An, Kp, Ec, Sm, Vc who are respectively: Aspergillus flavus, Fusarium moniliforme, Candidat albicans, trichophyton mentagrophytes, T. rubum, T.janusirius Microsporum canis, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Torulopsis etchellsii, Hansenula mariki, Pichia membranefaciens, Pedicoccus pentosaceus, Leuconostoc mesenteroides, Lactobacillus plantarum , Lactobacillus brevis, Listeria monocytogenes, Bacillus subtilis, Enterobacter aerogenes, Salmonella. Typhimurium, Torulopsis etchellsii, Hansenula mariki, Aspergillus niger, Klebsiella pneumoniae C, Enterobacter cloacae C, Clostridium cholerse MMTSO; Méthylmethanethiosulfonate, AITC: Allyisothyocyanate, MMTSO; Méthylmethanethiosulfonat. SRFC: Company of Forest Reserve of Cameroon; HNC: Cameroon National Herbarium.
(PAßN) (Sigma-Aldrich) were used as bacterial growth indicator and efflux pumps inhibitor respectively. Dimethylsulf-oxide 10% (DMSO) was used as solvent for all extracts.

**Bacterial susceptibility determinations**

The minimal inhibitory concentrations (MIC) of the plant extracts against the studied bacteria were determined by rapid INT colorimetric assay [19,20]. Briefly, the test samples were first dissolved in DMSO/MHB. The solution obtained was then added to MHB in a 96-well microplate followed by a two fold serial dilution. One hundred microliters (100 μL) of inoculum (1.5 × 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 ranges were 8-1024 μg/mL for plant extracts and 2-512 μg/mL for reference antibiotic chloramphenicol (CHL). Wells containing MHB (100 μL), 100 μL of inoculum and DMSO at a final concentration of 2.5% served as negative growth inhibition control. MIC was detected after 18 h of incubation at 37°C, following addition (40 μL) of 0.2 mg/mL INT and incubation at 37°C for 30 min. 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 bacterial growth [21]. The minimal bactericidal concentrations (MBC) of the samples was determined by taking 50 μL of the suspensions from the wells which did not show any growth after incubation during MIC assays to a new 96-well microplate containing 150 μL of fresh broth per well. The plate was further re-incubated at 37°C for 48 hours the addition of INT. The MBC was defined as the lowest concentration of samples which completely inhibited the growth of bacteria. Samples were tested alone and in the presence of PAßN at 30 μg/mL final concentration [22].

To evaluate the potentiating effect of tested extracts, a preliminary combination at their sub-inhibitory concentrations (MIC/2, MIC/5, MIC/10 and MIC/20) with antibiotics was assessed against *P. aeruginosa* PA124 strain. The appropriate sub-inhibitory concentrations were then selected on the basis of their ability to improve the activity

