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Comparative Chemical Characterization and in Vitro Antimicrobial Evaluation of Essential Oils from Species of the Genus *Senecio* From Patagonia, Argentina

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ABSTRACT

Species of the genus *Senecio* have the largest medicinal value for the native Patagonian communities, and studies on the biological activities attributed to them are scarce.This article reports on the chemical composition of four species from genus *Senecio. Senecio mustersii* var.*mustersii Senecio subpanduratus* O.Hoffm. *Senecio filaginoides* De Candolle and *Senecio coluhuapiensis* Spegazzini respectively, from Patagonia Argentina and the evaluation of its antibacterial activity. Essential oils were obtained by hydrodistillation from fresh leaves of all species and were analyzed by GC-MS. In the essential oils from the leaves of the four species, a total of 24 compounds were identified in *S. mustersii,* 21 in *S. subpanduratus*, 25 in *S. filaginoides,* and 28 in *S. coluhuapiensis*. These compounds collectively accounted for more than 95.2%, 92.9%, 93.5%, and 94.2% of the total oil composition, respectively. The major constituents were α-pinene (53.3 %) in *S.mustersii,* sabinene (23.8 %) in *S.subpanduratus* and Terpinen-4-ol (19.6%) in S.*coluhuapiensis*. In all analysis it was observed that essential oil from *S.filaginoides* have (53.7 %) of a compound called 8-hydroxy-9-keto-10αH- eremophil-7(11)-en-8,12-olide. The essential oils of *S.mutersii, S. subpanduratus, S.filaginoides* and *S.coluhuapiensis* showed antimicrobial activity against *Staphylococcus aureus* with (MIC 3.9 μg/mL). The essential oils of *S. subpanduratus* and *S.filaginoides* showed antimicrobial activity against *Candida albicans* (MIC 1.9 μg/mL). The essential oils of *S.filaginoides* and *S.coluhuapiensis* showed antimicrobial activity against C*andida guillermondi* (MIC 1.9 μg/mL).

KEYWORDS: Asteraceae, *Senecio,* terpenoids, antibacterial activity.

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I. INTRODUCTION

Regarding *Senecio* genus, there are about 3000 species around the world, mainly in hilly areas. In Argentina there are more than 270 species: most of them in the Andes Mountain and in Patagonia (Cabrera,1971). The chemical composition of the essential oils of some *Senecio* species including *S. trapezuntinus, S. platyphyllus, S. vernalis, S. glaucus,S. leucostachys, S. squalidus, S. aegyptius, S. graveolens,S farfarifolius, S. nutans, S. rufinervis,* and *S. longipenicillatus* are reported (Kahriman et al.,2011; Mishra et al,.2011).We studied the herbal use into a natural area of the Patagonian steppe inhabited predominantly by descendants of the mapuche - tehuelche indigenous people. *Senecio mustersii* var.*mustersii, Senecio subpanduratus* O.Hoffm. and *Senecio filaginoides* De Candolle are native species that grows spontaneously near Comodoro Rivadavia city, in the southeast of Patagonia Argentina. *Senecio coluhuapiensis* Spegazzini a species endemic to the Petrified Forest of Sarmiento, Chubut, Argentina, is associated with the Salamanca formation, characterized by a barely developed ground and an extremely acid pH. This work emphasizes the versatility of medicinal species and their possible contribution to self-reliance of rural populations. Qualitative phytochemical analysis showed the presence of polyphenols, tannins, flavonoids, coumarins, anthraquinones, terpenoids, and steroids. The results obtained support the unpalatability of these species (Gratti et al., 2014). The pharmacological activities have been summarized of different species including antimicrobial, antioxidant, repellent, antifungal, acaricidal, anti-inflammatory, cytotoxicity, phytotoxic, anticholinesterase, allelopathic, nematicidal, antimalarial, antileishmanial, α-glucosidase, anticorrosive, analgesic, and toxicity (V. AB Siddiq Salihu et al.,2025; Richeri et al., 2013) The most used species, due to its wide distribution in our country, is *Senecio filaginoides*. It is a perennial herbaceous plant known by the common name "yuyo moro". Its aerial parts are traditionally used for the so-called Cultural Syndrome (Arancibia et al., 2018). Essential oils from *S. mustersii* and *S.subpanduratus* have been studied because of their chemical composition and antimicrobial activity (Arancibia et al., 2010). In *S.coluhuapiensis*, 1-heneicosanol was isolated. This compound, identified in this angiosperm, has demonstrated significant antibacterial properties (Arancibia et al., 2016). The aim of this study was to investigate the possible variation of the chemical constituents of essential oils from leaves in four species, *S.mustersii, S.subpanduratus, S. filaginoides and S.coluhuapiensis* and to evaluate its antibacterial activity.

