Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria

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Abstract

Background: In response to the propagation of bacteria resistant to many antibiotics also called multi-drug resistant (MDR) bacteria, the discovery of new and more efficient antibacterial agents is primordial. The present study was aimed at evaluating the antibacterial activities of seven Cameroonian dietary plants (Adansonia digitata, Aframomum albiovilaceum, Aframomum polyanthum, Anonidium mannii, Hibiscus sabdarifa, Ocimum gratissimum and Tamarindus indica).

Methods: The phytochemical screening of the studied extracts was performed using described methods whilst the liquid broth micro dilution was used for all antimicrobial assays against 27 Gram-negative bacteria.

Results: The results of the phytochemical tests indicate that all tested extracts contained phenols and triterpenes, other classes of chemicals being selectively present. The studied extracts displayed various degrees of antibacterial activities. The extracts of A. digitata, H. sabdarifa, A. polyanthum, A. albiovilaceum and O. gratissimum showed the best spectra of activity, their inhibitory effects being recorded against 81.48%, 66.66%, 62.96%, 55.55%, and 55.55% of the 27 tested bacteria respectively. The extract of A. polyanthum was very active against E. aerogenes EA294 with the lowest recorded minimal inhibitory concentration (MIC) of 32 μg/ml.

Conclusion: The results of the present work provide useful baseline information for the potential use of the studied edible plants in the fight against both sensitive and MDR phenotypes.

Keywords: Antibacterial, Multi-drug resistant bacteria, Dietary plants

Background

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though pharmaceutical companies have produced a number of new antibacterials in the last years, resistance to these drugs has increased and has now became a global concern [1]. The global emergence of multi-drug resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure [2]. Bacterial resistance to chemically unrelated antimicrobial agents is public health concern [3] and may be caused by over-expression of MDR efflux pumps [4]. In Gram-negative bacteria, the effect of the efflux pumps in combination with the reduced drug uptake (due to the presence of a double membrane barrier) is responsible for the high inherent and acquired antibiotic resistance often associated with this group of organisms [5]. Among Gram-negative bacteria, many of these MDR efflux pumps belong to the RND (resistance-nodulation-cell division) type family of tripartite efflux pumps.

Due to the increase of resistance to antibiotics, there is a pressing need to develop new and innovative antimicrobial agents. Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be of interest in therapeutic. Because of their low toxicity, there is a long tradition of using dietary plants in the treatment of infectious disease in Cameroonian folk medicine.

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Consequently, we focused one of the objective of our research group at investigating the antibacterial potentials of such plants against MDR phenotypes. In previous studies we demonstrated the antimicrobial activity of many Cameroonian dietary plants against MDR bacteria [6-9]. In our continuous search of the antibacterial activities of Cameroonian edible plants, we designed the present work to determine the activity of seven selected Cameroonian dietary plants (Adansonia digitata, Aframomum alboviolaceum, Aframomum polyanthum, Anonidium mannii, Hibiscus sabdarifa, Ocimum gratissimum and Tamarindus indica) against MDR Gram-negative bacteria.

**Methods**

**Plant materials and extraction**

The herbal sample consisted of seven different Cameroon edible plants namely the leaves of Adansonia digitata and Anonidium mannii, the rhizomes of Aframomum alboviolaceum, Aframomum polyanthum, the whole plants of Hibiscus sabdarifa and Ocimum gratissimum, and the fruits of Tamarindus indica. The plants were purchased from markets in the West region of Cameroon in January 2011. They were further identified at the National Herbarium (Yaoundé, Cameroon) where the voucher specimens were deposited under reference numbers (Table 1). Each plant was dried at room temperature and the powdered material was then weighed (300 g), soaked in 1 L of methanol (MeOH) for 48 h and filtered using Whatman N°1 filter paper. The filtrate obtained was concentrated under reduced pressure (at 68°C) in a rotary evaporator to obtain the crude extract. The crude extracts were kept at 4°C until further uses.

