Composition and evaluation of the antimicrobial activity of the essential oil of *Senecio selloi* Spreng DC.

SILVA, G.N.S.[1]; SPADER, T.B.[2]; ALVES, S.H.[2]; MALLMANN, C.A.[3]; HEINZMANN, B.M.[2]*

[1]Departamento de Farmácia Industrial, Centro de Ciências da Saúde,Universidade Federal de Santa Maria, Avenida Roraima, Prédio 26, Campus Universitário, 97105-900, Santa Maria, RS, Brasil. [2]Departamento de Microbiologia e Parasitologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Avenida Roraima, Prédio 20, Sala 4139, Campus Universitário, 97105-900, Santa Maria, RS, Brasil. [3]Departamento de Medicina Veterinária Preventiva, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Avenida Roraima, Prédio 44, Campus Universitário, 97105-900, Santa Maria, RS, Brasil.

ABSTRACT: The essential oil of the aerial parts of *Senecio selloi* Spreng. DC. was extracted by hydrodistillation and analyzed by GC/MS. Nineteen compounds were identified, representing 99.9% of the total. The main compounds were found to be sesquiterpene hydrocarbons (71.3%), most of them with a bisabolane skeleton (59.4%). The major constituent was α-zingiberene (54%), followed by monoterpene α-isolimonene (16%). The essential oil was also tested against two Gram-positive and two Gram-negative bacterial species, three yeasts, and an algae. From the strains assayed, only *Bacillus subtilis* ATCC 6633 showed susceptibility (MIC and MBC = 4400 µg/mL) to the essential oil.

Key words: Antimicrobial activity, essential oil, *Senecio selloi* Spreng. DC.

RESUMO: Composição e avaliação da atividade antimicrobiana do óleo essencial de *Senecio selloi* Spreng DC. O óleo essencial das partes aéreas de *Senecio selloi* Spreng DC. foi extraído por hidrodestilação e analisado por CG/EM. Dezenove constituintes foram identificados, representando 99,9% do total. Os principais compostos fornecidos foram sesquiterpenos hidrocarbonetos (71,3%), a maioria destes com esqueleto bisabolano (59,4%). O constituinte majoritário foi a-zingibereno (54%), seguido do monoterpeno a-isolimoneno (16%). O óleo essencial foi testado contra duas cepas Gram-positivas e duas Gram-negativas, três fungos e uma alga. De todas as linhagens testadas somente *Bacillus subtilis* ATCC 6633 mostrou suscetibilidade (CIM e CBM = 4400 µg/mL) para o óleo essencial.

Palavras-chave: Atividade antimicrobiana, óleo essencial, *Senecio selloi* Spreng. DC.

INTRODUCTION

According to Bolzan et al (2007), 46 Latin American *Senecio* species are used for therapeutic purposes, including the treatment of fungal skin infections (Portillo *et al*., 2001), pneumonia (Hammond *et al.*, 1998) and antiseptic (Bah *et al.*, 1994). This information suggests antifungal and/or antibacterial activities for preparations obtained from this species.

The genus *Senecio* (Asteraceae) is constituted of about 2000 species worldwide, 85 of which are found in southern Brazil and of these, 33 are native of the state of Rio Grande do Sul (Cabrera & Klein, 1975). Despite the large number of species and its classification in a botanical family which is rich in essential oils, there are only few reports on the composition and biological activities of this group of compounds for the genus.

Essential oils are a mixture of volatile components with different biological functions, which are necessary for the survival of the produced plant species. They often play a crucial role in the defense against microorganisms (Siqui *et al.*, 2000). The essential oils have been used for bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, medicinal and cosmetic applications, yet in pharmaceutical, sanitary, cosmetic, agricultural and food industries (Bakkali *et al.*, 2008).

This paper reports the chemical analysis of the essential oil obtained from aerial parts of *Senecio selloi* Spreng. DC. and its antimicrobial evaluation.

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This species is popularly known as catião-melado and grows as shrub in southern Brazil (Rücker *et al.*, 1999). For this species, the literature describes peroxides with sesquiterpenic structure (Rücker *et al.*, 1996; Heinzmann, 1996) as well as triterpenoids (Rücker *et al.*, 1999; Heinzmann, 1996; Rücker *et al.*, 2003).

MATERIAL AND THE METHODS

Plant material

Aerial parts of *Senecio selloi* Spreng. DC. were collected in November 2006 in Guaíba, state of Rio Grande do Sul, Brazil. The plant material was identified by Prof. N. I. Matzenbacher, Dr. (Graduate Botany Program, UFRGS). A voucher specimen (SMDB 10206) was deposited in the herbarium of the Department of Biology, UFSM.

