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Antimicrobial Activity of *Senecio desiderabilis* Vellozo (Asteraceae)

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SUMMARY. CH_2Cl_2 and EtOH extracts from *S. desiderabilis* Vellozo were tested concerning their antimicrobial activity by broth microdilution method. The strongest effect was manifested by the CH_2Cl_2 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 multirresistant *Pseudomonas aeruginosa* were not inhibited by any extract.

RESUMEN. "Actividad Antimicrobiana de *Senecio Desiderabilis* Vellozo (Asteraceae)". Los extractos diclorometánico y etanólico 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* multiresistente 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 N° 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 CH_2Cl_2 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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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 subcultured 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 [8,9].

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 ranging from 80% to 82% transmittance at 530 nm. The suspensions were finally diluted at 1:50 on RPMI 1640 broth, according M38-A document [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 for *Candida* species, 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 CH_2Cl_2 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* [10]; species that require long treatments like *Trichophyton rubrum, T. tonsurans, Microsporum canis, M. gypseum* and *M. nanum* [11]; species already related with resistance to polyenes as *Trichosporon* spp. [12], *Candida lusitaniae* [13], *C. parapsilosis* and *Cryptococcus neoformans* [14]; species related with significant development of resistance to azoles such as *Candida albicans, C. dubliniensis, C. krusei* [15-17] and *Cryptococcus neoformans* [18]. 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 [19].

Microorganism	CH_2Cl_2 extract	EtOH extract
	MIC (µg/mL)	MIC (µg/mL)
Candida albicans ATCC 44313	2500	-
C. dubliniensis [a]	500	-
C. krusei FMUSP 1023	250	-
C. lusitaneae EPM 50	2500	-
C. parapsilosis [a]	2500	-
Cryptococcus neoformans var. *neoformans* sorotype D ATCC 28957	50	1000
Cryptococcus neoformans var. *gatti* sorotype B ATCC 56990	50	2500
Microsporum canis [a]	25	2500
M. gypseum [a]	1000	2500
M. nanum [a]	500	2500
Trichophyton rubrum [a]	250	2500
T. tonsurans [a]	500	5000
Trichosporum inkin [a]	250	5000
T. ovoides [a]	500	2500
Saccharomyces cerevisiae ATCC 2601	25	1000
Sporothrix schenckii [a]	500	2500
Staphylococcus aureus ATCC 25923	1000	2500
Micrococcus luteus ATCC 10240	500	5000
Escherichia coli ATCC 25922	-	-
Pseudomonas aeruginosa ATCC 27850	1000	5000
Multirresistant *Pseudomonas aeruginosa*	-	-
Prototheca zopfii [a]		2500

Table 1. MIC values for CH_2Cl_2 and EtOH extracts of *Senecio desiderabilis* (µg/mL). [a] clinical isolates.

Table 1 shows that both extracts have some activity against all species, except *E. coli* and a multirresistant *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* may be responsible for the antimicrobial activity of the CH_2Cl_2 extract. This activity was previously described for this class of constituents [5,25]. The terpenoids frequently exert their antimicrobial effects at the cytoplasmic membrane by altering its structure and function [26,27]. Another event which can explain its antimicrobial activity is the interference with the energy (ATP) generation system in the cell by enzyme inhibition [27].

The ethanolic extracts of *Senecio* species often 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 CH_2Cl_2 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

Senecio species, and also shows that *S. culcitioides* 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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