MATERIALS AND METHODS

A. Materials

The plant material from *S.mustersii, S.subpanduratus* and *S. filaginoides,* were collected to 3 km from Comodoro Rivadavia city (45°50'17"S, 67°30' 49"W) and *S.coluhuapiensis* was obtained in Sarmiento city (45°47′0″ S, 68°57′0″ W), all of them in the province of Chubut, Argentina.The species were identified by botanist (M.E.Arce) and kept in the Patagonia Regional Herbarium (Universidad Nacional de la Patagonia San Juan Bosco) under the following herbarium numbers: *S. filaginoides* HRP N° 6159-6170, *S.subpanduratus* HRP N° 6867, *S.mustersii* HRP N° 6866 and *S.coluhuapiensis* HRP N°7165. Phenological stages of the plants were carried out in the pre-flowering state between 2016 and 2018. The antibacterial and antifungal activities of the essential oils were assayed against nine microorganisms including Gram-positive and Gram-negative bacteria and yeast: *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25299), *Candida albicans* (NIM 982879), *Candida tropicalis* (ATCC 2000956), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), *Candida guilliermondi* (ATCC 5276) and *Candida glabrata* (ATCC 15126) all donated by ANLIS Malbrán Institute, Buenos Aires, Argentina.

B. Methods

Extraction of the essential oils (EO)

Crude essential oils were obtained by hydrodistillation in a Clevenger-type apparatus (Drioiche et al.,2022; Usta et al., 2009) with a cooling bath system 4 h. After extraction, the EO were collected and dried over anhydrous sodium sulphate. It was stored at − 4 °C prior to further usage.

Analysis of the components of essential oils by GC-MS

The essential oils were analyzed by GC-MS using a Perkin-Elmer Clarus 500 instrument with a modified configuration, equipped with a single split/splitless injector connected by a flow splitter to 2 capillary columns: a polyethylene glycol MW ca. 20 000 Da column and a 5%-phenyl-95%-methyl silicone column, both 60 m × 0.25 mm id with 0.25 µm of fixed phase (J&W Scientific). Helium was used as the carrier gas (flow rate: 1.87 mL/min). The column temperature was programmed according to the gradient: 90°C for 5 minutes, increasing at a rate of 3°C/min-230°C and maintained for 13 minutes. The injector temperature was 255°C. Mass range (m/z) and scan time were 40-350 Da and 1 second, respectively. The manual sample injection volume was 0.2 mL of the oils, and the split ratio was 80:1. A mixture of aliphatic hydrocarbons (C6-C24, Sigma Aldrich) in n-hexane was coinjected to calculate the RI using a generalized equation. Compound identification was done comparing the RI relative to C8-C24 n-alkanes, obtained in both columns, with those of reference compounds and from the literature (Adams,2007; Wiley,2008). Additionally, each experimental MS was compared with those from comercial libraries (Babushok et al., 2011) and from our own data basis.

Antimicrobial activity

The antibacterial and antifungal activities of the essential oils were assayed against nine microorganisms including Gram-positive and Gram-negative bacteria and yeast: *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25299), *Candida albicans* (NIM 982879), *Candida tropicalis* (ATCC 2000956), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), *Candida guilliermondi (*ATCC 5276) and *Candida glabrata* (ATCC 15126) all donated by ANLIS Malbrán Institute, Buenos Aires, Argentina. The minimum inhibitory concentration (MIC) was determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2008) protocols. All tests were performed in Mueller-Hinton (MHB) broth for bacteria and 4% glucose-flavored broth for yeast. Microorganism suspensions were adjusted in a spectrophotometer with sterile physiological saline solutions to obtain a final organism density of 0.5 on the McFarland scale (1–5 × 10^5 CFU/mL). EO stock solutions in DMSO were diluted to obtain serial dilutions, which were added to each medium to achieve final concentrations ranging from 989 to 1.9 µg/mL. The final DMSO concentration in the assay did not exceed 1%. The antimicrobial agents Chloramphenicol and Nystatin (Argentina Pharmaceutica) were used as positive controls. The tests were performed in triplicate, and the MIC values are expressed in µg/mL (Runke et al.,1996; Wright et al.,1983)