Essential oil extraction and analysis

The essential oil was obtained from the fresh aerial parts (2.507 kg) by hydrodistillation using Clevenger type apparatus for 2 h (Farmacopéia Brasileira, 2000) and then stored at -4 ºC in amber glass bottles. Its yield was calculated as the ratio between the weight of the fresh plant and weight of the essential oil obtained (% w/w).

GC-MS TIC analysis was performed using an Agilent-6890 gas chromatograph coupled with an Agilent 5973 mass selective detector, under the following conditions: HP5-MS column (5%-phenyl - 95%-methylsiloxane, 30 m x 0.25 mm x 0.25 mm); EIMS: 70 eV. Operating conditions: split inlet 1:100; temperature program, 40-260 ºC; 40 °C for 4 min; ramp rate, 4 ºC/min; carrier gas He; flow rate, 1 mL/min; injector and detector temperature, 220 ºC; interface temperature 250 °C; Databank NIST, 1998.

The constituents of the essential oils were identified based on retention index (RI), determined using a calibration curve of a homologous series of n-alkanes (C_8-C_{32}) injected under the same chromatographic conditions of samples and models fragmentation of mass spectra, both compared with literature data. (Adams, 2001) (Table 1).

The concentration of constituents was calculated using the full area of their peaks, related to the total area of all the constituent sample, obtained by analysis using gas chromatograph.

Antimicrobial activity

The microorganisms used in the test are cataloged strains and clinical isolates, and they are listed in Table 2. The antimicrobial activity of the essential oil was determined by the broth microdilution method (NCCLS, 1997; NCCLS, 2002). Initially, the essential oil was dissolved in methanol in order to obtain a stock solution which was diluted (1:100) in the appropriate medium; Mueller-Hinton broth for bacterial strains and buffered RPMI 1640 broth for yeasts. From these, serial dilutions were prepared in 96-well microtiter trays.

Bacterial strains and yeasts were grown in Mueller-Hinton (24 h / 35 ºC) and Sabouraud dextrose agar (48 h / 35 ºC), respectively. Bacterial suspensions of 0.5 McFarland standard turbidity (1 x 10^8 CFU/mL) were prepared by dilution with saline, and later diluted (1:100) with Mueller-Hinton broth (1 x 10^6 CFU/mL). Aliquots of 10 mL (1 x 10^4 CFU/mL) of inoculum were added to each well of the microtiter tray already containing 200 mL of the medium plus different concentrations of the essential oil. In parallel, yeast suspensions were prepared by dilution with 0.85% NaCl solution and adjusted to 0.5 McFarland standard turbidity (1 x 10^6 to 5 x 10^6 CFU/mL). These suspensions were diluted with sterilized water (1:50) and buffered RPMI 1640 broth (1:20), resulting in 1.0 x 10^3 to 5.0 x 10^3 CFU/mL. 100 µL of the inoculum was added to each well of the microtiter plate already containing 100 µL of the essential oil solution in different concentration. The microplates with bacterial strains were incubated for 24 h at 35 °C. Those with yeasts were incubated for 48 h at 35 °C. The same tests were performed simultaneously for inoculum growth and sterility control. The final concentration of methanol did not exceed 1% and did not affect the growth of the microorganisms. The algae *Prototeca zopfii* was assayed in the same way as yeasts were described.

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of essential oil that completely inhibited visible growth of microorganisms. To determine the minimum bactericidal and fungicidal concentration (MBC/MFC), 10 mL broth was taken from each well without microbial growth and inoculated in Mueller-Hinton and Sabouraud dextrose agar, respectively. The plates were incubated for 48 h at 35 °C until they were read. MBC and MFC were defined as the lowest concentration of the essential oil capable of killing the inoculum.

RESULTS AND DISCUSSION:

The essential oil of the fresh aerial parts of *S. selloi* gave a yield of 0.0035%, which can be considered low if compared with the yields obtained for the essential oils of the aerial parts of other *Senecio* species (Francescato, 2007). Results of the chemical analysis of the essential oil are shown in Table 1. In the oil, we observe the prevalence of sesquiterpenes, fact that was also reported for the essential oil of several species of *Senecio* (Francescato, 2007; Murari *et al.*, 2008). Among

TABLE 1. Composition of the essential oil of the aerial parts of *S. selloi*. KI = Literature Kovats index (Adams, 2001) KI_C = calculated Kovats index

