Antimicrobial Activity
of Senecio desiderabilis Vellozo (Asteraceae)

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SUMMARY. CH2Cl2 and EtOH extracts from S. desiderabilis Vellozo were tested concerning their antimicrobial activity by broth microdilution method. The strongest effect was manifested by the CH2Cl2 extract against Cryptococcus neoformans var. neoformans and C. neoformans var. gatti (MIC 50 µg/mL), Saccharomyces cerevisiae (25 mg/mL) and Microsporum canis (25 mg/mL). Escherichia coli and multiresistant Pseudomonas aeruginosa were not inhibited by any extract.

RESUMEN. “Actividad Antimicrobiana de Senecio Desiderabilis Vellozo (Asteraceae)”. Los extractos diclorometánico y etánolico de S. desiderabilis Vellozo fueron analizados en relación a su actividad antimicrobiana por el método de microdilución en caldo. El efecto más fuerte ocurrió con el extracto diclorometánico frente a Cryptococcus neoformans var. neoformans y C. neoformans var. gatti (MIC 50 µg/mL), Saccharomyces cerevisiae (25 µg/mL) y Microsporum canis (25 µg/mL). Pseudomonas aeruginosa multirresistente y Escherichia coli no fueron inhibidos por ninguno de los extractos.

INTRODUCTION
Resistance to antimicrobial agents has become an increasingly important and pressing global problem 1, and is the main reason for an extended search for new drugs to treat microbial infections. The Senecio genus has around 2000 species 2, which are rich in secondary metabolites like terpenes and flavonoids 3,4. These constituents have been largely investigated and have frequently exhibited antimicrobial activities. Also significant is the fact that some Senecio species have antimicrobial activities described 5-7. This work reports the in vitro antimicrobial evaluation of the dichloromethanic and ethanolic extracts from S. desiderabilis Vellozo (Pentacalia desiderabilis (Vell.) Cuatrec.), a shrub which grows in South of Brazil.

MATERIALS AND METHODS
Plant Material
Senecio desiderabilis Vellozo was identified and collected by Marcos Sobral (Faculdade de Farmácia da UFRGS/RS), in São José dos Ausentes, RS, Brazil, in February 2002. Voucher specimen No SMDB 8936 is preserved in the Herbarium of the Departamento de Botânica, UFSM, RS, Brazil.

Extraction
Fresh aerial parts (645.4 g) were divided and extracted successively with CH2Cl2 and EtOH by maceration, two times, 8 days for each solvent. Both macerates were concentrated, obtaining 42.7 g and 48.3 g, respectively.

Microorganisms
The microorganisms were reference strains and clinical isolates and are listed in Table 1.

Antimicrobial assay
The antimicrobial activity of both extracts were determined by microdilution method, based on documents M27-A2 for yeasts, M-38A for filamentous fungi, and M7-A4 for bacteria, all

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PALABRAS CLAVE: Actividad antimicrobiana, Asteraceae, Pentacalia desiderabilis, Senecio desiderabilis.

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preconized by CLSI (formerly NCCLS). The alga Prototheca zopfii was also assayed by M27-A2 methodology.

Previous to test, the bacterial cultures were activated by subculture on Muller-Hinton agar during 24 h at 35 °C; the yeasts and Prototheca isolates were subcutured on Sabouraud dextrose agar during 48 h at 30 °C and filamentous fungi were subcutured on potato dextrose agar during 8 days at 32 °C.

After subculture, the bacterial inoculum was prepared so that the turbidity of the suspension was similar to 0.5 Mc Farland standard (1 x 10^8 cfu/ml). Immediately this suspension was diluted 1:10 (1 x 10^7 cfu/ml) in sterile saline and volumes of 10 µL were deposited in the wells containing 200 µL of the medium plus different concentrations of the extracts, resulting in a final inoculum concentration varying from 2 x 10^5 to 5 x 10^5 cfu/mL. In a similar way, the initial inoculum suspension of yeasts was obtained and afterwards, in order to be adjusted according to the turbidity of Mc Farland # 0.5 standard, the suspensions were successively diluted at 1:50 and 1:20 on the RPMI buffered broth 0.9.

