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Antibacterial and antifungal potential of *Haplopappus* species in Chile

Potencial antibacteriano y antifúngico de las especies *Haplopappus* en Chile

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Abstract

Introduction: Plant extracts have traditionally been used to treat different conditions, and the genus *Haplopappus* stands out among those to which antimicrobial properties are attributed. *Haplopappus* sp. infusions are used to treat abdominal pain, urinary tract diseases, and clean wounds and skin ulcers. Considering that infection prevention is a critical aspect of wound care and the urgent need for novel antimicrobial agents, in this review, we aimed to analyze the antimicrobial properties of extracts from plants of the genus *Haplopappus* in Chile. **Material and methods:** Twelve articles were selected in this review that described the analysis of the antibacterial and antifungal activity of 15 *Haplopappus* species studied using different types of extracts, such as infusions and exudates. **Results:** The extracts were studied in 30 bacterial species, highlighting their antimicrobial effect on gram-positive bacteria, led by *Staphylococcus aureus*. The most potent activities were found in *Bacillus cereus* and *Bacillus subtilis* for the resinous exudates of *H. litoralis* and *H. chrysanthemifolius*. The effect of the extracts against fungal species was also studied, but to a lesser extent, with inhibitory activity reported against *Acremonium falciforme* and *Fusarium oxysporum*. **Conclusion:** The antimicrobial effect of *Haplopappus* species extracts requires more standardized studies to strengthen the current evidence and to explore new anti-infective properties, as well as to specifically assess the inhibitory activity of isolated compounds, particularly clerodanes.

Keywords: *Haplopappus; Haplopappus baylahuen*; antimicrobial activity; natural extracts; antibacterial; antifungal.

Resumen

Introducción: Los extractos de plantas han sido usados tradicionalmente para tratar diferentes afecciones y el género *Haplopappus* destaca entre aquellos a los que se le han atribuido propiedades antimicrobianas. Las infusiones de *Haplopappus* sp. se usan para tratar el dolor abdominal, enfermedades del tracto urinario y para limpiar heridas. Considerando que la prevención de infecciones es un aspecto crítico del cuidado de heridas y de la necesidad urgente de nuevos agentes antimicrobianos, esta revisión tiene como objetivo analizar las propiedades antimicrobianas de extractos de plantas del género de *Haplopappus* en Chile. **Material y método:** Doce artículos fueron seleccionados en esta revisión y describen el análisis de las propiedades antibacterianas y antifúngicas de quince especies de *Haplopappus* estudiadas como diferentes tipos de extractos, tales como infusiones y exudados. **Resultados:** Los extractos fueron estudiados en 30 especies bacterianas, destacando sus propiedades inhibitorias contra bacterias gram positivo como *Staphylococcus aureus*. Las actividades más potentes fueron contra *Bacillus cereus* y *Bacillus subtilis* para el exudado resinoso de *H. litoralis* y *H. chrysanthemifolius*. El efecto de los extractos contra especies fúngicas también fue estudiado, pero en menor medida, con actividad inhibitoria descrita contra *Acremonium falciforme* y *Fusarium oxysporum*. **Conclusión:** El efecto antimicrobiano de los extractos de especies de *Haplopappus* requiere estudios más estandarizados que fortalezcan la evidencia actual y permitan explorar actividades antiinfecciosas, así como evaluar de manera específica la acción inhibitoria de compuestos aislados, en particular los clerodanos.

Palabras clave: *Haplopappus; Haplopappus baylahuen*; actividad antimicrobiana; extractos naturales; antibacterianos; antifúngicos.