Statistical analysis

A Hierarchical Cluster Analysis (HCA) was carried out to identify species with similar chemical composition, followed by a Principal Component Analysis (PCA) to compare the chemical composition across species. In both cases, these statistical tests were carried out using the language R (Core Team .2020)

II. RESULT AND DISCUSSION

The essential oil yields obtained from *S. mustersii*, *S. subpanduratus, S. filaginoides,* and *S. coluhuapiensis* were 0.53%, 0.62%, 0.78%, and 0.21% (v/w), respectively. Analysis of the oil composition revealed 24 compounds identified in *S. mustersii*, 21 in *S. subpanduratus*, 25 in *S. filaginoides,* and 28 in *S. coluhuapiensis.* These identified compounds accounted for more than 95.2%, 92.9%, 93.5%, and 94.2% of the total oil composition of the leaves, respectively. Quantitative data expressed as percentage values of the principal constituents in the essential oil are listed in **Table 1.** The principal constituents identified were α-pinene (53.3%) in *S.mustersii,* sabinene (23.8%) in *S. subpanduratus*, and terpinen-4-ol (19.6%) in *S. coluhuapiensis.* All analyses indicated that the essential oil from *S. filaginoides* contains (53.7%) of the compound 8-hydroxy-9-keto-10αH-eremophil-7(11)-en-8,12-olide.

Antimicrobial activity

Different microbes, bacteria and yeast are associated with the antimicrobial activity of EO derived from several species of *Senecio*. Details of the activity are summarized in **Table 2.** The EO from various *Senecio* species exhibit notable antimicrobial activity, particularly against significant bacterial and fungal pathogens.The EO contains bioactive compounds like terpenoids and phenolic acids, which are effective against both Gram-positive and Gram-negative bacteria. These compounds work by disrupting bacterial membranes, interfering with enzymes, and preventing biofilm formation. *Senecio* EO are particularly effective against Gram-positive bacteria such as *Staphylococcus aureus*, which have simpler cell membranes that are easily disrupted. They are also active against Gram-negative bacteria like *E. coli* and *P. aeruginosa*. Although Gram-negative bacteria have an extra outer membrane for protection, the oils can penetrate and damage their cell structure. A fact that may become relevant, given the pathogenic properties of these strains (Magwa et al., 20026). This difference can be explained by the chemical composition of the EO. The antimicrobial activity of EO is difficult to attribute to a specific compound, probably because of the complexity of their composition as well as for the synergistic effects that may exist between the main components (Uribe et al.,1985). Despite this, there are studies that explain the antimicrobial activity of some of the components of essential oils. It has been demonstrated that α- and β-pinene are able not only to destroy cellular integrity, but also to inhibit respiration and ion transport processes (López et al., 2018). These compounds also increase membrane permeability in yeast cells (Galveza et al.,2020)