**Preliminary phytochemical screening**

The plant materials were screened for the presence of different classes of secondary metabolites including alkaloids, flavonoids, phenols, saponins, tannins, anthocyanins, anthraquinones, sterols, and triterpenes using previously described methods [34].

**Bacterial susceptibility determinations**

The minimal inhibitory concentrations (MICs) of the seven plant extracts were determined using a rapid broth microdilution method as described previously [37]. Bacterial strains tested respectively. The microorganisms of P. aeruginosa (PA01 and PA124), known for their resistance against 81.48% of the bacterial strains, followed by the extracts of H. sabdarifa (66.66%), A. polyanthum (62.96%), A. alboviolaceum (55.53%) and O. gratissimum (55.55%). The extract of A. polyanthum showed the highest activity against E. aerogenes EA294 with a MIC value of 32 μg/mL. The extracts of T. indica and A. mannii did not show antibacterial activity against the majority of the bacteria tested, their inhibitory effect being noted against 6/27 (22.22%) and 7/27(25.92%) bacterial strains tested respectively. The microorganisms of the species P. aeruginosa (PA01 and PA124), known for...
| Species (family); Voucher number* | Traditional uses | Parts used traditionally | Bioactive or potentially bioactive components | Bioactivities |
|----------------------------------|------------------|--------------------------|-----------------------------------------------|---------------|
| **Adansonia digitata** (Malvaceae); 42417/HNC | Analgesic, anti-diarrheal, smallpox, rubella [10], antipyretic, fever, dysentery, anti-inflammatory, astringent [11] | Pulps, Fruits, leaves, Pip, Bark | / | Ethanol and aqueous extract: Ec [12] Sa, Se, Stm, Pa [13]; [14,15] Hs [11]. |
| **Aframomum alboviolaceum** (Zingiberaceae); 34888/HNC | Diuretic, anthelmintic, fever, antiparasitic [16]. Roots | Methyl (E)-14Ksi,15-epoxylabd-8(17), 12-dien-16-oate; (E)-labda-8(17),12-diene-15,16-dial and (E)-8beta,17-epoxylabd-12-ene-15,16-dial [17] | Hc [18]. |
| **Aframomum polyanthum** (Zingiberaceae) 3981/SRFK | / | Fruits | Aframodial [19]. Sa, Scp, Ha, Cu [19]. |
| **Anonidium mannii** (Annonaceae); 1918/SRFK | Spider and snake bites, bronchitis, dysentery, gastroenteritis [20], syphilis, [21]; diarrea, malaria [22]. | Stem | Prenylatedbisindole [23]. / | |
| **Hibiscus sabdarifa** (Malvaceae); 42795/HNC | Diuretic, stomachic, laxative, aphrodisiac, antiseptic, astringent, cholagogue, sedative, hypertension and other cardiac diseases [24]. Flowers | Protocatechic acid, [25]; [26], hydroxycitricacid. | Ethanol, methanol and aqueous extracts: Ec, Pa, Kp, Hi, Sa, Spy, Sp [27]. Methanolic extract: Bs, Ml, Sm, Cs, Bc [24]. |
| **Ocimum gratissimum** (Lamiaceae); 42738/HNC | Respiratory tracts diseases, diarrhea, anti-hypertensive, malaria [28]. | leaves, Roots, Buds | (β-caryophyllene,y-terpinène, (Z)-α-bisabolone, thymol, p-cymene, eugenol, limonène, α-terpinolene, α-terpinol [29]. | Essential oil: Af, AB1, Hc [30] Ethanol extract: Ec, Sa [31]. |
| **Tamarindus indica** (Caesalpiniaiceae); 26326/SRFC | Fever, gastric ulcer, diarrhea, jaundice [32], conjunctivitis, hemornoid, astringent, asthma, eye inflammation [33]. | Fruits Bark | / | Ethanol and aqueous extracts: Ec [12] |

