ANTIMICROBIAL EVALUATION OF SESQUITERPENE α-CURCUMENE AND ITS SYNERGISM WITH IMIPENEM

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ABSTRACT

α-Curcumene was isolated from the fresh aerial parts of Senecio selloi Spreng. DC. and its activity against bacteria, yeasts and an alga was inspected by the applying the microdilution method. The strongest effect was manifested against Saccharomyces cerevisiae with estimated values of MIC and MFC 0.8 mg/mL. The α-curcumene synergism in the concentrations of 1 mM and 5 mM, respectively, with selected antibiotics (ciprofloxacin, imipenem, ceftazidim and a combination of amoxicillin and clavulanic acid) was investigated against Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Staphylococcus haemolyticus and Klebsiella pneumoniae by the disk diffusion assay. The results have shown the occurrence of synergism of α-curcumene with imipenem against the clinical isolate E. cloacae with a significance level of p > 0.05. Based on these informations it can be concluded that fungal strains are more sensitive for α-curcumene than the bacterial ones and the synergism of α-curcumene with imipenem can improve the antibiotic efficiency against the E. cloacae.

Keywords: Senecio selloi; α-curcumene; antimicrobial activity; Enterobacter cloacae; imipenem, synergism

INTRODUCTION

In accordance with World Health Organization (WHO), the antimicrobial resistance threatens, the effective prevention and treatment of an ever-increasing range of infections caused for instance by bacteria and fungi. A high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) or multidrug-resistant Gram-negative bacteria. Furthermore, the resistance to one of the most widely used antibacterial drugs for the oral treatment of urinary tract infections caused by Escherichia coli, is very widespread (WHO, 2014). Therefore greater efforts must be made toward the discovery of new antimicrobial compounds. Constituents isolated from plants may be an alternative such as species from the genus Senecio that even crude extracts are known to possess an antimicrobial activity, the antibacterial and the antifungal ones (Yang et al., 2011). The genus Senecio (Asteraceae) is constituted of about 2000 species worldwide, 85 of which are found in southern Brazil and of these, 25 are native of the state of Rio Grande do Sul (Matzenbacher, 1998). Besides pyrrolizidine alkaloids (Tundis et al., 2007), Senecio species are rich in diterpenes (Ndoum et al., 2010), sesquiterpenes (Xie et al., 2010), triterpenes (Rücker et al., 1999) and flavonoids (Niu et al., 2013). Some of these are components of the essential oils, which are goals of synergic studies with antibiotics, in a view to evaluating and enhancing antimicrobial efficacy (Rosato et al., 2007). The ability of sesquiterpenes to increase the bacterial susceptibility to a number of clinically important antibiotics was investigated with nerolidol, bisabolol, and apitrene as well enhancing the activities of all six antibiotics tested against S. aureus. Nerolidol and farnesol also sensitized E. coli to polymyxin B (Brehm-Stecher and Johnson, 2003). In this context, the present report describes the isolation, the identification, an antimicrobial evaluation and the synergism with antibiotics of α-curcumene (Figure 1), a sesquiterpene obtained from the aerial parts of Senecio selloi Spreng DC.

Figure 1 Chemical structure of sesquiterpene α-curcumene.

MATERIAL AND METHODS

Plant material

Plant material was identified by Prof. Dr. Nelson Ivo Matzenbacher (Graduate Botany Program, UFRGS). The aerial parts were collected in Guaíba, RS, Brazil. Voucher specimen Nº SMDB 10206 is preserved in the Herbarium of the Department of Botany, UFSM, RS, Brazil.