Statistical analysis

The analysis of the chemical composition of the essential oils of *S.mustersii, S.subpanduratus, S.filaginoides.* and *S.coluhuapiensis* shows a strong diversity of the identified compounds both qualitatively and quantitatively. To obtain a statistical description of our sample and to be able to highlight possible chemical variability and identify possible relationships between the abundance of volatile organic compounds and geographical factors, we used two multivariate statistical methods: hierarchical cluster analysis (HCA) and a principal component analysis (PCA). Principal component analysis (PCA) further highlighted quantitative differences among species, supporting the HCA findings. Hierarchical cluster analysis (HCA). **Figure 1** and **Figure 2. Figure 1** shows the dissimilarity between the EO samples of the *Senecios* studied. The cut off set divides the samples into two clusters. The first cluster (I) includes one sample of *Senecios, S.filaginoides*. The second cluster (II) brings together the remaining *Senecios* samples (*S.mustersii*, *S.subpandur*atus and *S.coluhuapiensis)*.The sample in cluster I shows a distinct composition among the Senecios, while the three samples in cluster II share a common chemical profile.At the level of the dendrogram, we note that the EO of the group (III) includes two samples of EO and are grouped within the same class, so there is an intra-class homogeneity; this is confirmed by the small distance between the two populations, which explains that the two taxa are similar or very close at the level of the chemical profile. On the other hand, groups (III) and (I) are composed of *S.subpanduratus* and *S.coluhuapiensis* and *S.filaginoides*. The species of group (II) are heterogeneous concerning class (I), with a significant distance, the species *S.filaginoides* presents just 50% dissimilarity with the other species (*S.mustersii*).First, a hierarchical bottom-up analysis was performed to divide the sample into groups of homogeneous observations, employing simple linkage to form clusters and the euclidean distance as metric of dissimilarity. PCA highlights the main quantitative differences found between species and complements the results of the HCA. We deduce that the chemical composition of the samples of essential oils of *Senecios* collected in different regions of Patagonia presents a heterogeneity; indeed, some samples contain 8-hydroxy-9-keto-10αH-eremophil-7(11)-en-8,12-olide as the majority compound, while others contain α-pinene, β-pinene, Terpinen-4-ol and Spathulenol.The chemical composition of *Senecio* essential oils from different Patagonian regions exhibited heterogeneity; some samples contained 8-hydroxy-9-keto-10αH-eremophil-7(11)-en-8,12-olide as the dominant compound, while others had α-pinene, β-pinene, terpinen-4-ol, and spathulenol as major constituents. All the studies conducted indicate that essential oils contain common components, though in varying proportions. Some oils are composed mainly of terpenes, others of oxygenated terpene derivatives, and still others of compounds considered taxonomic markers of each species. One example is eremophilane from the species *S. filaginoides*, a terpene derivative with 15 carbon atoms to which a five-atom lactone is attached on the original decaline ring.This conclusion is of great value because this metabolite is a main constituent of the oil and eremophilanes are of taxonomic importance for this genus. The Senecio species in the region do not exhibit chemical homogeneity. The variability in their compounds depends on the growing conditions in areas disturbed by industrial activity and extreme climatic conditions. Principal component analysis (PCA). PCA highlights the main quantitative differences found between species and complements the results of the HCA. We deduce that the chemical composition of the samples of essential oils of *Senecios* collected in different regions of Patagonia presents a heterogeneity; indeed, some samples contain 8-hydroxy-9-keto-10αH- eremophil-7(11)-en-8,12-olide as the majority compound, while others contain α-pinene, β-pinene, Terpinen-4-ol and Spathulenol **Figure 2**

Table 1. Common compounds present in different *Senecio* species (expressed as percentages)

Family	Compounds	*S.mustersii*	*S.subpanduratus*	*S.filaginoides*	*S. coluhuapiensis*
Monoterpenes	α- thujene	0.1	1.7	1.2	0.4
	α-pinene	53.3	22.1	9.8	5.2
	β-pinene	21.2	11.9	3.8	15.1
	Sabinene	1.6	23.8	0.9	0.6
	Δ-3-carene	0.2	0.4	0.5	6.6
	Limonene	1.8	1.2	1.7	2.2
Oxygenated derivatives of monoterpenes	Terpinen-4-ol	0.5	10.2	0.4	19.6
Oxygenated derivatives of sesquiterpenoids	Spathulenol	0.2	0.4	53.7	4.1

Table 2. Antimicrobial activities of *Senecio* essential oils

Essentials oils	Descripcion
S.mustersii	Exhibited activity only against *S.aureus* (MIC 3.9 μg/mL)
S.subpanduratus (MIC 1.9 μg/mL)	Exhibited good activity against all bacteria tested (MIC 3.9 μg/mL) and *Candida albicans*
S.filaginoides	Exhibited excelent activity ((MIC 1.9 μg/mL) against *P. aeruginosa*, *C. albicans, C. guillermondii* and good activity for *C. krusei* and *S.aure*us (MIC 3.9 μg/mL)
S.coluhuapiensis	Exhibited excellent activity against *C. albicans* and *C. guillermondii* (MIC 1.9 μg/mL) and tested and against *S.aureus* (MIC 3.9 μg/mL)

