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Antibacterial activities of the methanol extracts and compounds from *Erythrina sigmoidea* against Gram-negative multi-drug resistant phenotypes

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Abstract

Background: In the present study, the methanol extracts from the leaves, as well as compounds namely sigmoidin I (1), atalantoflavone (2), bidwillon A (3), neocyclomorusin (4), 6 α -hydroxyphaseollidin (5) and neobavaisoflavone (6) (from the bark extract) were tested for their activities against a panel of Gram-negative bacteria including multi-drug resistant (MDR) phenotypes.

Methods: Broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of the extracts as well as compounds 1–6.

Results: The MIC results indicated that the crude extracts from the leaves and bark of this plant were able to inhibit the growth of 96.3 % of the 27 tested bacteria. Compounds 2–6 displayed selective activities, their inhibitory effects being obtained on 8.3 %, 41.7 %, 58.3 %, 58.3 % and 66.7 % of tested bacteria respectively for 2, 3, 5, 6 and 4. The lowest MIC value of 8 μ g/mL was obtained with 6 against *Escherichia coli* ATCC8739, *Enterobacter cloacae* ECC169, *Klebsiella pneumoniae* KP55, *Providencia stuartii* NAE16 and *Pseudomonas aeruginosa* PA01.

Conclusion: The present study demonstrates that *Erythrina sigmoidea* is a potential source of antibacterial drugs to fight against MDR bacteria. Neobavaisoflavone (6) is the main antibacterial constituents of the bark crude extract.

Keywords: Antibacterial, *Erythrina sigmoidea*, Compounds, Multidrug resistance, Neobavaisoflavone

Background

Medicinal plants have been used since ancient times in the management of human including microbial infections. Approximately 60 % of world's population still relies on medicinal plants for their primary healthcare [1]. The African mainland has between 40,000-60,000 plant species, of which approximately 35,000 are endemic [2, 3]. Cameroon has a rich biodiversity, with about 8,620 plants species [4]. Several Camerooninan medicinal plants were previously reported for their antibacterial activities against multi-drug resistant Gram-negative bacteria [5–8]. Some of the them include

Beilschmiedia cinnamomea and *Echinops giganteus* [5], *Beilschmiedia obscura*, *Pachypodanthium staudtii* and *Peperomia fernandopoiana* [9] or *Capsicum frutescens* [10]. The antimicrobial activities of many secondary metabolites from Cameroonian plants were also reported [11, 12]. In our continuing search of new herbal drug from the Cameroon flora, the present study was designed to demonstrate the antibacterial activity of the extracts and compounds from *Erythrina sigmoidea* Hua (Fabaceae). *Erythrina sigmoidea* is a tree of up to 6 m high, with stems armed with stout found in Senegal, Nigeria, Cameroon, Chad and Central African Republic [13]. The plant is traditionally used as antidotes (venomous stings, bites, etc.), diuretic, febrifuge and to treat arthritis, rheumatism, pulmonary troubles, stomach troubles, infectious diseases and kidney diseases [13]. In the Western

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Region of Cameroon, the aqueous extracts from leaves, bark and roots are used to treat gastrointestinal infections, venereal diseases and leprosy [14]. Previously phytochemical study this plant led to the isolation of sigmoidin I (**1**), atalantoflavone (**2**), bidwillon A (**3**), neocyclomorusin (**4**), 6 α -hydroxyphaseollidin (**5**), and neobavaisoflavone (**6**) [15]. They displayed good cytotoxicity towards drug-sensitive and drug resistant cancer cell line [15]. In addition, they showed low cytotoxicity against the normal AML12 hepatocytes [15].

Methods

Plant material and extraction

The leaves and bark of *Erythrina sigmoidea* (Fabaceae) were collected in April 2013 in Bangangté (West Region of Cameroon). The plant was identified by a botanist of the National Herbarium in Yaoundé, Cameroon and compared with voucher kept under the registration number N°24470/HNC.