After incubation during 8 days at 32 °C, the inocula of filamentous fungi were standardized as follows. 1 mL of sterile saline with one drop of Tween 20 was added to the cultures, covering the colonies. The resulting fungal suspension of conidia and hyphal fragments were transferred to a sterile tube. After leaving heavy particles to settle for 3 to 5 min, the upper homogenous suspension was collected and mixed with a vortex for 15 s. The turbidity of conidia suspension was adjusted to an optical density with a vortex for 15 s. The turbidity of Mc Farland # 0.5 standard, the suspensions were successively diluted at 1:50 and 1:20 on the RPMI buffered broth 0.9.

For the assay of antibacterial activity, 200 µL of the final concentration of each extract was deposited in the wells and then 10 µL of the standardized inoculum was added.

The assays were incubated at 35 °C for 48 h; 72 h for Cryptococcus neoformans strains and Prototheca zopfii; and at 32 °C during 7 days for filamentous fungi. The assays with bacteria were incubated at 37 °C for 24 h.

In all cases the results were visually compared with a positive growing control in RPMI 1640 broth or Muller-Hinton broth containing DMSO:Tween 80 1:4 diluted in the medium at the same proportion that could be found on the highest concentrations (5000, 2500, 1000 µg/mL) for crude extracts.

Both extracts were solubilized in DMSO: TWEEN 80 1:4 in order to obtain a stock solution of 25 mg/mL. The intermediate concentrations were prepared diluting the stock solution in the appropriated medium to obtain the final concentrations of 5000, 2500, 1000, 500, 250, 50 and 25 µg/mL. Volumes of 100 µL from intermediate concentrations of each extract were deposited in each well with 100 µL of inoculum. For the study of the antibacterial activity, volumes of 200 µL from final concentrations of each extract were deposited in the wells and then 10 µL of standardized inoculum was added. The MICs were determined as the lowest concentration which has completely inhibited the growth of microorganisms.

RESULTS
The antimicrobial activities of the CH2Cl2 and EtOH extracts of S. desiderabilis are summarized in the Table 1.

DISCUSSION
In order to test the extracts, we have chosen 22 species of microorganisms which represent great variability in susceptibility to antimicrobial agents. Thus, we have included 16 fungi species with different susceptibility profiles to antifungal agents: species usually sensitive such as Saccharomyces cerevisiae and Sporothrix schenckii; species that require long treatments like Trichophyton rubrum, T. tonsurans, Microsporum canis, M. gypseum and M. nanum; species already related with resistance to polyenes as Trichosporon spp.; Candida lusitaniae, C. parapsilosis and Cryptococcus neoformans; species related with significant development of resistance to azoles such as Candida albicans, C. dubliniensis, C. krusei and Cryptococcus neoformans. Among bacterial species we have included those usually recommended for studies with antibacterial agents, which were Gram-positive cocci (Staphylococcus aureus and Micrococcus luteus), a fermentative Gram-negative rod (Escherichia coli) and two non-fermentative Gram-negative rods (Pseudomonas aeruginosa); one of these was a clinical isolate with a multidrug profile of resistance (data not shown). Finally, we have included Prototheca zopfii, a unicellular algae which has been known as agent of disease in humans and animals, but there has been no consistency in the clinical response to antimicrobial agents employed.
Table 1 shows that both extracts have some activity against all species, except *E. coli* and a multiresistant *P. aeruginosa*. These strains are Gram-negative bacteria and according to literature reports, the Gram-negatives are usually less sensitive to natural products than Gram-positive 20-23. The assays clearly showed a difference between the sensibility of *P. aeruginosa* ATCC and the clinical isolate of the same species, and confirm the multiresistance of the latter. The complex envelope of Gram-negative bacteria (with a dual membrane) appears to afford protection, and in this group, *P. aeruginosa* shows the greatest resistance 24.