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Introduction

Antimicrobial resistance: A global public health challenge

Antimicrobial resistance (AR) poses a significant threat to public health globally, highlighting the urgent need for effective solutions, such as investing in research and development of new antibiotics. To support this initiative, the World Health Organization (WHO) has released priority lists of bacteria and fungi for which new antimicrobials are urgently needed. The list of antibiotic-resistant bacteria was described by Tacconelli *et al.* (2018) and includes 20 bacterial species, while in the list of fungi, *Cryptococcus neoformans, Aspergillus fumigatus, Candida auris,* and *Candida albicans* are considered as a critical priority group (WHO, 2022). Along with these lists, the United States Center for Disease Control and Prevention (CDC) categorizes AR as a health threat and classifies bacteria and fungi into four categories. Carbapenem-resistant *Acinetobacter* and *Candida auris* are examples of pathogens at the urgent level. At the same time, drug-resistant *Candida*, multidrug-resistant *Pseudomonas aeruginosa,* and methicillin-resistant *S. aureus* (MRSA) are classified as a serious threat level (CDC, 2019). There is also the acronym ESKAPE, which encompasses six pathogens that exhibit AR to multiple drugs and virulence: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter s*pp. (Mulani *et al*., 2019). AR imposes considerable costs on healthcare systems by increasing hospitalization time, medical visits, and prolonged recovery times (Ventola, 2015). Therefore, investigating new antimicrobial compounds is of utmost importance.

In addition to developing resistance to chemotherapy, bacteria and fungi can exhibit phenotypic adaptations that confer tolerance to antimicrobial treatments. One such adaptation is the formation of biofilms (Rather *et al*., 2021). Biofilms are microbial aggregates encased in a self-secreted extracellular polymeric substance that shields the microbial community from the effects of antibiotics by acting as a diffusion barrier (Flemming & Wingender, 2010). Additionally, bacteria located in the deeper regions of the biofilm exhibit a lower metabolic state due to limited access to nutrients and oxygen. This subpopulation, known as persisters, can survive antibiotic treatments but become susceptible when subcultured in fresh media (Moldoveanu *et al*., 2021). Biofilms rarely affect healthy individuals, but in cases where the immune response is compromised, biofilms can establish and lead to chronic infections. Biofilms are responsible for recurrent urogenital and lung infections, among others (Wu *et al*., 2020; Naziri *et al*., 2021; Kolpen *et al*., 2022). Therefore, there is a need not only for new inhibitory agents but also for agents that can prevent biofilm formation or disrupt pre-existing biofilms.

Natural extracts are a promising source of new bioactive compounds with antimicrobial potential

Plants have played a significant role in traditional medicine among various cultures for thousands of years. Phytocompounds such as alkaloids, terpenes, and polyphenols are of significant interest in the study of antimicrobial activity, along with plant extracts, which exhibit considerable diversity in their composition (Álvarez-Martínez *et al*., 2020). This diversity is influenced by the solvents used and the harvesting season, but also by genetic factors, ecological conditions, phenological stage, and environmental stress, which modulate the biosynthesis of secondary metabolites (Faini *et al*., 2011; Mitsi & Echeverría, 2024). In *Haplopappus remyanus*, quantitative differences in chemical composition have been reported between populations, attributed to environmental influences and suggesting the presence of distinct ecotypes (Faini *et al*., 2011). Such variability directly impacts the abundance of secondary metabolites, including phenolic compounds, alkaloids, and terpenes, which are closely associated with antimicrobial activity. Phenolics, such as flavonoids, have been shown to impair bacterial membrane integrity, increase permeability, and induce oxidative stress. Alkaloids display antibacterial activity by targeting enzymes, nucleic acids, and efflux pumps; and terpenes, due to their lipophilic nature, integrate into microbial membranes and destabilize them. Together, these mechanisms contribute to the antimicrobial potential observed in *Haplopappus* and other medicinal plants (Cushnie *et al*., 2014; Jubair *et al*., 2021)

Moreover, the antimicrobial activity of extracts represents a complex mixture of compounds that may exert synergistic interactions among various phytochemicals against bacteria (Álvarez-Martínez *et al*., 2020). Some plant extracts commonly used in antimicrobial phytotherapy include liquid preparations of cranberry (*Vaccinium macrocarpon*), bearberry infusions (*Arctostaphylos uva-ursi*), and thyme essence (*Thymus vulgaris*), which are frequently employed to treat urinary tract infections (UTI) (Lopes *et al*., 2019). It has been hypothesized that one strategy to prevent or slow the emergence of AR in bacteria is using extracts such as essential oils. These oils, comprising multiple compounds, can target various molecular sites. The combination of multiple mechanisms of action may compel bacteria to develop several resistance mechanisms simultaneously, posing a greater and less likely adaptive challenge (Álvarez-Martínez *et al*., 2020).