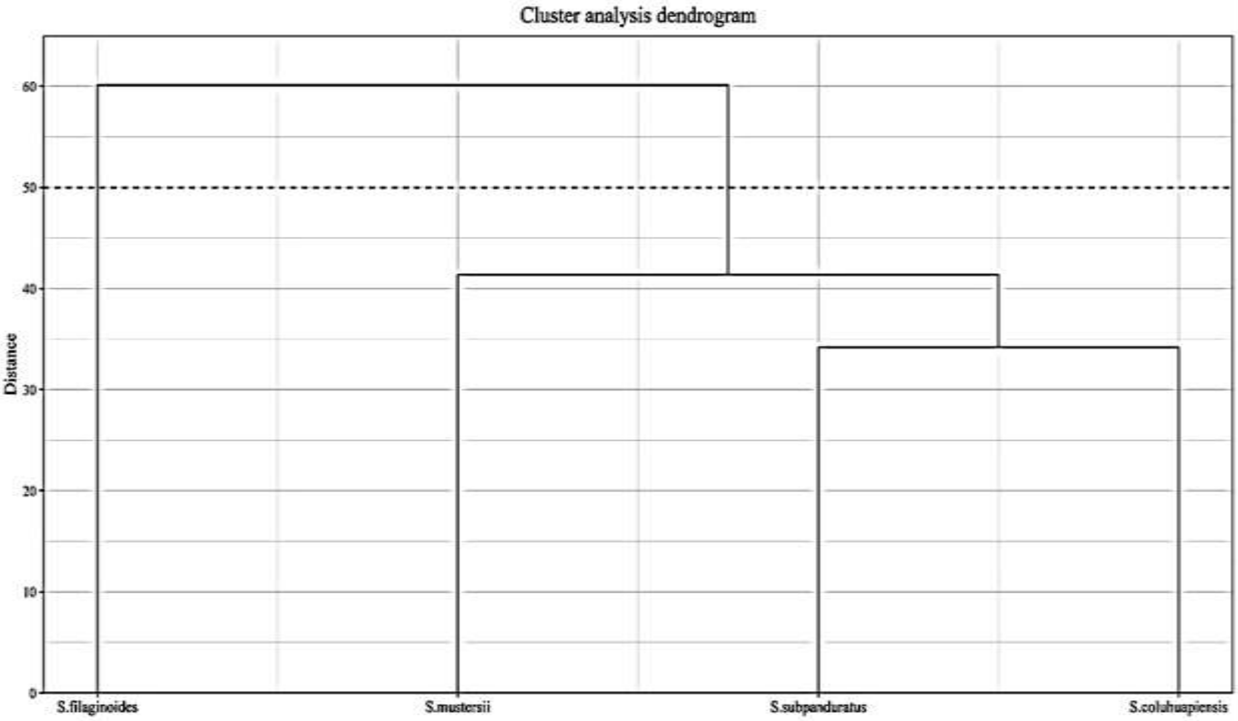

Figure 1. Phylogenetic tree of the studied *Senecio* EO.