**Table 3** Bacterial strains and features

| Bacteria and strains | Features | References |
|----------------------|----------|------------|
| *Escherichia coli*   | ATCC 8739 References strains |           |
|                      | ATCC 10536 References strains |           |
| AG100 Ate  | AG 100 sur-expressing AcrAB pumps, containing TET<sup>+</sup> gene acrF | [14]        |
| AG100    | Wild-typeE. ColiK-12 | [15]        |
| AG102    | AG100 Sur-expressingAcrAB pumps. | [17]        |
| MC4100   | Wild typeE. coli |           |
| *Enterobacter aerogenes* | ATCC 13048 References strains |           |
| EA27     | Clinical MDR isolate exhibiting energy-dependent norfloxacin and chloramphenicol efflux with KAN<sup>+</sup>, AMP<sup>+</sup>, NAL<sup>+</sup>, STR<sup>+</sup>, TET<sup>+</sup> | [18]        |
| EA-3     | Clinical MDR isolate CHL<sup>+</sup>, NOR<sup>+</sup>, OFX<sup>+</sup>, SPX<sup>+</sup>, MOX<sup>+</sup>, CFT<sup>+</sup>, ATM<sup>+</sup>, FEP<sup>+</sup> | [18]        |
| EA 289   | KAN sensitive derivative d’EA27 | [18]        |
| EA 294   | EA289 sur-expressing AcrA pumps Exhibiting KAN<sup>+</sup> | [18]        |
| EA 298   | EA289 TolC KAN<sup>+</sup> | [18]        |
| CM64     | CHL<sup>+</sup>resistant variant obtained from ATCC13048 over-expressing the AcrAB pump | [18]        |
| *Klebsiella pneumoniae* | ATCC 11296 References strains |           |
| K-2      | Clinical MDR isolate exhibiting energy-dependent norfloxacin and chloramphenicol efflux with KAN<sup>+</sup>, AMP<sup>+</sup>, NAL<sup>+</sup>, STR<sup>+</sup>, TET<sup>+</sup> | Laboratory collection of UNR-MD1, University of Marseille, France |
| K-24     | AcrAB-TolC |           |
| KP 55    | Clinical isolate MDR, TET<sup>+</sup>, AMP<sup>+</sup>, ATM<sup>+</sup>, CEF<sup>+</sup> | [17]        |
| KP 63    | Clinical isolate du MDR, TET<sup>+</sup>, CHL<sup>+</sup>, AMP<sup>+</sup>, ATM<sup>+</sup> | [17]        |
| *Pseudomonas aeruginosa* | PA01 References strains |           |
| PA124    | MDR clinical isolate | [15]        |
| NAE16    | MDR clinical isolate AcrAB-TolC | [15]        |
| *Providencia stuartii* | ATCC 29916 References strains |           |
| *AMP, ATM*, CEF*, CFT*, CHL*, FEP*, KAN*, MOX*, STR*, TET*. Resistance to ampicillin, aztreonam, cephalothin, cefadroxil, chloramphenicol, cefepime, kanamycin, moxalactam, streptomycin, and tetracycline; OMPC and OMPF: Outer Membran Protein F and C respectively. AcrAB-TolC: Efflux pump of type AcrAB associated to one porine of type TolC.* | [15]        |

*AMP, ATM*, CEF*, CFT*, CHL*, FEP*, KAN*, MOX*, STR*, TET.* Resistance to ampicillin, aztreonam, cephalothin, cefadroxil, chloramphenicol, cefepime, kanamycin, moxalactam, streptomycin, and tetracycline; OMPC and OMPF: Outer Membran Protein F and C respectively. AcrAB-TolC: Efflux pump of type AcrAB associated to one porine of type TolC.*
of the maximum antibiotic. These sub-inhibitory concentrations for selected extracts were further tested in combination with antibiotics against more MDR bacteria. The Fractional inhibitory concentration (FIC) of each combination was then calculated as the ratio of MIC of Antibiotic in combination versus MIC of Antibiotic alone [23,24].

### Results

#### Phytochemical composition of the tested plant’s extracts

The results of the qualitative phytochemical analysis showed that each of the studied extract contained alkaloids, phenols, flavonoids, triterpenes and sterols. None of them contained anthocyanins and anthraquinones. Other phytochemical classes have been selectively detected as shown in Table 2.

#### Antibacterial activity of the plant’s extracts

Bacterial strains and MDR isolates were tested for their susceptibility to plant extracts and chloramphenicol. The results summarized in Table 4 the selectivity of the extracts towards the tested bacteria, with MIC values ranging from 256 to 1024 μg/mL on the majority of the 22 tested microorganisms. *Capsicum frutescens* extract displayed the largest spectrum of activity, 73% (16/22) against the tested bacteria; followed by *Brassica oleacea* var. *italica*, 50% (11/22); *Basilicum polystachyon* 41% (9/22) and *Brassica oleacea* var. *butyris* 27% (6/22) extracts. The lowest MIC value (256 μg/mL) was recorded with *Basilicum polystachyon* extract against *P. stuartii* (ATCC 29916) and *E. aerogenes* (ATCC 13048). No significant MBC value was recorded.

Eight (8) of the twenty two (22) studied MDR bacteria were also tested for their susceptibility to the plant extracts in the presence of PAβN (Table 5). The largest spectrum of activity was recorded with *B. oleacea* var. *butyris* extract against 75% (6/8) tested MDR bacteria. This efflux pumps inhibitor (EPI) also improved the activity of *C. frutescens* extract against *E. coli* (AG100), *K. pneumoniae* (KP53) and *E. aerogenes* (EA27) as well as that of *B. polystachyon* against *P. stuartii* (NAE16).