Therapeutic uses of *Happlopappus baylahue*n in traditional Chilean medicine

In Chile, a group of plants of the *Haplopappus* genus, including *H. baylahuen, H. multifolius, H. remyanus,* and *H. foliosus,* are used interchangeably for their similar medicinal properties and are locally known as "bailahuén" with 62 species of *Haplopappus* found in Chile (Rodriguez *et al*., 2018).

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Haplopappus belongs to the family Asteraceae, which includes more than 1,000 species in Chile and is a strictly endemic genus of southern South America, with its diversity concentrated in Chile and some species also present in Argentina (Rodriguez *et al*., 2018; Mitsi & Echeverría, 2024). The members of this genus are perennial shrubs or subshrubs, usually resinous and aromatic, bearing yellow composite flowers typical of the Asteraceae family. The vernacular name "bailahuén" is widely used to designate *H. baylahuen* and several other related species, which are traditionally employed interchangeably due to their similar medicinal properties (MINSAL, 2010; Mitsi & Echeverría, 2024). In Chile, *Haplopappus* species have been used from Aymara communities in the north to Mapuche groups in the south, mainly for gastrointestinal ailments, wound healing, and other traditional purposes (Mitsi & Echeverría, 2024).

H. baylahuen, seen in Figure 1, is a shrub 25 to 40 cm tall with a lower, resinous, and hairless woody structure. The leaves are smooth with serrated, spiny margins, measuring about 1-3.5 cm long and 1-2 cm wide. In addition, the plant's distinctive scent can be attributed to its resin. These species are in the high mountains of central Chile (MINSAL, 2010). Among the different traditional uses of *Happlopappus* infusions recognized by the MINSAL, including abdominal colic and diseases of the urinary tract, these infusions are also used to clean wounds and skin ulcers.

Considering that the prevention of infections is a critical aspect of wound care (Ovington, 2001) and the urgent need for novel antimicrobial agents (Tacconelli *et al*., 2018; WHO, 2022), *Haplopappus* infusion and other extracts may have potential antimicrobial properties that merit further review as a potential source of antimicrobial compounds. Therefore, this review aimed to identify the evidence of antimicrobial properties studied on different extracts of Chilean *Haplopappus* species.

Figure 1: *Haplopappus baylahuen* (Fundación RA Philippi de estudios naturales, 2023)

Methodology

In this review, the literature search and selection were carried out by a single reviewer using PubMed and Google Scholar, covering all publications up to 20 August 2022, the date of the search. PubMed was used because it is the most widely recognized database in the biomedical field, providing access to peer-reviewed literature indexed in MEDLINE. Google Scholar was used as a complementary source to broaden the search. The keywords and Boolean phrases were "(Haplopappus OR baylahuen)" AND "(antimicrobial OR antibacterial OR bactericidal OR bacteriostatic OR antifungal OR fungicidal OR fungistatic)". After removing duplicates, titles and abstracts were screened, excluding those that did not meet the inclusion criteria. The inclusion criteria applied in this review were original articles related to the antimicrobial activity of *Haplopappus* spp. and native Chilean *Haplopappus* species. The proposed exclusion criteria for this review were studies in a language other than Spanish or English.

Out of 180 articles screened, 7 could not be retrieved, 45 were non-original articles, 107 were unrelated to the antimicrobial activity of *Haplopappus* spp., and 5 were about non-native Chilean *Haplopappus* species. Consequently, only 12 articles met the criteria for inclusion in this review (Figure 2).