Antimicrobial assays

Chemicals for antimicrobial assay

Compounds isolated from the bark of *Erythrina sigmoidea* included β - sigmoidin I (**1**), atalantoflavone (**2**), bidwillon A (**3**), neocyclomorusin (**4**), 6 α -hydroxyphaseollidin (**5**) and neobavaisoflavone (**6**) (Fig. 1). Their isolation and identification were previously reported [15]. Chloramphenicol ≥ 98 % (Sigma-Aldrich, St. Quentin Fallavier, France) was used as reference antibiotics (RA) against Gram-negative bacteria. *p*-Iodonitrotetrazolium chloride ≥ 97 % (INT, Sigma-Aldrich) was used as microbial growth indicator [16, 17].

Microbial strains and culture media

The studied microorganisms included sensitive and resistant strains of *Escherichia coli* (ATCC8739, AG100,

AG100A, AG100A_{TET}, AG102, MC4100, W3110), *Enterobacter aerogenes* (ATCC13048, CM64, EA27, EA289, EA294, EA298), *Enterobacter cloacae* (ECCI69, BM47, BM67), *Klebsiella pneumoniae* (ATCC12296, KP55, KP63, K24, K2), *Providencia stuartii* (NEA16, ATCC29916, PS2636, PS299645) and *Pseudomonas aeruginosa* (PA01, PA124) obtained clinically or from the American Type Culture Collection. Their bacterial features are summarized in Table 1. Nutrient agar was used for the activation of the tested bacteria [18].

INT colorimetric assay for MIC and MBC determinations

MIC determinations on the tested bacteria were conducted using rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay according to described methods [16] with some modifications [19, 20]. The test samples and chloramphenicol were first of all dissolved in DMSO/Mueller Hinton Broth (MHB) or DMSO/7H9 broth. The final concentration of DMSO was lower than 2.5 % and does not affect the microbial growth [21, 22]. The 96-wells microplate were used and the inoculum concentration was 1.5×10^6 CFU/mL [19, 20]. The plates were incubated at 37 °C for 18 h. The assay was repeated thrice. Wells containing adequate broth, bacterial inoculum and DMSO to a final concentration of 2.5 % served as negative control. The MIC of samples was detected after 18 h incubation at 37 °C, following addition (40 μ L) of 0.2 mg/mL of INT and incubation at 37 °C for 30 min. Viable bacteria reduced the yellow dye to a pink. MIC was defined as the sample concentration that prevented the color change of the medium and exhibited complete inhibition of microbial growth [16]. The MBC was determined by adding 50 μ L aliquots of the preparations, which did not show any growth after incubation during MIC assays, to 150 μ L of

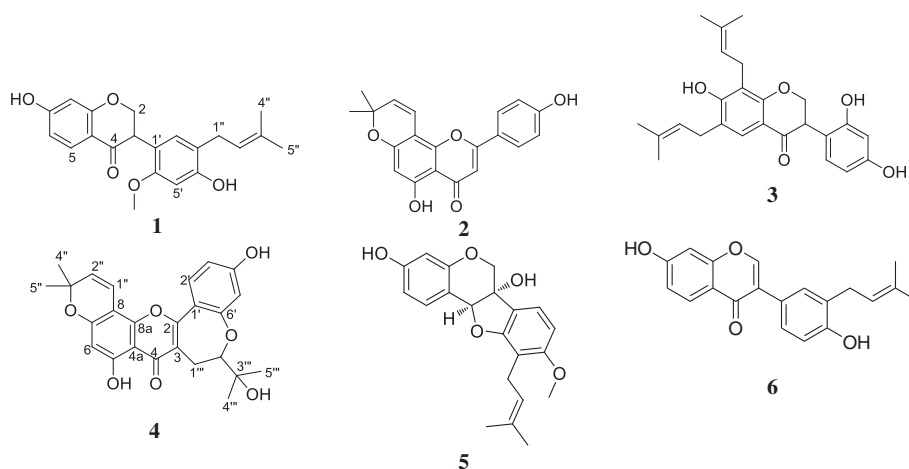


Fig. 1 Chemical structures of the compounds isolated from *Erythrina sigmoidea*. sigmoidin I (**1**); atalantoflavone (**2**); bidwillon A (**3**); neocyclomorusin (**4**); 6 α -hydroxyphaseollidin (**5**); neobavaisoflavone (**6**)