#### Antibacterial activity of extract-antibiotic combination

A preliminary assay against *P. aeruginosa* PA124 strain allowed selecting MIC/2 and MIC/5 as appropriate sub-inhibitory concentrations to be used on other bacteria Table 4 MIC and MBC of the tested plants extracts and CHL on the studied bacterial species

| Strains bacterial | Capsicum frutescens | Brassica oleacea var. varbutyris | Brassica oleacea var. italica | Basilicum polystachyon | Chloramphenicol |
|-------------------|---------------------|----------------------------------|-------------------------------|------------------------|----------------|
| Escherichia coli  | ATCC 8739           | 1024                             | 1024                          | 1024                   | 8              |
|                   | ATCC 10536          | -                                | -                             | -                      | 512            |
|                   | AG100 Aret          | 512                              | -                             | -                      | 2              |
|                   | AG100               | -                                | -                             | 512                    | 64             |
|                   | AG102               | 1024                             | 1024                          | 1024                   | 8              |
|                   | MC4100              | 1024                             | 512                           | 1024                   | 128            |
| Enterobacter aerogenes | ATCC 13048       | 1024                             | 1024                          | 1024                   | 256            |
|                   | EA27                | -                                | -                             | -                      | 8              |
|                   | EA294               | -                                | -                             | -                      | 256            |
|                   | EA298               | 512                              | 1024                          | -                      | 128            |
|                   | EA 289              | -                                | -                             | -                      | -              |
|                   | CM64                | 1024                             | -                             | 1024                   | 128            |
| Klebsiella pneumoniae | ATCC 11296        | 1024                             | 1024                          | 1024                   | 8              |
|                   | K-2                 | 512                              | 1024                          | 512                    | 512            |
|                   | K-24                | 1024                             | 1024                          | 1024                   | 16             |
|                   | KP 55               | 1024                             | 1024                          | 512                    | 32             |
|                   | KP 63               | 512                              | -                             | -                      | 128            |
| Pseudomonas aeruginosa | PA01              | -                                | -                             | -                      | 64             |
|                   | PA124               | -                                | -                             | -                      | 512            |
| Providencia stuartii | ATCC 29916        | 1024                             | 1024                          | 1024                   | 256            |
|                   | NAE16               | 1024                             | -                             | -                      | 256            |

NT: Not determined; -: superior to 1024 μL for extracts and superior to 512 μg/mL for antibiotics; CHL: Chloramphenicol; Values in Bold are the lowest MIC values for the plant extracts.
Table 5 Antibacterial activities of extracts alone and in the presence of PAβN

| Bacterial strains | Capsicum frutescens | Brassica oleacea var. butyris | Brassica oleacea var. italica | Basilicum polystachyon | CHL | PAβN |
|------------------|---------------------|-------------------------------|-------------------------------|------------------------|-----|------|
| AG100            | 1024 (256)         | - (1024)                      | - (1024)                      | - (1024)               | -  (1024) | 16 (4) >128 |
| AG100 Atet       | 512 (512)          | 1024 (512)                    | 1024 (1024)                   | - (-)                  | 64 (32) | >128 |
| CM64             | 1024 (1024)        | - (-)                         | 1024 (512)                    | - (1024)               | 128 (64) | >128 |
| EA27             | - (512)            | - (128)                       | - (512)                       | - (-)                  | 256 (64) | >128 |
| KP5              | - (-)              | - (1024)                      | - (1024)                      | - (-)                  | 64(8)  | >128 |
| KP63             | 512 (256)          | - (1024)                      | - (-)                         | - (-)                  | 128(16)| >128 |
| PA124            | - (-)              | - (-)                         | - (1024)                      | - (-)                  | 512(128)| >128 |
| NAE16            | - (-)              | - (-)                         | - (1024)                      | - (-)                  | 256(64)| >128 |

(Table 6). Synergistic effects were observed with all the tested extracts. Brassica oleacea var. italica and B. oleacea var. butyris extracts potentiate (0.125 < FIC < 0.5 and 0.031 < FIC < 0.5 respectively) the effects of the majority of antibiotics on most of the tested MDR bacteria (Table 7). Extracts from C. frutescens and B. polystachyon showed synergistic effects with six of the nine studied antibiotics, with 0.125 < FIC < 0.5 and 0.25 < FIC < 0.5 respectively.

