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Antimicrobial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri* (Moraceae)

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Abstract

The aim of this study was to evaluate the antimicrobial activity of the crude extract of the twigs of *Dorstenia barteri* (DBT) as well as that of four of the five flavonoids isolated from this extract. Gram-positive bacteria (six species), Gram-negative bacteria (12 species) and fungi (four species) were used. The agar disc diffusion test was used to determine the sensitivity of the tested samples while the well micro-dilution was used to determine the minimal inhibition concentrations (MIC) and the minimal microbicidal concentration (MMC) of the active samples. The results of the disc diffusion assay showed that DBT, isobavachalcone (**1**), and kanzonol C (**4**) prevented the growth of all the 22 tested microbial species. Other compounds showed selective activity. The inhibitory activity of the most active compounds namely compounds **1** and **4** was noted on 86.4% of the tested microorganisms and that of 4-hydroxylonchocarpin (**3**) was observed on 72.7%. This lowest MIC value of 19.06 μg/ml was observed with the crude extract on seven microorganisms namely *Citrobacter freundii, Enterobacter aerogens, Proteus mirabilis, Proteus vulgaris, Bacillus megaterium, Bacillus stearothermophilus and Candida albicans*. For the tested compounds, the lowest MIC value of 0.3 μg/ml (on six of the 22 organisms tested) was obtained only with compound **1**, which appeared as the most active compound. This lowest MIC value (0.3 μg/ml) is about 4-fold lower than that of the RA, indicating the powerful and very interesting antimicrobial potential of isobavachalcone (**1**). The antimicrobial activities of DBT, as well as that of compounds **1**, **3**, **4**, amentoflavone (**5**) are being reported for the first time. The overall results provide promising baseline information for the potential use of the crude extracts from DBT as well as some of the isolated compounds in the treatment of bacterial and fungal infections.

Keywords: Antimicrobial activity; *Dorstenia barteri*; Bureau; flavonoids; Moraceae

Abbreviations: CLED agar, cystine-lactose-electrolyte deficient agar; CFU, colony forming unit; CH_2Cl_2, dichloromethane; $CHCl_3$, chloroform; Compound **1**, isobavachalcone; Compound **2**, stipulin; Compound **3**, 4-hydroxylonchocarpin; Compound **4**, kanzonol C; Compound **5**, amentoflavone; DBT, *Dorstenia barteri*; DMSO, dimethylsulfoxide; EtOAc, ethyl acetate; IZ, inhibition zone; LMP, laboratory of applied microbiology and molecular pharmacology; MeOH, methanol; MHA, Mueller Hinton agar; MIC, minimal inhibition concentration; MMCm, inimal microbicidal concentration; MW, molecular weight; NA, nutrient agar; NBGP, nutrient broth containing 0.05% phenol red and supplemented with 10%; NMR, nuclear magnetic resonance; RA, reference antibiotics; SDA, sabouraud dextrose agar; PSM, pseudomonas selective medium; SS agar, *Salmonella-Shigella* agar; TLC, thin layer chromatography.

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1. Introduction

The plants of the genus *Dorstenia* are traditionally used in African and South American folk medicine in the treatment of many illnesses such as snakebite, rheumatic, infectious diseases, arthritis (Bouquet, 1969; Adjanohoun et al., 1996; Abegaz et al., 2000; Kuete et al., 2007a). In Cameroon, the leaves and twigs of *Dorstenia barteri* Bureau are used as decoction in the treatment of mumps, yaws and infected wounds (Thomas et al., 1989; Abegaz et al., 1998; Tsopmo et al., 1999). This genus is a rich source of flavonoids such as prenylated and geranylated chalcones, and coumarins (Abegaz et al., 2004; Ngameni et al., 2007). The antimicrobial potency of the above secondary metabolite classes has been demonstrated (Bruneton, 1999; Cowan, 1999). In our research group, many compounds with significant pharmacological effects have been isolated from the genus *Dorstenia.* Many Chalcones isolated from *Dorstenia barteri* namely isobavachalcone, paratocarpin C, stipulin, and dorsmannin A inhibited the proliferation of the brain tumour derived U87 glioblastoma cells (Ngameni et al., 2007). Stipulin and other flavonoids isolated from the twigs of *Dorstenia angusticornis* (gancaonin Q, angusticornin B, and bartericin A) were also found to be very active on bacteria and yeasts associated to human pathologies (Kuete et al., 2007a). In our continuous herbal drug research program from medicinal plants from the genus *Dorstenia*, we undertook to evaluate the antimicrobial potency of the crude extracts from the twigs of *Dorstenia barteri* Bureau *var. multiradiata* as well as that of the compounds isolated from this extract against the wide range of microorganisms implicated in infectious diseases.