Table 1 Bacterial strains used and their features

| Strains | Features and references |
|----------------------------------|--|
| <i>Escherichia coli</i> | |
| ATCC8739 | Reference strain |
| AG100 | Wild-type <i>E. coli</i> K-12 [34] |
| AG100A | AG100 Δ acrAB::KAN ^R [11, 34, 35] |
| AG100A _{TET} | Δ acrAB mutant AG100, with over-expressing <i>acrF</i> gene; TET ^R [34] |
| AG102 | Δ acrAB mutant AG100, owing <i>acrF</i> gene markedly over-expressed; TET ^R [12, 36] |
| MC4100 | Wild type <i>E. coli</i> [37] |
| W3110 | Wild type <i>E. coli</i> [37, 38] |
| <i>Enterobacter aerogenes</i> | |
| ATCC13048 | Reference strains |
| CM64 | CHL ^R resistant variant obtained from ATCC13048 over-expressing the AcrAB pump [39] |
| EA27 | Clinical MDR isolate exhibiting energy-dependent norfloxacin and chloramphenicol efflux with KAN ^R AMP ^R NAL ^R STR ^R TET ^R [40, 41] |
| EA289 | KAN sensitive derivative of EA27 [42] |
| EA294 | EA289 <i>acrA</i> ::KAN ^R [42] |
| EA298 | EA 289 <i>tolC</i> ::KAN ^R [42] |
| <i>Enterobacter cloacae</i> | |
| ECC169 | Clinical MDR isolates, CHL ^R [5] |
| BM47 | Clinical MDR isolates, CHL ^R [5] |
| BM67 | Clinical MDR isolates, CHL ^R [5] |
| <i>Klebsiella pneumoniae</i> | |
| ATCC12296 | Reference strains |
| KP55 | Clinical MDR isolate, TET ^R , AMP ^R , ATM ^R , CEF ^R [43] |
| KP63 | Clinical MDR isolate, TET ^R , CHL ^R , AMP ^R , ATM ^R [43] |
| K24 | AcrAB-TolC, Laboratory collection of UNR-MD1, University of Marseille, France [5] |
| K2 | AcrAB-TolC, Laboratory collection of UNR-MD1, University of Marseille, France [5] |
| <i>Providencia stuartii</i> [30] | |
| NEA16 | Clinical MDR isolate, AcrAB-TolC |
| ATCC29916 | Clinical MDR isolate, AcrAB-TolC |
| PS2636 | Clinical MDR isolate, AcrAB-TolC |
| PS299645 | Clinical MDR isolate, AcrAB-TolC |
| <i>Pseudomonas aeruginosa</i> | |
| PA 01 | Reference strains |
| PA 124 | MDR clinical isolate [44] |

^aAMP, ATM^R, CEF^R, CFT^R, CHL^R, FEP^R, KAN^R, MOX^R, STR^R, TET^R. Resistance to ampicillin, aztreonam, cephalothin, cefadroxil, chloramphenicol, cefepime, kanamycin, moxalactam, streptomycin, and tetracycline; MDR : Multidrug resistant

adequate broth. These preparations were incubated at 37 °C for 48 h. The MBC was regarded as the lowest concentration of extract, which did not produce a color change after addition of INT as mentioned above [19, 20].

Results and discussion

Compounds tested in this study included five isoflavonoids: atalantoflavone (2), bidwillon A (3), neocyclomorusin (4), 6 α -hydroxyphaseollidin (5), neobavaisoflavone

(6) and one flavonoid: sigmoidin I (1) (Fig. 1). Their isolation and identification from the bark of *Erythrina sigmoidea* were previously reported [15]. These compounds as well as the crude extracts from the leaves and bark of *Erythrina sigmoidea* were tested for their antibacterial activities on a panel bacterial strains and the results are reported in Tables 2 and 3.