*(HNC): Cameroon National Herbarium; (SRFC): Société des Réserves Forestières du Cameroun; AB: Aflatoxin; Af: Aspergillus flavus; Bc: Bacillus cereus; Bs: Bacillus stearothermophilus; Cu: Candida ufilis; Cs: Clostridium sporogenes; Ec: Escherichia coli; Ha: Hansenula anomala; Hc: Haemanchus contortus; Hs: Haemophilus influenza; Kp: Klebsiella pneumoniae; Mr: Micrococcus luteus; Pa: Pseudomonas aeruginosa; Sa: Staphylococcus aureus; Sc: Sacccharomyces cerevisiae; Se: Staphylococcus epidermidis; Scp: Schizosaccharomyces pombe; Sm: Serratia mance; Stm: Streptococcus mutans; Sp: Streptococcus pneumoniae; Spy: Streptococcus pyogenes; (/): not documented.
their multi-resistance to drugs, were resistant to all the plant extracts tested in this work (with MIC > 1024 μg/ml).

Some of the studied extracts showed bactericidal effects on few numbers of bacteria. These effects were observed with the crude extracts of A. digitata, against E. coli MC4100 and K. pneumoniae KP55 with the ratios minimal bactericidal versus minimal inhibitory concentrations (MBC/MIC) equal to 1 and 2 respectively. For A. polyanthum’s extract, the ratio MBC/MIC was equal to 2 on K. pneumoniae KP55. O. gratissimum also showed ratios MBC/MIC equal to 1 on E. coli AG 102. The crude extract of H. sabdarifa was also bactericidal against E. coli MC4100 and W3110 and against E. cloacae BM67 with the ratio equal to 1; 1 and 4 respectively. Chloramphenicol was bacteristatic on the majority of bacteria ranging from 2 to 512 μg/ml. These activities of chloramphenicol could explain their antibacterial activity against A. digitata on K. pneumoniae KP55, H. sabdarifa on E. cloacae BM 67 and O. gratissimum on E. cloacae ECCI69.

**Discussion**

Each of the extract tested in the present study displayed antibacterial activity on at least 6 of 27 bacterial strains tested. However differences were observed between antibacterial activities of the extracts. These differences could be due to the differences in the chemical composition of these extracts as the secondary metabolites of plants have many effects including antibacterial and antiviral properties [9,38]. The overall data of this study were in accordance with previous results. Apart from the phytochemicals found in A. digitata extract, previous studies showed the presence of an alkaloid namely adansonin [15]. The antibacterial activity of the aqueous and ethanol extracts of this plant has already been reported against E. coli [12]. Therefore, the inhibitory activity found herein against reference and multi-resistant strains of E. coli as well as other Gram-negative species is complementary to Yagoub’s [12] report.

Phytochemical screening results of H. sabdarifa was in accordance with the results previously obtained [24]. This latter suggested that the presence of alkaloids (which interfere with cell division) in H. sabdariffa could account for its antimicrobial activity. They demonstrated that methanol extract of H. sabdarifa possess inhibitory activities against E. coli, P. aeruginosa and S. aureus. In this report, the antibacterial activity was not observed against P. aeruginosa, but the results obtained herein are not in contradiction with those previously reported since the previous MIC of 1300 μg/ml was higher than the highest concentration used in this work. The results of the present work also bring additional data on the antibacterial activity of H. sabdarifa, since we report for the first time its activity against E. aerogenes, P. stuartii and K. pneumoniae.

To the best of our knowledge, phytochemical composition of A. alboviolaceum and A. polyanthum is described here for the first time. The different phytochemicals found here should then explain its antibacterial activity against different bacterial strains tested. The plants of the genus Aframomum was already found to possess flavonoids, diterpenoids and arylalkaloids which could explain their antibacterial activity [39].

