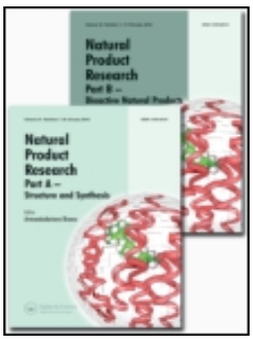

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Enhancement of the antibiotic activity of aminoglycosides by extracts from Anadenanthera colubrine (Vell.) Brenan var. cebil against multi-drug resistant bacteria

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SHORT COMMUNICATION

Enhancement of the antibiotic activity of aminoglycosides by extracts from *Anadenanthera colubrine* (Vell.) Brenan var. *cebil* against multi-drug resistant bacteria

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The aim of this work was to evaluate the antimicrobial activity of ethanol (EEAC) and hexane (HFAC) extracts from the stem bark of *Anadenanthera colubrina* (Vell.) Brenan var. *cebil* alone or in combination with aminoglycosides against multi-drug resistant (MDR) bacteria. Minimal inhibitory concentrations (MICs) of the extracts were determined by using microdilution assay. For the 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. Both EEAC and HFAC did not show antimicrobial activity against MDR strains tested. However, the addition of EEAC and HFAC enhanced the activity of neomycin and amikacin against *Staphylococcus aureus* SA10 strain. When the natural products were replaced by chlorpromazine, the same effect was observed. *Anadenanthera colubrine* var. *cebil* may be a source of phytochemicals able to potentiate the aminoglycoside activity against MDR *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

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1. Introduction

Infectious diseases caused by multi-drug resistant (MDR)bacteria remains as an important problem of public health worldwide (Kali et al. 2013). Because of this troubling scenario, several researches have been performed aiming to obtain new compounds actives against MDR strains (Kumar & Chopra 2013). Alternatively, synthetic or natural compounds have been tested for its ability to reverse the bacterial resistance to traditionally used antibiotics (Kristiansen et al. 2010).

Anadenanthera colubrina (Vell.) Brenan var. *cebil* (Fabaceae) is a tree known locally as 'angico', which is common in semi-arid regions of Brazil and other countries, including Argentina, Bolivia and Paraguay. In northeastern Brazil, the stem bark from this plant has been used for preparation of teas, infusions, decocts, dyeing and syrups for treatment of various diseases, including coughs and bronquitis (Pessoa et al. 2012).

Aminoglycosides are bactericide antibiotics which bind in the ribosomal subunit 30S, causing the synthesis of anomalous proteins, which insert on plasma membrane increasing its permeability (Mingeot-Leclercq et al. 1999). Resistance to aminoglycosides mediated by membrane proteins able to pump antibiotic molecules from cytoplasm to extracellular medium has been verified (Tintino et al. 2013). The aim of this study was to investigate antimicrobial activity of the ethanol (EEAC) and hexane (HFAC) extracts obtained from stem bark of *A. colubrina*, as well as investigate the modulatory activity on aminoglycoside resistance against MDR strains.

2. Results and discussion

The antibacterial activity of the EEAC and HFAC extracts was tested by micro-dilution method against *Staphylococcus aureus* and *Escherichia coli* strains. The MICs for these extracts tested against both Gram-positive and Gram-negative strains were ≥ 1024 μg/mL. These results show that the natural products did not have direct inhibitory activity at clinically relevant concentrations tested (Tanaka et al. 2005).

A reduction in the MIC for neomycin (from 625 to 312.5 μg/mL) was verified against the strain SA10 when EEAC or HFAC were added to the growth medium at sub-inhibitory concentration (Figures S1 and S2). For amikacin, the MIC was decreased from 442 to 98.4 μg/mL. Decrease in the MICs of aminoglycosides tested were also verified when the natural products were replaced by chlorpromazine (CPZ) (Figures S1 and S2).

Aminoglycosides are often used in association with antibiotic inhibitors of peptidoglycan synthesis in treatment of Gram-positive infections, once these agents are able to increase uptake of aminoglycosides into the bacterial cell (Bliziotis et al. 2005). Modulation of aminoglycoside resistance has already verified for other medicinal plants (Coutinho et al. 2009; Matias et al. 2011; Veras et al. 2012). However, this is the first time that natural products obtained from *Anadenanthera colubrine* var. *cebil* stem bark is described as enhancers of aminoglycoside activity against a methicillin resistant *S. aureus* strain. Bactericide effect of aminoglycosides is dependent of an effective transport through the plasma membrane, once its molecular targets are located in the bacterial cytoplasm (Gries et al. 2013).

Through phytochemical prospecting of the HFAC it was possible to determine the presence of diverse classes of secondary metabolites, as described in Pessoa et al. (2012). Pentacyclictriterpenes are amphiphilic compounds, able to form complex with proteins and phospholipids of the plasma membrane, changing its permeability or leading to its disruption. The combined use of pentacyclictriterpenoids which damage the cell membrane with antibiotics inhibitors of peptidoglycan biosynthesis (vancomycin and methicillin) have resulted in a synergistic effect against *S. aureus* (Chung et al. 2011). With regard to the modulatory effect found in this study, it could be attributed to an interaction of the lipophilic components of HFAC

with the plasma membrane, increasing the uptake of neomycin and amikacin molecules. Furthermore, this interaction could inhibit efflux systems dependent of proton-motive force, as *S. aureus* LmrS protein (Floyd et al. 2010), increasing the intracellular concentration of aminoglycosides, as verified when EEAC and HFAC were replaced by CPZ, an inhibitor of bacterial efflux systems (Schindler et al. 2013). This result suggests the presence of aminoglycoside resistance mediated by efflux pump in the SA10 strain, which may be inhibited by phytochemicals present in the natural products tested.

The extracts were not able to reduce the MICs for neomycin and amikacin against the MDR strain *E. coli* EC13. Gram-negative bacteria as *E. coli* have a lipopolysaccharide rich outer membrane, which confer a hydrophilic character to its surface, acting to a very effective barrier for spontaneous diffusion of lipophilic compounds (Nikaido 2003).

3. Conclusion

A. colubrine var. *cebil* extracts EEAC and its nonpolar fraction HFAC were able to potentiate the activity of neomycin and amikacin against MDRs strains of *S. aureus in vitro*. This plant could be a source of secondary metabolites for use in association with neomycin and amikacin in the antibiotic chemotherapy of infections caused by MDR strains of *S. aureus*.

Supplementary material

Supplementary material relating to this paper is available online at http://dx.doi.org/10.1080/14786419.2015.1049177.

Disclosure statement

No potential conflict of interest was reported by the authors.

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