Results of the MIC determinations indicate that crude extracts from leaves and bark of this plants were able to

Table 2 MICs and MBC ($\mu\text{g/mL}$) of the crude extracts from *Erythrina sigmoidea* and chloramphenicol on the panel of tested bacteria

| Bacterial strains | Tested plant samples, MIC and MBC ($\mu\text{g/ml}$) and ratio MBC/MIC | | | | | | | | |
|-------------------------------|--|-------|---------|---|-------|---------|-----------------|------|---------|
| | <i>Erythrina sigmoidea</i> leaves extract | | | <i>Erythrina sigmoidea</i> bark extract | | | Chloramphenicol | | |
| | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC |
| <i>Escherichia coli</i> | | | | | | | | | |
| ATCC8739 | 64 | 64 | 1 | 16 | 64 | 4 | 4 | 64 | 16 |
| AG100 | 32 | 256 | 8 | 32 | 128 | 4 | 8 | >512 | na |
| AG100A | 512 | 1024 | 2 | 256 | 1024 | 4 | 4 | >512 | na |
| AG100A _{TET} | 1024 | 1024 | 1 | 256 | 512 | 2 | 32 | >512 | na |
| AG102 | 512 | 1024 | 2 | 128 | 1024 | 8 | 8 | >512 | na |
| MC4100 | 1024 | 1024 | 1 | 512 | 512 | 1 | 32 | >512 | na |
| W3110 | 512 | 512 | 1 | 512 | 512 | 1 | 8 | >512 | na |
| <i>Enterobacter aerogenes</i> | | | | | | | | | |
| ATCC13048 | 128 | 256 | 2 | 128 | 1024 | 8 | 16 | 128 | 8 |
| CM64 | 1024 | >1024 | na | 1024 | | na | 512 | >512 | na |
| EA27 | 256 | 256 | 1 | 64 | 128 | 2 | 128 | >512 | na |
| EA289 | 1024 | >1024 | na | 512 | >1024 | na | 512 | >512 | na |
| EA298 | 512 | 512 | 1 | 512 | 1024 | 2 | 256 | >512 | na |
| EA294 | 64 | 512 | 8 | 16 | 128 | 8 | 4 | 32 | 8 |
| <i>Enterobacter cloacae</i> | | | | | | | | | |
| ECCI69 | 1024 | >1024 | na | 1024 | >1024 | na | 256 | >512 | na |
| BM47 | 1024 | 1024 | 1 | 1024 | 1024 | 1 | 512 | >512 | na |
| BM67 | 1024 | >1024 | na | 1024 | 1024 | 1 | 256 | >512 | na |
| <i>Klebsiella pneumoniae</i> | | | | | | | | | |
| ATCC11296 | 256 | 256 | 1 | 64 | 512 | 8 | 16 | 128 | 8 |
| KP55 | 512 | >1024 | na | 256 | >1024 | na | 64 | 256 | 4 |
| KP63 | 128 | >1024 | na | 16 | 128 | 8 | 128 | >512 | na |
| K24 | 256 | 512 | 2 | 128 | >1024 | na | 16 | >512 | na |
| K2 | 128 | 1024 | 8 | 64 | 512 | 8 | 16 | 256 | |
| <i>Providencia stuartii</i> | | | | | | | | | |
| ATCC29916 | 128 | >1024 | na | 32 | 128 | 4 | 8 | 128 | 16 |
| NAE16 | 128 | 128 | 1 | 32 | >1024 | na | 8 | 256 | 32 |
| PS2636 | 1024 | >1024 | na | 1024 | 1024 | 1 | 64 | >512 | na |
| PS299645 | 512 | 1024 | 2 | 64 | 128 | 2 | 32 | >512 | na |
| <i>Pseudomonas aeruginosa</i> | | | | | | | | | |
| PA01 | 1024 | 1024 | 1 | 256 | 256 | 1 | 16 | 256 | 8 |
| PA124 | >1024 | >1024 | na | >1024 | >1024 | na | 64 | 256 | 4 |