Discussion

The Pharmacological potencies of plants' secondary metabolites are well demonstrated. The qualitative phytochemical screening of the plant extracts showed the presence of several classes of secondary metabolites, such as alkaloids, flavonoids, phenols, triterpenes, sterols, saponins, tannins and coumarins. Several antibacterial activities associated to the presence of compounds belonging to these various classes were shown [25-27]. It should however be mentioned that the detection of an alleged bioactive class of secondary metabolite in a plant is not a guarantee for any biological property, as this will depend on the nature of the compounds as well as their concentrations and the possible interactions with other constituents [12]. The differences observed between the antibacterial activities of the extracts as observed in the present work could be due to the differences in their phytochemical composition [9]. According to the criteria of classification of the antibacterial activity of the phytochemicals [28], the extracts used in this study were moderately and/or weak active (256 ≤ MIC < 1024 μg/mL). Their direct

Table 6 MICs of antibiotics in combination with plant extracts against P. aeruginosa PA124

| Plants’ extracts | CEF | AMP | CIP | ERY | KAN | TET | STR | CHL | NOR |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATB ALONE        | - (-) | - (-) | 64 | 512 | 128 | 64 | 64 | 512 | 256 |
| Capsicum frutescens | | | | | | | | | |
| MIC2             | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 128 (1) 1 | 32 (0,5) 5 | 256 (4) 1 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC5             | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 128 (1) 1 | 64 (1) 1 | 256 (4) 1 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC10            | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 128 (1) 1 | 64 (1) 1 | 256 (4) 1 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC20            | - (-) | - (-) | 64 (1) 1 | 256 (0,5) 5 | 256 (2) 1 | 64 (1) 1 | 256 (4) 1 | 256 (0,5) 5 | 128 (0,5) 5 |
| Brassica oleacea var. butyris | | | | | | | | | |
| MIC2             | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 16 (0,125) 5 | 16 (0,25) 5 | 32 (0,5) 5 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC5             | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 16 (0,125) 5 | 16 (0,25) 5 | 32 (0,5) 5 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC10            | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 16 (0,125) 5 | 16 (0,25) 5 | 32 (0,5) 5 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC20            | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 32 (0,25) 5 | 32 (0,25) 5 | 64 (1) 1 | 256 (0,5) 5 | 128 (0,5) 5 |
| Brassica oleacea var. italica | | | | | | | | | |
| MIC2             | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 128 (1) 1 | 32 (0,25) 5 | 32 (0,5) 5 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC5             | - (-) | - (-) | 64 (1) 1 | 256 (0,5) 5 | 128 (1) 1 | 32 (0,25) 5 | 32 (0,5) 5 | 256 (0,5) 5 | 128 (0,5) 5 |
| MIC10            | - (-) | - (-) | 64 (1) 1 | 256 (0,5) 5 | 128 (1) 1 | 32 (0,25) 5 | 64 (1) 1 | 512 (1) 1 | 256 (1) 1 |
| MIC20            | - (-) | - (-) | 64 (1) 1 | 256 (0,5) 5 | 128 (1) 1 | 64 (1) 1 | 64 (1) 1 | 512 (1) 1 | 256 (1) 1 |
| Basilicum polystachyon | | | | | | | | | |
| MIC2             | - (-) | - (-) | 32 (0,5) 5 | 128 (0,25) 5 | 256 (2) 1 | 64 (1) 1 | 64 (1) 1 | 256 (0,5) 5 | 256 (1) 1 |
| MIC5             | - (-) | - (-) | 32 (0,5) 5 | 256 (0,5) 5 | 256 (2) 1 | 64 (1) 1 | 64 (1) 1 | 256 (0,5) 5 | 256 (1) 1 |
| MIC10            | - (-) | - (-) | 64 (1) 1 | 256 (0,5) 5 | 256 (2) 1 | 64 (1) 1 | 64 (1) 1 | 256 (0,5) 5 | 256 (1) 1 |
| MIC20            | - (-) | - (-) | 64 (1) 1 | 256 (0,5) 5 | 256 (2) 1 | 64 (1) 1 | 64 (1) 1 | 256 (0,5) 5 | 256 (1) 1 |