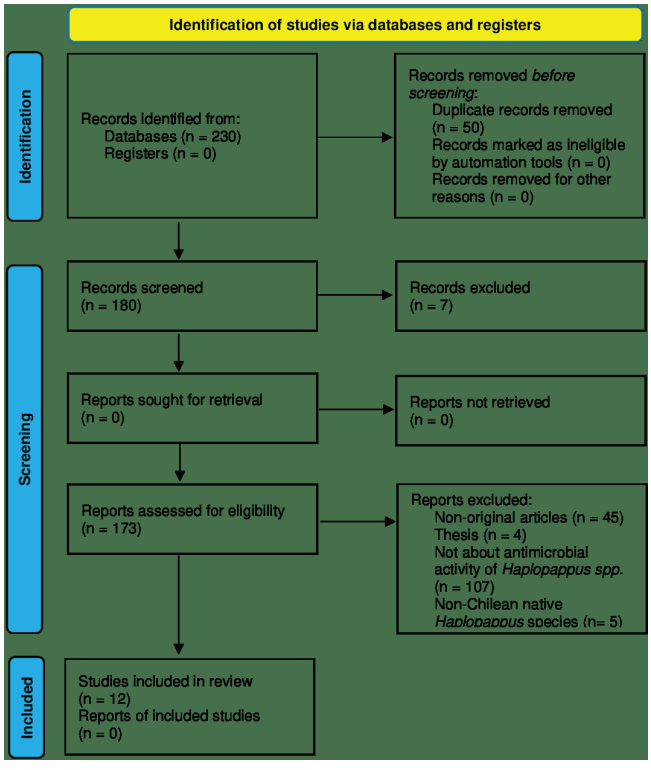

Figure 2: PRISMA diagram of the article selection.

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Results

Twelve articles that met the established inclusion criteria were selected, detailing the antimicrobial effects of *Haplopappus* spp. extracts. These articles examine the studied species, the types of extracts, the tested microbial species, and the techniques used for antimicrobial evaluation.

Haplopappus species studied in the literature

Fifteen *Haplopappus* species were studied and their frequency across the reviewed articles is the following: 4 articles for *H. baylahuen,* 3 articles for *H. uncinatus* and *H. foliosus,* 2 articles for *H. rigidus* and *H. multifolius,* and 1 article for *H. velutinus, H. anthylloides, H. chrysanthemifolius, H. diplopappus, H. illintus, H. litoralis, H. pusillus, H. schumannii, H. scrobiculatus, and H. taeda*. It should be noted that *H. cuneifolius*, as described in some articles, is a synonym for *H. pusillus* and will be referred to as such in this article (Rodriguez *et al*., 2018). Furthermore, most reviewed articles include the study of more than one *Haplopappus* species.

The bailahuén complex is widely distributed across continental Chile, considering the distribution of *Haplopappus* species (Rodriguez *et al*., 2018) and the collection regions documented in the reviewed articles. *H. baylahuen* is the species most frequently studied for its antimicrobial activity, and it is mainly distributed in the Atacama and Coquimbo regions of Chile (Rodriguez *et al*., 2018). In only the two articles, samples were collected from different regions. One collection took place in the Metropolitan Region and the other in the Biobío Region, with the identification conducted by the Department of Biology at Universidad de Santiago de Chile and the Herbarium CONC from the Department of Botany of Universidad de Concepción of Chile, respectively (Becerra *et al*., 2010; Elgueta *et al*., 2021). The second most frequently studied species is *H. foliosus*, distributed from the Atacama Region to the Maule Region (Rodriguez *et al*., 2018). In the three reports on this species, collections were made in the Valparaíso Region (Urzúa *et al*., 1995; Urzúa & Mendoza, 2001; Urzúa *et al*., 2006). Meanwhile, *H. uncinatus* is distributed between the Coquimbo and Maule regions (Rodriguez *et al*., 2018), with three articles reporting collections from the Metropolitan Region (Urzúa *et al*., 1995; Urzúa & Mendoza, 2001; Urzúa *et al*., 2006).

Extracts of Chilean *Haplopappus species*

Natural plant extracts are the result of extraction processes, which involve the separation of compounds based on their differences in solubility, a technique commonly used to isolate or purify natural products (Elufioye & Badal, 2017). For *Haplopappus* spp., extracts were generally prepared using the whole plant as raw material, although in some studies, only the leaves were used (Becerra *et al*., 2010; Elgueta *et al*., 2021), and one study relied on plant material sourced from local pharmacies and herbal shops (Lazo, 1990). The chemical composition and bioactivity of plant extracts can vary due to several factors related to material handling and extraction. Harvest time, drying conditions, and plant processing (e.g., grinding) affect metabolite stability and extraction efficiency. Likewise, solvent polarity and type of extract (aqueous, ethanolic, hexanic, etc.) determine the selectivity for different classes of compounds, influencing the extract's chemical profile and biological properties (Do *et* al., 2014; Azwanida, 2015; Abubakar & Haque, 2020). Careful control and reporting of these variables are essential to ensure reproducibility in phytochemical studies.