na: not applicable

inhibit the growth of 26 of the 27 (96.3 %) tested Gram-negative bacteria, and the obtained MIC values ranged from 16 to 1024 $\mu\text{g/mL}$ (Table 2). Compound **1** was not active whilst 2–6 displayed selective activities (Table 3), the MIC values below or equal to 512 $\mu\text{g/mL}$ being noted on 1/12 (8.3 %), 5/12 (41.7 %), 7/12 (58.3 %), 7/12 (58.3 %) and 8/12 (66.7 %) tested bacteria respectively for **2**, **3**, **5**, **6** and **4**. The lowest MIC value of 16 $\mu\text{g/mL}$ for crude extracts was obtained with the bark extract

against *Escherichia coli* ATCC8739, *Enterobacter aerogenes* EA294 and *Klebsiella pneumoniae* KP63. The corresponding value for the tested compounds (8 $\mu\text{g/mL}$) was obtained with **6** against *E. coli* ATCC8739, *Enterobacter cloacae* ECCI69, *K. pneumoniae* KP55, *Providencia stuartii* NAE16 and *Pseudomonas aeruginosa* PA01. The antimicrobial activity of a phytochemical (crude extract) has been defined as significant when MIC is below 100 $\mu\text{g/mL}$, moderate when 100 $\mu\text{g/mL}$ < MIC < 625 $\mu\text{g/mL}$ or low

Table 3 MICs and MBC of compounds from the bark of *Erythrina sigmoidea* on selected bacteria

| Bacterial strains | Tested compounds, MIC and MBC ($\mu\text{g/ml}$) and ratio MBC/MIC | | | | | | | | | | | | | | | | | | |
|-------------------------------|--|-----|---------|-----|-----|---------|-----|-----|---------|-----|-----|---------|-----|-----|---------|-----|-----|---------|--|
| | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | | 6 | | | |
| | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC | |
| <i>Escherichia coli</i> | | | | | | | | | | | | | | | | | | | |
| ATCC8739 | - | - | na | - | - | na | 512 | - | na | 256 | - | na | 512 | - | na | 8 | - | na | |
| AG100A _{TET} | - | - | na | 128 | - | na | - | - | na | 256 | - | na | 512 | - | na | 32 | - | na | |
| AG102 | - | - | na | - | - | na | 512 | - | na | 128 | 512 | 4 | 512 | 512 | 1 | - | - | na | |
| <i>Enterobacter aerogenes</i> | | | | | | | | | | | | | | | | | | | |
| ATCC13048 | - | - | na | - | - | na | - | - | na | 512 | - | na | - | - | na | - | - | na | |
| EA289 | - | - | na | - | - | na | - | - | na | - | - | na | - | - | na | - | - | na | |
| <i>Enterobacter cloacae</i> | | | | | | | | | | | | | | | | | | | |
| ECCL69 | - | - | na | - | - | na | - | - | na | - | - | na | - | - | na | 8 | 512 | 64 | |
| <i>Klebsiella pneumoniae</i> | | | | | | | | | | | | | | | | | | | |
| ATCC11296 | - | - | na | - | - | na | - | - | na | - | - | na | - | - | na | - | - | na | |
| KP55 | - | - | na | - | - | na | 256 | - | na | 256 | 512 | 2 | 512 | - | na | 8 | - | na | |
| <i>Providencia stuartii</i> | | | | | | | | | | | | | | | | | | | |
| ATCC29916 | - | - | na | - | - | na | 256 | - | na | 256 | - | na | 512 | - | na | - | - | na | |
| NAE16 | - | - | na | - | - | na | - | - | na | 256 | - | na | 512 | - | na | 8 | - | na | |
| <i>Pseudomonas aeruginosa</i> | | | | | | | | | | | | | | | | | | | |
| PA01 | - | - | na | - | - | na | 256 | - | na | 256 | - | na | 512 | - | na | 8 | - | na | |
| PA124 | - | - | na | - | - | na | - | - | na | - | - | na | - | - | na | 256 | - | na | |