s: Synergy; I: Indifference; A: Antagonism; (;): fractional inhibitory concentration or FIC; -: MIC > 512 μg/mL; ATB: Antibiotic; CIP: Ciprofloxacin; NOR: Norfloxacin; CHL: Chloramphenicol, STR: Streptomycin, TET: Tetracycline, KAN: Kanamycin, ERY: Erythromycin, AMP: Ampicillin and CEF: Cefepime; The values in bold represent the cases of synergy between extract and antibiotic.
| Antibiotics | Bacterial strains | Capsicum frutescens | Brassica oleracea var. butyris | Brassica oleracea var. Italica | Basilicum polystachyon |
|-------------|-------------------|---------------------|-------------------------------|-------------------------------|------------------------|
|             | MIC               | MIC/2               | MIC/5                         | MIC/2                         | MIC/5                  |
| CEF         |                   |                     |                               |                               |                        |
| AG100       | -                 | -                   | -                             | -                             | -                      |
| EA27        | 256               | -                   | -                             | -                             | -                      |
| CM64        | -                 | -                   | -                             | -                             | -                      |
| KP55        | -                 | -                   | -                             | -                             | -                      |
| KP63        | -                 | -                   | -                             | -                             | -                      |
| NAE16       | -                 | -                   | -                             | -                             | -                      |
| PA124       | -                 | -                   | -                             | -                             | -                      |
| AMP         |                   |                     |                               |                               |                        |
| AG100       | -                 | -                   | -                             | -                             | -                      |
| EA27        | -                 | -                   | -                             | -                             | -                      |
| CM64        | -                 | -                   | -                             | -                             | -                      |
| KP55        | -                 | -                   | -                             | -                             | -                      |
| KP63        | -                 | -                   | -                             | -                             | -                      |
| NAE16       | -                 | -                   | -                             | -                             | -                      |
| PA124       | -                 | -                   | -                             | -                             | -                      |
| CIP         |                   |                     |                               |                               |                        |
| AG100       | 32                | 32 (1)              | 64 (2)                        | 8 (0.25)                      | 8 (0.25)               |
| EA27        | 16                | 32 (2)              | 32 (1)                        | 4 (0.25)                      | 4 (0.25)               |
| CM64        | 16                | 16 (1)              | 16 (1)                        | 8 (0.5)                       | 8 (0.5)                |
| KP55        | 16                | 4 (0.25)            | 8 (0.5)                       | 4 (0.25)                      | 4 (0.25)               |
| KP63        | 8*                | 4 (0.5)             | 4 (0.5)                       | 1* (0.125)                    | 1* (0.125)             |
| NAE16       | 8*                | 2 (0.25)            | 2 (0.25)                      | 2* (0.25)                     | 2* (0.25)              |
| PA124       | 64                | 32 (0.5)            | 32 (0.5)                      | 32 (0.5)                      | 32 (0.5)               |
| ERY         |                   |                     |                               |                               |                        |
| AG100       | 32                | 16 (0.5)            | 16 (0.5)                      | 8 (0.25)                      | 8 (0.25)               |
| EA27        | 32                | 32 (1)              | 64 (2)                        | 64 (2)                        | 64 (2)                 |
| CM64        | 32                | 64 (2)              | 16 (0.5)                      | 32 (1)                        | 32 (1)                 |
| KP55        | 128               | 128 (1)             | 64 (0.5)                      | 64 (0.5)                      | 128 (1)                |
| KP63        | 128               | 32 (0.25)           | 64 (0.5)                      | 64 (0.5)                      | 128 (1)                |
| NAE16       | 128               | 16 (0.125)          | 16 (0.125)                    | 32 (0.25)                     | 128 (1)                |
| PA124       | 512               | 256 (0.5)           | 256 (0.5)                     | 256 (0.5)                     | 256 (0.5)              |
| KAN         |                   |                     |                               |                               |                        |
| AG100       | 32                | 32 (1)              | 64 (2)                        | 64 (2)                        | 64 (2)                 |
| EA27        | 32                | 8 (0.25)            | 8 (0.25)                      | 8 (0.25)                      | 8 (0.25)               |
| CM64        | 64                | 64 (1)              | 16 (0.25)                     | 32 (0.5)                      | 32 (0.5)               |
| KP55        | 64                | 16 (0.25)           | 16 (0.25)                     | 16 (0.25)                     | 16 (0.25)              |
| KP63        | 64                | 16 (0.25)           | 16 (0.25)                     | 16 (0.25)                     | 16 (0.25)              |
| NAE16       | 64                | 16 (0.125)          | 16 (0.125)                    | 16 (0.125)                    | 16 (0.125)             |
| PA124       | 512               | 256 (0.5)           | 256 (0.5)                     | 256 (0.5)                     | 256 (0.5)              |
| TET         |                   |                     |                               |                               |                        |
| AG100       | 32                | 8 (0.25)            | 8 (0.25)                      | 8 (0.25)                      | 8 (0.25)               |
| EA27        | 128               | 64 (0.5)            | 16 (0.125)                    | 16 (0.125)                    | 16 (0.125)             |
| CM64        | 64                | 128 (2)             | 4 (0.062)                     | 8 (0.125)                     | 64 (1)                 |
| KP55        | 16                | 2 (0.125)           | 4 (0.062)                     | 1 (0.062)                     | 1 (0.062)              |
| KP63        | 32                | 8 (0.25)            | 8 (0.25)                      | 16 (0.5)                      | 16 (0.5)               |
| NAE16       | 128               | 64 (0.5)            | 128 (1)                       | 128 (1)                       | 64 (0.5)               |
| PA124       | 64                | 32 (0.5)            | 64 (1)                        | 16 (0.25)                     | 32 (0.5)               |
| STR         |                   |                     |                               |                               |                        |
| AG100       | 64                | 256 (4)            | 256 (4)                       | 128 (1)                       | 64 (0.5)               |