Table 1 details the types of *Haplopappus* extracts studied and their extraction methods.

The choice of solvents primarily depends on their polarity, with dichloromethane having a low dipole moment, while solvents such as methanol and water possess high dipole moments (Computational Chemistry Comparison and Benchmark Database, 2022). The differences in polarity are important, as they influence the type of compound being extracted, such as chloroform for terpenoids, fats, and oils (Abubakar & Haque, 2020). A distinction should be made among infusions, decoctions, and essential oil extraction, all of which employ water as a solvent. Infusion involves immersing plant material in cold or hot water for a short period, whereas decoction requires boiling water plus heat during an extraction period typically lasting 15 minutes (Abubakar & Haque, 2020). Essential oils are typically obtained through hydrodistillation or steam distillation, in which volatile compounds are carried by water vapor, condensed, and separated into hydrosol and essential oil fractions (Sadgrove & Jones, 2015; Kant & Kumar, 2022).

The difference between maceration and resinous exudate extraction lies in the plant material used. Maceration involves grinding the plant material into small pieces to ensure adequate contact with the solvent, thereby facilitating the recovery of larger amount of extract solution (Azmir *et al*., 2013). In contrast, resinous exudate extraction uses fresh plant material that is briefly submerged in the solvent. This method is specifically designed to extract resins from the surface of the plant (Dilworth *et al*., 2017).

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Table 1: Frequency of the extraction methods used for the evaluation of antimicrobial properties of *Haplopappus*. The frequency is given by the number of articles where the extracts are used.

Type of extract	Solvents	Frequency per article	*Haplopappus* species studied	Source	Extraction methods	References
Infusion	Distilled water	1	*H. multifolius* *H. taeda*	Harvest	Prepared by placing the dried leaves in boiling distilled water, then filtered and freeze-dried.	(Padilla *et al*., 2021)
Decoction	Distilled water	2	*H. baylahuen* *H. rigidus*	Herbal store	Herbal sachets are boiled in a water bath in sterile distilled water, allowed to cool and adjusted to pH 6 if necessary.	(Lazo, 1990; Pérez & Anesini, 1994)
Ethyl acetate extract	Ethyl acetate	1	*H. baylahuen*	Herbal store	The prepared decoctions were subjected to extraction with ethyl acetate for 24 hours.	(Lazo, 1990)
Chloroform extract	Chloroform	1	*H. rigidus*	Harvest	The dried and ground plant was mixed with chloroform, which was boiled by reflux for ten minutes, then the dry residue was obtained with a rotary evaporator and lyophilized.	(Morales *et al*., 2003)
Dichloro-methane extract	Dichlorome-thane	1	*H. baylahuen*	Herbal store	The dried plant was subjected to dichloromethane for a week, filtered into the vacuum, and dried in a rotary devaporator.	(Brodkiewicz *et al*., 2017)
Ethanolic extract	Ethanol	4	*H. baylahuen* *H. rigidus* *H. multifolius* *H. taeda*	Harvest and herbal store	The dried plant was subjected to ethanol at different concentrations (from 50% to 95%) and then the extract was dried and concentrated.	(Elgueta *et al*., 2021; Lazo, 2019; Morales *et al*., 2003; Padilla *et al*., 2021)
Methanolic extract	Methanol	1	*H. baylahuen*	Herbal store	The dried plant was soaked in methanol at room temperature for seven days each, with discontinuous stirring, and vacuum filtered, and then the extract was dried by a rotary evaporator.	(Brodkiewicz *et al*., 2017)
Resinous exudate	Dichlorome-thane	4	*H. diplopappus* *H. anthylloides* *H. schumannii* *H. cuneifolius* *H. velutinus* *H. pusillus* *H. multifolius* *H. illinitus* *H. foliosus* *H. litoralis* *H. chrysanthemifolius* *H. scrobiculatus*	Harvest	The fresh plant was immersed in cold dichloromethane for 15 to 20 seconds, and then concentrated in a sticky residue.	(Urzúa *et al*., 1995; Urzúa *et al*. 2003; Urzúa *et al*. 2006; Urzúa *et al*. 2012)
Methanolic solution of resinous exudate	Methanol	1	*H. uncinatus* *H. foliosus*	Harvest	The resinous exudate was diluted in methanol.	(Urzúa & Mendoza, 2001)
Essential oil	Distilled water	1	*H. baylahuen*	Harvest	The plant was hydrodistilled in a Clevenger type apparatus for 4 hours, the resulting oil was dried with anhydrous sodium sulfate.	(Becerra *et al*., 2010)