All the phytochemical constituent found in the extract of O. gratissimum was previously reported by Akinmoladun et al. [40] who also found flavonoids in the same extract. Nevertheless, the antibacterial activity of this extract is in agreement with the findings of Obinna et al. [31] who showed the inhibitory activity of O. gratissimum against E. coli and S. aureus. Moreover the present work brings

| Table 2 Parts used, extraction yields, and phytochemical composition of the plant extracts |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Extracts**    | **A. digitata** | **A. alboviolaceum** | **A. polyanthum** | **A. mannii** | **H. sabdarifa** | **O. gratissimum** | **T. indica** |
| **Parts used**  | Leaves          | Fruits           | Fruits           | Leaves         | Twigs           | Twigs           | Fruits         |
| **Yield* (%)** | 12.17           | 6.45             | 3.23             | 3.39           | 4.94            | 4.75            | 37.98          |
| Alkaloids       | -               | +                | -                | +              | +               | +               | +              |
| Anthocyanines   | -               | -                | +                | -              | -               | -               | -              |
| Anthraquinones  | -               | -                | -                | -              | -               | -               | -              |
| Flavonoids      | -               | +                | -                | -              | +               | -               | +              |
| Phenols         | +               | +                | +                | +              | +               | +               | +              |
| Polyphenols     | +               | +                | +                | +              | +               | +               | +              |
| Saponines       | +               | -                | +                | +              | +               | -              | +              |
| Tannins         | +               | -                | -                | +              | -               | +              | -              |
| Sterols         | +               | -                | -                | +              | +               | +              | +              |
| Triterpenes     | +               | +                | +                | +              | +               | +              | +              |

(∗): Present; (−): Absent; * yield calculated as the ratio of the mass of the obtained methanol extract/mass of the plant powder.
| Bacteria      | Adansonia digitata | Aframomum alboviolaceum | Aframomum polyanthum | Anonidium mannii |
|--------------|--------------------|-------------------------|----------------------|-----------------|
|              | MIC  | MBC  | MBC/MIC | MIC  | MBC  | MBC/MIC | MIC  | MBC  | MBC/MIC |
| **E. coli**  |      |      |         |      |      |         |      |      |         |
| ATCC8739    | 1024 | -    | -       | -    | -    | -       | 1024 | -    | -       |
| ATCC10536   | -    | -    | -       | -    | -    | -       | -    | -    | -       |
| AG100       | 512  | -    | -       | 1024 | -    | -       | -    | -    | -       |
| AG100A      | 128  | -    | 1024    | -    | -    | -       | -    | -    | -       |
| AG100A_MIN  | 1024 | 256  | -       | 1024 | 512  | -       | -    | -    | -       |
| AG102       | 256  | 512  | 2       | 1024 | -    | -       | 512  | -    | -       |
| MC4100      | 1024 | 1024 | 1       | -    | -    | -       | 1024 | -    | -       |
| W311O       | 512  | -    | -       | -    | -    | -       | 512  | -    | -       |
| **E. aerogenes** |      |      |         |      |      |         |      |      |         |
| ATCC13048   | 128  | 512  | 4       | 512  | -    | -       | -    | -    | -       |
| CM64        | 1024 | -    | -       | -    | -    | -       | 1024 | -    | -       |
| EA27        | -    | -    | -       | 1024 | -    | -       | -    | -    | 1024    |
| EA289       | 512  | -    | 1024    | -    | -    | -       | -    | -    | 1024    |
| EA298       | 1024 | 1024 | 1       | -    | -    | -       | 1024 | -    | -       |
| EA294       | 1024 | 256  | -       | 32   | 512  | 16      | -    | -    | -       |
| **E. cloacae** |      |      |         |      |      |         |      |      |         |
| ECC69       | 512  | -    | -       | -    | -    | -       | 1024 | -    | 1024    |
| BM47        | 1024 | -    | -       | 1024 | -    | -       | 1024 | -    | -       |
| BM67        | 1024 | -    | 1024    | -    | 512  | -       | 1024 | -    | -       |
| **K. Pneumonia** |      |      |         |      |      |         |      |      |         |
| ATCC11296   | 512  | -    | -       | -    | -    | 256     | 1024 | 4    | -       |
| KPSS        | 128  | 256  | 2       | 512  | 1024 | 2       | -    | -    | -       |
| KP63        | 1024 | -    | -       | -    | -    | 1024    | -    | -    | -       |
| IO4         | 512  | -    | 512     | -    | -    | 1024    | -    | -    | -       |
| K2          | 1024 | -    | 512     | -    | -    | 1024    | -    | -    | 1024    |
| **P. Sturtii** |      |      |         |      |      |         |      |      |         |
| ATCC29914   | -    | -    | -       | 512  | -    | -       | -    | -    | -       |
| PS2636      | 1024 | -    | 1024    | -    | -    | 1024    | -    | -    | -       |
| PS299645    | 1024 | -    | -       | -    | -    | 1024    | -    | -    | -       |
| **P. aer.-ginosa** |      |      |         |      |      |         |      |      |         |
| PA01        | -    | -    | -       | -    | -    | -       | -    | -    | -       |
| PA124       | -    | -    | -       | -    | -    | -       | -    | -    | -       |
### Table 3 Minimal inhibitory concentration (MIC), minimal bactericidal (MBC) and MBC/MIC ratios of the plant extracts and CHL on the studied bacterial species (Continued)