sigmodin I (**1**); atalantoflavone (**2**); bidwillon A (**3**); neocyclomorusin (**4**); 6 α -hydroxyphaseollidin (**5**); neobavaisoflavone (**6**); (-): MIC or MBC >512 $\mu\text{g/mL}$; nt: not tested as MIC was >512 $\mu\text{g/mL}$

when MIC > 625 µg/mL [4, 23]. On this basis, the crude extracts from *Erythrina sigmoidea* could be considered as promising herbal drug. In fact, MIC values below 100 µg/mL were obtained with leaves and bark extracts respectively against 3/27 (11.1 %) and 10/27 (37.0 %) tested bacteria. Compound **6** can also be considered as a good antimicrobial agent, as MIC values below 10 µg/mL were obtained on 5/12 (41.7 %) tested bacteria. Interestingly, the bark extract was more active (lower MIC value) than chloramphenicol on some MDR strains such as *E. aerogenes* EA27, *K. pneumoniae* KP63, highlighting its good antimicrobial potency. Minimal bactericidal concentration (MBC) values below or equal to 1024 µg/mL were also obtained on 18/27 (66.7 %) and 20/27 (74.1 %) tested bacterial strains respectively for leaves and bark extracts. Data from Tables 2 and 3 indicated that some MBC/MIC ratios were below 4, indicating that the studied extracts exerted bactericidal effects on certain Gram negative bacteria [24–26]. However, a keen look of the MICs and MBCs of compounds indicated that they rather exerted bacteriostatic effects (MBC/MIC > 4) [24–26]. It should be noted that the antibacterial spectra of compounds were lower than that of the bark extract. This suggested that a possible synergistic effect between the constituents of this extract could be expected. It should also be noted that the bark extract was not active on the resistant *P. aeruginosa* PA124 strains contrary to the isolated compound **6**. This can either be due to the fact that this active compound (**6**) is less concentrated in the initial crude extract or to the possible interactions with other constituent. Regarding the clinical involvement of MDR bacteria in treatment failures [11, 12, 27, 28], the antibacterial activity of the crude extracts as well as that of compound **6** could be considered promising. *Pseudomonas aeruginosa* is an important nosocomial pathogen, highly resistant to clinically used antibiotics, leading to substantial morbidity and mortality [29]. MDR Enterobacteriaceae, including *K. pneumoniae*, *E. aerogenes*, *E. cloacae* and *P. stuartii* and *E. coli* have also been classified as antimicrobial-resistant organisms of concern in healthcare facilities [11, 12, 30].

To the best of our knowledge, the antibacterial activity of the crude extracts from the *Erythrina sigmoidea* as well as compounds **2–6** against MDR bacteria is being reported for the first time. However, the antibacterial activities of compounds belonging to the classes flavonoids and isoflavonoids are well known [31]. In addition, a preliminary antibacterial study of flavonoids from the stem bark of *Erythrina burttii* showed that bidwillon A was active against *E. coli* and *Staphylococcus aureus* [32]. Neobavaisoflavone also displayed antifungal activity against *Aspergillus fumigatus* and *Cryptococcus neoformans* [33]. The present study provides additional information on the antimicrobial potency of neobavaisoflavone (**6**).

Conclusions

The results of the present study are interesting, taking in account the medical importance of the studied microorganisms. These data provided evidence that the crude extracts from *Erythrina sigmoidea* as well as some of its constituents, and mostly neobavaisoflavone (**6**) could be potential antimicrobial drugs to fight MDR bacterial infections.

Competing interests

The authors declare that there are no competing interest.

Authors' contributions

DEJ, JAKN, LPS and LKO carried out the study; VK designed the experiments, wrote the manuscript, and provided the bacterial strains; BTN and VK supervised the work; all authors read and approved the final manuscript.

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