Extraction, isolation and identification

Fresh aerial parts of Senecio selloi (550 g) were divided and extracted with CH2Cl2 by the maceration for 8 days (d), twice. The macerate was concentrated in vacuo, yielding 15 g of crude extract. The amount 5 g of them was chromatographed on a silica gel column (335 g) using CH2Cl2, yielding 41 fractions. The fractions 5-8 (400 mg) were rechromatographed on a silica gel column with 10% AgNO3 using hexane, obtaining 50 fractions. The fractions 11-13 (17 mg of α-curcumene) were analyzed by NMR and MS spectra. The extraction, isolation and identification were done based on previous research of Silva et al. 2010. General experimental: Optical rotation in CHCl3: Perkin-Elmer 241; EI-MS at 70 eV: Kratos MS 50; NMR Spectra in CDCl3: Varian XL 300 MHz.
α-Curcumene: R, 0.35 (Petrol), [α]D + 59.95° (c = 8.18). EI-MS m/z (%): 202 (30), 187 (21), 159 (4.5), 145 (29), 132 (93), 119 (100), 105 (50.9), 95 (6.6), 91 (29.4), 77 (11.3), 69 (16), 65 (7.2), 54 (26.4), 41 (51.1). 1H NMR (300 MHz, CDCl3), δ ppm: 7.14 (4 H, m, H-2, H-3, H-5, H-6), 5.15 (1 H, dsept, 1 J, 5.7 and 7 Hz, H-10), 2.72 (1 H, t, J 7 and 7 Hz, H-7), 2.38 (3 H, s, Me-15), 1.94 (2 H, m, H-2, H-9), 1.74 (3 H, d, J 1.5 Hz, Me-13), 1.66 (2 H, m, H-2, H-8), 1.58 (3 H, d, J 1.5 Hz, Me-12), 1.28 (3 H, d, J 7 Hz, Me-14). 13C NMR (75 MHz, CDCl3), δ ppm: 144.5 (C4), 135.0 (C1), 131.2 (C11), 128.9 (C2), 128.9 (C6), 126.8 (C3), 126.8 (C5), 124.5 (C10), 39.1 (C7), 38.5 (C8), 26.3 (C9), 25.8 (C13), 22.6 (C14), 21.1 (C15), 17.8 (C12).

The in vitro antimicrobial assay

The antimicrobial activity of α-curcumene was determined by the microdilution method, based on documents M27-A2 for yeasts (Candida albicans ATCC 44773 and Pseudoomonas aeruginosa ATCC 27852) and antibiotic susceptibility (ciprofloxacin, ceftazidime, Imipenem, a β-lactam, was the first carbapenem antibiotic selected for development. Other antibiotics did not act by forming an enzyme adduct formed between C. curcumene and E. clavulanic acid and, finally, for a general role for the use of α-curcumene as the enhancers of non-specific bacterial permeability to impenem. The synergism of antibiotic impenem and α-curcumene against E. cloacae is presented in Table 2. Other applied antibiotics did not act by forming an enzyme adduct formed between C. curcumene and E. clavulanic acid and, finally, for a general role for the use of α-curcumene as the enhancers of non-specific bacterial permeability to impenem.

Table 1 MIC values of the α-curcumene against bacteria, yeasts and an algae.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (mg/mL)</th>
<th>MBC (mg/mL)</th>
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</thead>
<tbody>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>&gt;6.4</td>
<td>ND</td>
</tr>
<tr>
<td>Alga</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. zopfii</td>
<td>&gt;6.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

Legend: ND=Not Determined.
Table 2 Evaluation of the synergism of α-coumene with antibiotics by the disk diffusion against E. cloacae (a clinical isolate).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
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<tr>
<td></td>
<td>Control 1 mM</td>
</tr>
<tr>
<td>Imapenem</td>
<td>24</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>R</td>
</tr>
<tr>
<td>Combination of amoroxilin and clavulanic acid</td>
<td>R</td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>R</td>
</tr>
</tbody>
</table>

Legend: R-Resistant. Different letters superscripts are significantly different (p < 0.05).

CONCLUSION

In this study, a sesquiterpene, α-coumene, was obtained from aerial parts of Senecio selloi. Following the antimicrobial evaluation, the best efficiency was found against yeasts, S. cerevisiae and C. albicans. The activity of α-coumene against in vitro tested bacteria was observed only in the highest concentration. It can be concluded that fungal strains are more sensitive for α-coumene than the bacterial ones. Since relatively high MIC values for bacteria species, the investigation of the α-coumene synergism with antibiotics was inspected. Among the four antibiotics (or their combination) and five bacteria tested; only the synergism of α-coumene with imipenem occurred against the clinical isolate of E. cloacae. Current study can contribute to information for the development of potent antimicrobial compounds derived from natural sources with antibiotic synergism.

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REFERENCES