| Bacteria       | Hibiscus sabdarifa | Ocimum gratissimum | Tamarintus indica | Chloramphenicol* |
|----------------|--------------------|--------------------|-------------------|------------------|
|                | MIC    | MBC  | MBC/MIC | MIC    | MBC  | MBC/MIC | MIC    | MBC  | MBC/MIC | MIC    | MBC  | MBC/MIC |
| E. coli        |        |      |         |        |      |         |        |      |         |        |      |         |
| ATCC8739       | -      | -    | -       | -      | -    | -       | 4      | -    | -       | 4      | -    | -       |
| ATCC10536      | -      | -    | -       | 1024   | -    | -       | 2      | 128  | 64      | 2      | 128  | 64      |
| AG100          | 1024   | -    | -       | -      | -    | -       | -      | -    | -       | -      | -    | -       |
| AG100A         | 512    | -    | -       | -      | -    | -       | 2      | 64   | 32      | 2      | 64   | 32      |
| AG100A_TET     | 1024   | -    | -       | -      | -    | -       | -      | -    | -       | 64 (8) | 256 (64)| 4 (8)   |
| AG102          | 1024   | -    | -       | 1024   | 1024 | 1       | 8      | -    | -       | -      | -    | -       |
| MC4100         | 1024   | 1024 | 1       | 1024   | -    | -       | 64     | -    | -       | -      | -    | -       |
| W3110          | 1024   | 1024 | 1       | -      | -    | -       | -      | -    | -       | 4      | 32   | 8       |
| E. aerogenes    |         |      |         |        |      |         |        |      |         |        |      |         |
| ATCC13048      | 1024   | -    | -       | -      | -    | -       | -      | -    | -       | -      | -    | -       |
| CM64           | 1024   | -    | -       | 1024   | -    | -       | -      | -    | -       | 256 (64)| 64   | (64)   |
| EA27           | 1024   | -    | -       | -      | -    | -       | -      | -    | -       | 516 (32)| 512 (256)| 2 (8) |
| EA289          | 1024   | -    | -       | 512    | -    | -       | -      | -    | -       | 512 (32)| 512 (128)| (14)  |
| EA298          | 512    | -    | -       | 512    | -    | -       | 1024   | 1024 | 1       | 128 (64)| 64   | (64)   |
| EA294          | 1024   | -    | -       | 1024   | -    | -       | -      | -    | -       | 4      | 16   | 4       |
| E. cloacae     |         |      |         |        |      |         |        |      |         |        |      |         |
| ECC169         | -      | -    | -       | 512    | -    | -       | 1024   | 1024 | 1       | 512    | 1024 | 1       |
| BM47           | -      | -    | -       | -      | -    | -       | -      | -    | -       | 512    | 1024 | 1       |
| BM67           | 256    | 1024 | 4       | -      | -    | -       | 1024   | -    | 256     | -      | -    | 1024    |
| K. Pneumonia   |         |      |         |        |      |         |        |      |         |        |      |         |
| ATCC11296      | 1024   | -    | -       | 1024   | -    | -       | -      | -    | -       | -      | -    | -       |
| KPS5           | 512    | -    | -       | 512    | -    | -       | -      | -    | -       | 128    | 128  | 1       |
| KP63           | -      | -    | -       | 1024   | -    | -       | -      | -    | -       | 64 (16)| 256  | (16)   |
| K24            | 1024   | -    | -       | 1024   | -    | -       | -      | -    | -       | 16 (1) | 64   | (64)   |
| K2             | 512    | -    | -       | 1024   | -    | -       | -      | -    | -       | 32     | -    | -       |
| P. Stwartii    |         |      |         |        |      |         |        |      |         |        |      |         |
| ATCC29914      | -      | -    | -       | -      | -    | -       | -      | -    | -       | 8      | 128  | 16      |
| PS2636         | -      | -    | -       | 128    | -    | -       | 32     | 256  | 8       |
| PS299645       | 512    | -    | -       | -      | -    | -       | 16     | 512  | 32      |
| P. aeruginosa  |         |      |         |        |      |         |        |      |         |        |      |         |
| PA01           | -      | -    | -       | -      | -    | -       | -      | -    | -       | 16 (8) | 256  | (32)   |
| PA124          | -      | -    | -       | -      | -    | -       | 32 (16)| -    | (16)    |