Terpenoids, which occur in essential oils of *Senecio* species of- ten contain flavonoids, which have the ability to inhibit spore germination and have therefore antifungal activity. This class of secondary metabolites also possesses antibacterial activity. They act as bacteriostatic by three distinct mechanisms: inhibition of nucleic acid synthesis, damage of bacterial membranes and inhibition of energy metabolism 1.

The CH₂Cl₂ extract showed antimicrobial activity for 19 species (86.4% of the tested microorganisms), and the ethanolic extract inhibited the growth of 68.2%. The best results were obtained for *Microsporum canis* and *Cryptococcus neoformans*.

These results permit to postulate that fungal strains were more sensitive than the bacterial ones, and it seems that the active metabolites of this extract are more specific for this kind of microorganism. The literature describes the evaluation of the antimicrobial activities of different

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>CH₂Cl₂ extract</th>
<th>EtOH extract</th>
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<tbody>
<tr>
<td></td>
<td>MIC (µg/mL)</td>
<td>MIC (µg/mL)</td>
</tr>
<tr>
<td><em>Candida albicans</em> ATCC 44313</td>
<td>2500</td>
<td>-</td>
</tr>
<tr>
<td><em>C. dubliniensis</em> (a)</td>
<td>500</td>
<td>-</td>
</tr>
<tr>
<td><em>C. krusei</em> FMUSP 1023</td>
<td>250</td>
<td>-</td>
</tr>
<tr>
<td><em>C. lusitaneae</em> EPM 50</td>
<td>2500</td>
<td>-</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> (a)</td>
<td>2500</td>
<td>-</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em> var. <em>neofor+ms</em> sorotype D ATCC 28957</td>
<td>50</td>
<td>1000</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em> var. <em>gatt</em> sorotype B ATCC 56990</td>
<td>50</td>
<td>2500</td>
</tr>
<tr>
<td><em>Microsporum canis</em> (a)</td>
<td>25</td>
<td>2500</td>
</tr>
<tr>
<td><em>M. gypseum</em> (a)</td>
<td>1000</td>
<td>2500</td>
</tr>
<tr>
<td><em>M. nanum</em> (a)</td>
<td>500</td>
<td>2500</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em> (a)</td>
<td>250</td>
<td>2500</td>
</tr>
<tr>
<td><em>T. tonsurans</em> (a)</td>
<td>500</td>
<td>5000</td>
</tr>
<tr>
<td><em>Trichosporum inkin</em> (a)</td>
<td>250</td>
<td>5000</td>
</tr>
<tr>
<td><em>T. ovoides</em> (a)</td>
<td>500</td>
<td>2500</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> ATCC 2601</td>
<td>25</td>
<td>1000</td>
</tr>
<tr>
<td><em>Sporothrix schenckii</em> (a)</td>
<td>500</td>
<td>2500</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>1000</td>
<td>2500</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em> ATCC 10240</td>
<td>500</td>
<td>5000</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27850</td>
<td>1000</td>
<td>5000</td>
</tr>
<tr>
<td>Multiresistant <em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Prototheca zopfii</em> (a)</td>
<td></td>
<td>2500</td>
</tr>
</tbody>
</table>

Table 1. MIC values for CH₂Cl₂ and EtOH extracts of *Senecio desiderabilis* (µg/mL). (a) clinical isolates.
Senecio species, and also shows that S. culcitoides has stronger antifungal properties 7.

Plants can produce antifungal compounds to protect themselves from biotic attack that could be essential for fungi resistance 28. These results suggest a potential importance for the use of active constituents from this plant as leads to develop new drugs for the treatment of fungal infections. A bioassay-guided phytochemical research is going on to identify the active compounds responsible for the antimicrobial effects of the extracts.

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REFERENCES