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Antimicrobial properties of *Haplopappus* extracts

The antibacterial properties of agents are traditionally studied using the zone of inhibition (ZOI) method or the determination of the minimum inhibitory concentration (MIC). For ZOI analysis, a bacterial culture is grown in a Petri dish, where a drop or disk loaded with the agent under study is applied. If the agent has an antimicrobial effect against the microorganism at a given concentration, bacterial growth will be inhibited where the agent concentration exceeds the effective level. The ZOI is the region around the drop or disk where no microbial growth occurs. ZOI can be measured using computational methods or metric scales (Bhargav *et al*., 2016).

The MIC is the minimum concentration of an antibacterial agent that completely inhibits visible growth of an organism under *in vitro* conditions. It is commonly determined by serially diluting the agent under study. Various dilution solutions of a standard antibacterial agent are prepared, typically in water, and bacterial growth is compared to a negative control (without the antibacterial agent) and the dilutions of the agent or extract being studied (Kowalska-Krochmal & Dudek-Wicher, 2021). One method of measuring MIC involves assessing the turbidity of the medium via optical density/absorbance using a spectrophotometer (Buss da Silva *et al*., 2019). When the dilution is carried out in test tubes, the method is referred to as microdilution, whereas when it is performed in multiwell plates, it is referred to as microdilution.

Although ZOI and MIC are indicators of antimicrobial activity, they cannot be directly compared across different antimicrobials to determine which is most effective against a particular microorganism, as each microorganism has different susceptibility values for each antimicrobial agent (Sabu *et al*., 2018). In the study of plant extracts, these extracts can be evaluated directly and diluted, with the MIC expressed in terms of the extract concentration. Alternatively, the extracts can be standardized based on their content of a specific compound, allowing the MIC to be expressed in terms of the concentration of that reference compound in the extract (Araya *et al*., 2022).

Antibacterial properties of *Haplopappus* extracts

The antibacterial activity was evaluated across 35 microbial species, including 30 bacterial species and 5 fungal species. Inhibitory activity was primarily observed in bacteria such as *S. aureus, Bacillus subtilis,* and *Bacillus cereus,* as shown in Table 2. Additional bacterial species evaluated, along with their respective results, are detailed in the Supplementary material.

The inhibitory effect of the different extracts was observed predominantly in gram-positive bacterial species. Most of the evidence for the inhibitory activity of *Haplopappus* extracts (9 research articles) was reported against *Staphylococcus aureus*, with resinous exudates of *H. litoralis* (MIC 1.25 µg/mL) and *H. rigidus* (ZOI 11–20 mm). The ZOI obtained with *H. rigidus* was the largest among all the extracts and bacterial species studied. Resinous exudates of *H. foliosus* (MIC 2.5 µg/mL) and *H. chrysanthemifolius* (MIC 0.625 µg/mL), along with ethanolic extracts of *H. rigidus* (ZOI 11–20 mm), exhibited inhibitory activity against *B. subtilis*. The resinous exudate of *H. foliosus* demonstrated the most potent inhibitory activity in terms of MIC per µg/mL, followed by its inhibitory effect on *Bacillus coagulans* (MIC 5 µg/mL) (Urzúa *et al*., 2003). Furthermore, the most potent inhibitory activity overall, measured as MIC per µg, was observed against *B. cereus* with resinous exudates of *H. litoralis* and *H. chrysanthemifolius* (MIC 0.32 µg/mL) (Urzúa *et al*., 2012).