*Note: The MIC values are given in micrograms per milliliter (µg/mL). The MIC/2 and MIC/5 values are calculated as the MIC divided by 2 and 5, respectively. The values in parentheses represent the actual concentration of the antibiotic in µg/mL. The superscript 's' indicates sensitivity, and 'i' indicates intermediate sensitivity.
use in the control of MDR bacterial infections could therefore be of limited importance. None-the-less, the obtained results can be considered as interesting when considering the fact that the extracts are obtained directly from edible plant materials.

Efflux pumps are responsible for the reduction of intracellular concentration of antibiotic compounds [29]. To tackle problems related to this phenomenon, an intensive search of efflux pumps inhibitors (EPI) is welcome [30]. The EPI blocks the efflux pumps and leads to the increase of the intracellular concentration of active principle (MIC < FIC < 0.5) with the majority of the tested antibiotics against the studied MDR strains. This suggests that the extracts might contain bioactive compounds that, combined with antibiotics, acted at different sites by various mechanisms [33,34]. These data indicate that a combination of these extracts with antibiotics could be envisaged to fight MDR bacteria.

**Conclusion**

These results provide promising baseline information for the potential use of *Capsicum frutescens, Brassica oleracea* var. *italica, Basilicum polystachyon* and *Brassica oleracea* var. *butyris*, independently or in combination with some commonly used antibiotics in the fight against MDR Gram-negative bacteria.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

FTK carried out the study; VK designed the experiments. FTK, AJS, AGF and NV carried out the microbiological studies. VK was involved in data analysis. FTK and NV drafted the manuscript.

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**Table 7 MIC of antibiotics in combination with plant at their MIC/2 and MIC/5 against selected MDR bacteria strains (Continued)**

|                | EA27 | CM64 | KP55 | KP63 | NAE16 | PA124 |
|----------------|------|------|------|------|-------|-------|
| MIC                      | 32 (1) | 32 (1) | 32 (1) | 32 (1) | 32 (1) | 32 (1) |
| FIC                      | 4 (0.25) | 4 (0.25) | 4 (0.25) | 4 (0.25) | 4 (0.25) | 4 (0.25) |
| MIC                      | 8 (1) | 16 (1) | 16 (1) | 16 (1) | 16 (1) | 16 (1) |
| FIC                      | 32 (0.5) | 32 (0.5) | 32 (0.5) | 32 (0.5) | 32 (0.5) | 32 (0.5) |
| MIC                      | 64 (1) | 128 (1) | 128 (1) | 128 (1) | 128 (1) | 128 (1) |
| FIC                      | 64 (0.5) | 64 (0.5) | 64 (0.5) | 64 (0.5) | 64 (0.5) | 64 (0.5) |
| MIC                      | 128 (1) | 256 (1) | 256 (1) | 256 (1) | 256 (1) | 256 (1) |
| FIC                      | 256 (1) | 256 (1) | 256 (1) | 256 (1) | 256 (1) | 256 (1) |

The extracts of *B. oleracea* var. *butyris*, *B. oleracea* var. *italica, Basilicum polystachyon* and *C. frutescens* showed significant synergistic effects (0.031 < FIC < 0.5) with the majority of the tested antibiotics against the studied MDR strains. This suggests that the extracts might contain bioactive compounds that, combined with antibiotics, acted at different sites by various mechanisms [33,34]. These data indicate that a combination of these extracts with antibiotics could be envisaged to fight MDR bacteria.

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