2. Methodology

2.1. Plant material

The twigs of *Dorstenia barteri* Bureau *var. multiradiata* were collected in March 2003 in Kumba, South West province of Cameroon. The botanical identification of the plants was done by the National Herbarium in Yaounde, where the voucher specimen is conserved under the reference number 44016/HNC.

2.2. Isolation and general procedures

The air-dried and powdered twigs of *Dorstenia barteri* Bureau *var. multiradiata* (1 kg) were macerated in either a mixture of CH_2Cl_2/MeOH (1:1) or in MeOH for 24 and 2 h, respectively, at room temperature. These two extracts were then combined. Removal of the solvent from the combined extracts under reduced pressure yielded 45 g of a dark green residue that constituted the crude extract. A mass of 40 g of this organic extract was submitted to flash liquid chromatography on silica gel 60 (220 g) and eluted with hexane-ethyl acetate gradients: (3:1), (1:1), (1:3), and finally with pure EtOAc to give 40 fractions of 250 ml each. These fractions were monitored by TLC and the fractions showing similar ^{1}H NMR spectra were combined. Fractions 1–20 (10 g), obtained with (3:1, v/v) hexane–EtOAc were subjected to column chromatography over silica gel using hexane–EtOAc as eluent with a continuous gradient (95:5 to 7:3, v/v), followed by gel filtration chromatography over Sephadex LH-20 using $CHCl_3$–MeOH (2:1) as eluent. The post-chlorophyll fractions were combined and purified successively on column chromatography followed by preparative TLC to yield: isobavachalcone (**1**, 98 mg, MW: 324, mp: 171–172) (Abegaz et al., 1998) and stipulin (**2**, 20 mg, MW: 392, mp: 122–123) (Abegaz et al., 1998). Combined fractions 21–40 (25 g) obtained from the (1:1) to (1:3) hexane–EtOAc mixtures and pure EtOAc were subjected successively to silica gel column chromatography and preparative TLC, eluting with solvent mixtures of increasing polarity (from CH_2Cl_2 to 96:4 (v/v) CH_2Cl_2–MeOH). The fractions eluted with CH_2Cl_2 gave, after repeated preparative TLC, 4-hydroxylonchocarpin (**3**, 45 mg, MW: 322, mp: 207–208) (Ngadjui et al., 2000). Those eluted with CH_2Cl_2–MeOH (96:4) gave kanzonol C (**4**, 125 mg, MW: 392, mp: 190–194) (Fukai et al., 1994), and amentoflavone (**5**, 40 mg, MW: 554, mp: 247–248) (Goh et al., 1992; Shih et al., 2004), after repeated preparative TLC.

Aluminium sheet pre-coated with silica gel 60 GF_{254} Merck was used for thin layer chromatography and the isolated spots were visualized using both ultra-violet light (254 and 366 nm) and by spraying with ammonium molybdate solution and heating. The chemical structures of each of the isolated compounds was determined on the basis of spectral data produced by one and two-dimensional nuclear magnetic resonance (NMR), recorded on Brüker AMX-500 or on Varian Gemini-300 spectrometers. ESIMS were recorded on a Micromass Quattro LC mass spectrometer. The chemical structures of the compounds isolated from *Dorstenia barteri* Bureau *var. multiradiata* are given in Fig. 1.

2.3. Microbial strains

Twenty-two of microorganisms namely *Bacillus cereus*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis* (Gram-positive bacteria), *Escherichia coli*, *Shigella dysenteriae*, *Proteus vulgaris*, *Proteus mirabilis*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Morganella morganii*, *Enterobacter aerogens*, *Citrobacter freundii*, *Enterobacter cloacae* (Gram-negative bacteria), *Candida albicans*, *Candida gabrata*, *Microsporum audorium*, *Trichophyton rubrum* (fungi) were used in this study. 'Institut Appert de Paris' provided three Bacillus species, while the A.F.R.C Reading Laboratory of Great Britain provided Bacillus cereus. Other strains were clinical isolates from 'Centre Pasteur of Cameroon', Yaoundé. The microbial isolates were maintained on agar slant at 4 °C in the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP) (Faculty of Science, University of Yaoundé I) where the antimicrobial tests were performed. The strains were sub-cultured on a fresh appropriate agar Plate 24 h prior to any antimicrobial test.