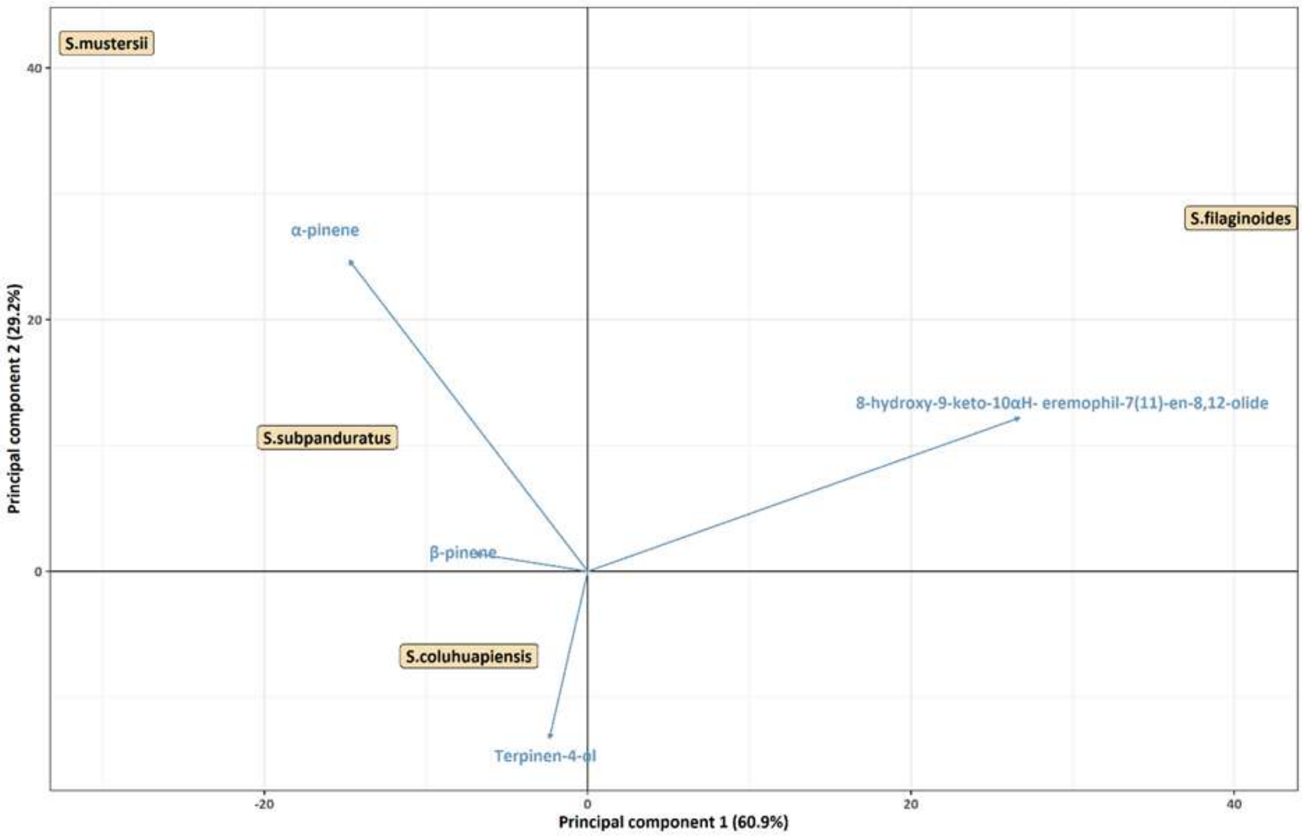

Figure 2. Principal components of the studied *Senecio* EO.

The herbal remedies used by the communities of the central plateau of Chubut represent, on the one hand, a relic of local traditional knowledge from the indigenous peoples and, on the other, the result of processes of cultural hybridization with other societies. This comprehensive and flexible body of knowledge linked to the structure and dynamics of the natural environment remains current and active. The plants represent an efficient and self-sustaining alternative for the home treatment of the most common ailments faced by isolated communities with limited economic resources. We have collected data on the composition of essential oils and publication of the *Senecio* genus from 2010 to the present (Ayomadewa et al.,2024; Arancibia et al.,2013). This is the first report about the comparative study of the chemical composition and biological activity from essentials oils of species the *Senecio* genus from East Central Area of Patagonia Argentina (Assem El-Shazly et al.,2005). The high percentage of monoterpenes and monoterpenoids in two species *(S.mustersii,* and *S.Subpanduratus*) and sesquiterpenoids in *S. coluhuapiensis* could play an important role in defensive mechanism and adaptation to dessert area. The differences in the antimicrobial activity between the species of the genus *Senecio* studied in our work could be related to the oxygenated derivatives, which are found in different percentages in all of them. Eugenol, with aromatic ring and exocyclic double bond (*S. Coluhuapiensis)*, indicates the probable presence of a different chemotype (Cock et al., 2022). Eremophilans, a group of terpenoids found in Eremophila species, are responsible for significant biological activity (Beattie et al.,2010; Bisht et al., 2011). They have demonstrated substantial inhibitory activity against Gram-negative bacteria (Buděšínský et al.,1984) and are also effective against *Candida albicans.* The action against *C. albicans* primarily involves the inhibition of key virulence factors, such as hyphal formation and host interaction. While the exact mechanism of action for eremophilans is not yet fully elucidated, their established broad-spectrum efficacy highlights their therapeutic potential *S. filaginoides* exhibits 53% of an eremofilane called 8-hydroxy-9-keto-10αH- eremophil-7(11)-en-8,12-olide as (Arancibia et al.,2018). This would explain the antimicrobial activity of this oil.The essential oils obtained have been tested and evaluated for their biological activity, primarily antimicrobial. However, most of the scientific studies consulted have isused on testing the essential oils through in vitro studies, while in vivo biological methods and biochemical investigations into the mechanism of action of the analyzed essential oils are lacking. Several in vitro and in vivo assessments could be developed to evaluate their ability to combat numerous diseases. Since the potential toxicological impacts of the *Senecio* species have not yet been evaluated, these characteristics should be considered in future reports.

CONCLUSIONS

This study contributes to the understanding of the *Senecio* genus and its species, which are highly abundant in the Patagonian region. The results highlight the remarkable specificity of biosynthetic processes in nature, which produce distinct stereochemical structures depending on the species. The use of classical separation and analytical methods remains a valuable strategy for the accurate identification of compounds present in a plant's volatile fraction. Moreover, the pleasant aroma of this plant's essential oil suggests its potential application in the fragrance industry, while its biological activity against clinically relevant bacteria and yeasts indicates that the combination of several low–molecular-weight compounds, acting synergistically, could be effective against certain opportunistic infections

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