(-): >1024 μg/ml for extracts and >512 μg/ml for chloramphenicol and not calculated for MBC/MIC.

*(): for chloramphenicol in the presence of PABN.
additional information of the antibacterial activities of this plant against multi-resistant bacteria.

Previous reports showed good antibacterial effect of \textit{T. indica} against \textit{E. coli} strains isolated from urine and water samples. Another plant of the present work namely \textit{A. manni} is used traditionally for treatment of different ailments including different infectious diseases like gastroenteritis and syphilis. PAßN, is a potent inhibitor of the RND efflux systems is especially active on AcrAB-TolC and MexAB-OprM. The wide range enhancement (on all the strains) of the antibacterial activity by PAßN observed herein with chloramphenicol confirmed that an active efflux system expressed by tested bacteria is responsible for their resistance to chloramphenicol. The wide substrate specificity of these pumps could allow them to provoke extrusion of various active antibacterial compounds, preventing their inhibitory effects [9]. Therefore, the low antibacterial activities of these plants shown in the present work should thus be due to the resistance of bacteria strains tested (see Additional file 1: Table S1). The contrast between high number of secondary metabolite classes found in these extracts reinforces the idea that the detection of the classes of phytochemicals in plants is not a guarantee for a good antibacterial properties [9]. A sample is bactericidal when the ratio MBC/MIC ≤ 4 and bacteriostatic when this ratio is >4 [9]. It therefore appeared that bactericidal effects were obtained with the extract from \textit{A. albivioleacrum, T. indica} and \textit{O. gratissimum} against 1 of the 27 tested bacterial strains and \textit{A. digitata} against 5/27 (Table). No bactericidal activity was obtained with \textit{A. manni} extract on all the studied bacteria. This shows that the studied extract mostly exhibited bacteriostatic effects.

Conclusion
The results of the present study support the traditional use of the studied plants in the treatment of bacterial infections. They also provide an important basis for the use of methanol extract of the edible plants used to control infectious diseases caused by Gram-negative bacteria including MDR strains.

Additional file

Additional file 1: Table S1. Bacterial strains and features.

Abbreviations

ATCC: American Type Culture Collection; CFU: Colonies forming unit; CHL: Chloramphenicol; DMpSO: Dimethylsulfoxide; INT: p-Iodonitrotetrazolium chloride; MDR: Multidrug Resistant; MHB: Mueller Hinton Broth; MIC: Minimal Inhibitory Concentration; PAßN: Phenylalanine Arginine ß-Naphthylamide; RND: Resistance Nodulation-cell Division.

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

Authors’ contributions

DED, JAHK, AGF, IKY, SBT, AHNJ and AJJS carried out the study; VK designed the experiments, supervised the work; JAHK and VK wrote the manuscript; VK provided the bacterial strains; All authors read and approved the final manuscript.

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