SUPPLEMENTARY MATERIAL

Enhancement of the antibiotic activity of aminoglycosides by extracts from *Anadenanthera colubrina* var. *cebil* against multi-drug resistant bacteria

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Abstract

The aim of this work was evaluate the antimicrobial activity of ethanol (EEAC) and hexane (HFAC) extracts from the stem bark of *Anadenanthera colubrina* var. *cebil* alone or in combination with aminoglycosides against multi-drug resistant bacteria. Minimal inhibitory concentrations (MICs) of the extracts were determined by microdilution assay. For evaluation of extracts as modulators of antibiotic resistance, MICs of neomycin and amikacin were determined in presence or absence of each compound at sub-inhibitory concentrations. All assays were performed in triplicate and the results were analyzed by the geometric mean with $P < 0.001$ using the Program GraphPad Prisma. Both EEAC and HFAC did not show antimicrobial activity against multi-drug resistant strains tested. However, the addition of EEAC and HFAC enhanced the activity of neomycin and amikacin against *S. aureus* SA10 strain. When the natural products were replaced by chlorpromazine, the same effect was observed. *Anadenanthera colubrina* var. *cebil* may be a source of phytochemicals able to potentiate the aminoglycoside activity against multi-drug resistant *S. aureus* by the inhibition of efflux pump.

Keywords: *Anadenanthera colubrina* var. *cebil*; *Staphylococcus aureus*; *Escherichia coli*; antimicrobial activity; modifying-resistance activity; efflux pump; chlorpromazine

1. Experimental

1.1. Strains and drugs

All tests were performed with the multi-drug resistant bacterial strains of *Staphylococcus aureus* SA10 (resistant to cephalothin, cephalexin, cefadroxil, penicillin G, ampicillin, amoxicillin, oxacillin, erythromycin, clarithromycin, azithromycin, clindamycin, moxifloxacin, ciprofloxacin, levofloxacin, nalidixic acid, tetracycline, neomycin, gentamicin and amikacin) and *Escherichia coli* EC13 (resistant to lincomycin, erythromycin, ciprofloxacin, norfloxacin, nalidixic acid, tetracycline, neomycin, gentamicin, amikacin and tobramycin). Standard strains (*S.aureus* ATCC25923 and *E. coli* ATCC 25922) were used as positive controls. All strains were maintained on Nutrient Agar (Himedia, India) slant at 4°C and prior to assay the cells were grown overnight at 37°C in Brain Heart Infusion (BHI, Himedia, India). All strains were gently supported by Dr. Henrique D. M. Coutinho, Head of the Laboratory of Microbiology and Molecular Biology of the Regional University of Cariri

– URCA. Neomycin, amikacin and chlorpromazine were obtained from Sigma Chemical Corp., St. Louis, MO, USA. Antibiotics and chlorpromazine were dissolved in sterile water.

1.2. Plant material

Stem bark of *Anadenanthera colubrine* var. *cebil* was collected in the City Park in the county of Teresina (latitude 5,056797 south and longitude 42,811083 west), Piauí, Brazil. The plant material was identified by Dr. Gardene Maria de Sousa and a voucher specimen was deposited with the number TEPB 27881 at the Herbarium “Graziela Barroso” of the Universidade Federal do Piauí – UFPI.

1.3. Extracts preparation

The stem bark was dried at room temperature and powdered. The powdered material was extracted by maceration using ethanol as solvent in the ratio of 1:3 (m/v) and the homogenate was allowed to stand for 72 h at room temperature. This procedure was realized in triplicate. The supernatants were then filtered, gathered and concentrated under vacuum in a rotary evaporator (model Q-344B – Quimis, Brazil) and ultrathermal bath (model Q-214M2 – Quimis, Brazil), resulting in the ethanol extract (EEAC). A part of this extract was suspended in ethanol/water (1:1, v/v) and partitioned in solvents with increasing polarity, obtaining the fraction hexane (HFAC) used in the present study. The fractions were concentrated under vacuum in a rotary evaporator and lyophilized.

1.4. Evaluation of the antibacterial activity

Stock solutions of EEAC and HFAC were prepared by dissolving 10 mg of each product in 1 mL of dimethyl sulphoxyde (VITEC, Brazil), thus starting with an initial concentration of 10 mg/mL. The resulting solution was then diluted to 1024 µg/mL in sterile water. Minimal inhibitory concentrations (MICs) of the EEAC, HFAC and chlorpromazine (CPZ) were determined by the micro-dilution assay in BHI broth 10% with bacterial suspensions of 10⁵ CFU/mL. The final concentrations of the natural products and CPZ ranged from 512 to 8 µg/mL (Javadpour et al. 1996). MICs of neomycin and amikacin were determined for the same method with antibiotic concentrations ranging from 2500.0 to 2.4 µg/mL.

1.5. Modulation of the antibiotic activity

For evaluation of EEAC and HFAC as antibiotic resistance modulators, MICs of the antibiotics were determined in the presence or absence of sub-inhibitory concentrations (1/8 MIC) of the extracts (128 µg/mL) and chlorpromazine (8 µg/mL) (Coutinho et al., 2010). Plates were incubated at 37 °C for 24 h.

1.8. Statistical analysis

Each experiment was performed six times and the results were normalized by calculation of geometric mean values. Error deviation and standard deviation of the geometric mean were revealed. Statistical analyses were performed using GraphPad Prism, version 5.02. Differences between treatment with antibiotics in the absence and in the presence of EEAC, HFAC and CPZ were examined using Two-way analysis of variance (ANOVA). The differences mentioned above were analyzed by Bonferroni post-test and they were considered statistically significant when $P < 0.05$.

Figure S1 - MIC of antibiotics in absence or presence of EEAC and chlorpromazine (CPZ) for *S. aureus* strain SA10. *** Value statistically significant ($p < 0.0001$) compared with the control (antibiotic alone).

Figure S2 - MIC of antibiotics in absence or presence of HFAC and chlorpromazine (CPZ) for *S. aureus* strain SA10. *** Value statistically significant ($p < 0.0001$) compared with the control (antibiotic alone).

References

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