Constituents	KI	KIc	Percentage (%)
Monoterpenes			
Acyclic derivatives			
Z-β-Ocimene	1037	1044	1.8
Monocyclic derivatives			
Camphene	954	958	3.4
α-Isolimonene	985	992	16
γ-Terpinene	1060	1064	1.7
[p-Mentha-3,8-diene]	1073	1075	5.7
Σ (% total of monoterpenes)			28.6
Sesquiterpenes			
Acyclic derivatives			
E-Nerolidol	1563	1561	0.2
Bisabolane derivatives			
α-Curcumene	1481	1489	4.1
α-Zingiberene	1494	1498	54
β-Curcumene	1516	1508	1.3
Cadinane derivatives			
α-Cubebene	1351	1351	0.04
α-Copaene	1377	1377	0.40
Zonarene	1530	1526	1.3
Caryophyllane derivatives			
Caryophyllene Oxide	1583	1591	0.6
E-β-Caryophyllene	1419	1421	4.1
Humulane derivatives			
α-Humulene	1455	1456	0.6
Germacrane derivatives			
β-Elemene	1391	1394	0.1
Germacrene D	1485	1484	4.4
Other			
Isocomene	1388	1387	0.05
Acorenol	1633	1638	0.15
Σ (% total of sesquiterpenes)			71.3
UC	-	1476	0.1
UC	-	1479	0.12

UC: Unidentified Compound

TABLE 2. Minimum inhibitory concentration values of the essential oil of *Senecio selloi* against bacteria, yeasts and algae.

Microorganisms	Essential oil		Antibiotics control	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	Ampicillin
Staphylococcus aureus ATCC 25923	> 4400	> 4400	2	
Escherichia coli ATCC 25922	> 4400	> 4400	8	
Bacillus subtilis ATCC 6633	4400	4400	1	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	Ceftiofur
Pseudomonas aeruginosa ATCC 27853	> 4400	> 4400	32	
	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	Fluconazole
Candida albicans ATCC 44373	>2200	>2200	8	
Candida glabrata ATCC 10231	>2200	>2200	16	
Saccharomyces cerevisiae ATCC 2601	>2200	>2200	2	
	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	Amphotericin B
Prototheca zopfii (clinical isolated)	>2200	>2200	4	

the sesquiterpenes, the derivatives of bisabolane skeleton predominate with 59.4% of the composition of oil, being *a*-zingiberene the major component, with 54%.

Results concerning the evaluation of the antimicrobial activity are shown in Table 2.

The essential oil of the aerial parts of *S. selloi* presented activity only against one Gram-positive strain, *Bacillus subtilis* ATCC 6633, with values for MIC and MBC of 4400 mg/mL. *B. subtilis* is a microorganism of interest because of its wide industrial application, its similarity to pathogenic strains, and its role as a model organism for Gram-positive sporulating bacteria (Henry *et al.*, 2009).

The absence of susceptibility of Gram-negative bacteria against the essential oil evaluated is not surprising because of the dual membrane presented by these microorganisms, which prevents the penetration of various antimicrobial agents. Additionally, their periplasmic space contains enzymes that are capable of breaking down foreign molecules introduced into the environment (Duffy & Power, 2001).

Different authors attribute the ability of essential oils to inhibit the growth of microorganisms to the hydrophobicity of their constituents. This feature would allow their partition in the bilipid layer cell membrane, increasing permeability and leading to loss of vital cell content (Burt, 2004; Juven *et al.*, 1994; Kim *et al.*, 1995). However, other studies have reported the relevance of an oxygen function in the molecule for this activity. According to Dorman & Deans (2000), alcohols have recognized bactericidal activity against vegetative cells, acting as protein denaturing agents, solvents or dehydrating agents. Other authors have reported that components of essential oils containing phenolic or alcoholic groups exhibit more pronounced inhibitory effects on microbial growth, followed by aldehydes and ketones (Griffin *et al.*, 1999). When hydrophobicity is crucial for the antimicrobial activity, a good antimicrobial activity for the essential oil of *S. selloi* would be expected, since the major constituents are hydrocarbons. In contrast, the antibacterial activity was detected only for a single strain of Gram-positive bacteria and only in high concentrations.

Some peculiarities of essential oils such as volatility, low solubility in water and their complexity, significantly interfere with the results of tests for evaluation of antimicrobial activity. Other important factors to take into consideration are the characteristics of each microorganism and the oil to be analyzed. In experiments with fungi, for example, we must take into account the incubation time of 48 hours, which may interfere with the results due to the decomposition or evaporation of oil during the test period (Nascimento *et al.*, 2007).

The absence of oxygenated compounds in the oil is another likely explanation for the high values of MIC and MBC detected in this study (Murari *et al.*, 2008). Therefore, the results described in this work reinforce the importance of the oxygenated function to the antimicrobial activity of essential oils. Although we did not evaluate the antimicrobial activity of the essential oil of *S. selloi* against phytopathogens, the results obtained suggest that its components do not act in plant defense against microorganisms, however, it must have other biological functions. In fact, the literature describes different biological activities for its major constituent, zingiberene, which shows insecticide, repellent and antifeedant activities (Antonious & Kochhar, 2003).

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