The evaluation of different extracts from *Haplopappus* species on *Escherichia coli*, a gram-negative bacterium, was reported in eight articles; however, no inhibitory or only slight inhibitory effects were observed (Lazo, 1990; Urzúa *et al*., 1995; Urzúa & Mendoza, 2001; Morales *et al*., 2003; Urzúa *et al*., 2003; Urzúa *et al*., 2006; Urzúa *et al*., 2012; Padilla *et al*., 2021). Similarly, slight or null inhibitory effects were reported for the gram-negative bacteria *Proteus vulgaris* and *Pseudomonas aeruginosa* in four and six articles, respectively (Urzúa *et al*., 1995; Urzúa & Mendoza, 2001; Morales *et al*., 2003; Urzúa *et al*., 2006; Urzúa *et al*., 2012; Brodkiewicz *et al*., 2017; Padilla *et al*., 2021).

Table 2 excluded studied microbial species not inhibited by *Happlopapus* extracts, mostly gram-negative bacteria. No inhibitory effect could be observed for *Klebsiella pneumoniae* and *Salmonella paratyphi B* (both gram-negative) with extracts of resinous exudate and methanol solution of resinous exudate (Urzúa & Mendoza, 2001; Urzúa *et al*., 2003; Urzúa *et al.,* 2006, Urzúa *et al.,* 2012), *Erwinia carotovora* (gram-negative) with extracts of resinous exudate (Urzúa & Mendoza, 2001; Urzúa *et al*., 2006; *et al*., 2012), *Salmonella typhi* (gram-negative) with extracts by decoction, infusions, and ethanol and chloroform (Pérez & Anesini, 1994; Morales *et al*., 2003; Padilla *et al*., 2021), *Acinetobacter baumannii* (gram-negative) with extracts by infusion, and ethanol and chloroform (Morales *et al*., 2003; Padilla *et al*., 2021) and *Enterobacter cloacae* (gram-negative) with resinous exudate extract (Urzúa & Mendoza, 2001; Urzúa *et al*., 2003). This lack of inhibitory effect was also observed in *Enterococcus faecium* (gram-positive), *Listeria monocytogenes* (gram-positive), *Morganella morganii* (gram-negative), *Proteus mirabilis* (gram-negative), *Providencia stuartii* (gram-negative), and *Shigella sonnei* (gram-negative) with infusions and ethanol extracts (Padilla *et al*., 2021).

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Table 2: Antibacterial effect of extracts of *Haplopappus* species and the frequency per article in which this activity was described for each bacterial species. The frequency is given by the number of original research articles that describe inhibitory effects for these microbial species. This table only shows the bacterial species studied in which inhibitory effects were reported in at least one article.

Bacterial specie	Frequency per article	*Haplopappus* species	Extract	Results	Reference
Staphylococcus aureus	9	*H. uncinatus*	Resinous exudate	No inhibition	(Urzúa *et al*., 2006)
				Inhibition zone 11-13 mm	(Urzúa *et al*., 1995)
		H. foliosus		MIC 1000 µg/mL	(Urzúa & Mendoza, 2001)
				MIC 500 µg/mL	
				MIC 2.5 µg	(Urzúa *et al*., 2003)
		H. diplopappus		Inhibition zone 9-10 mm	(Urzúa *et al*., 1995)
		H. anthylloides			
		H. schumannii			
		H. pusillus			
		H. velutinus			
		H. illinitus			
		H. multifolius		Inhibition zone 11-13 mm	
			Ethanolic extract	Inhibition zone 9.3 mm	(Padilla *et al*., 2021)
			Infusion	Inhibition zone 12.4 mm	
		H. taeda	Ethanolic extract	Inhibition zone 7.9 mm	
			Infusion	Inhibition zone 7.2 mm	
		H. rigidus	Ethanolic extract	Inhibition zone 11-20 mm	(Morales *et al*., 2003)
			Chloroform extract	Inhibition zone 6-10 mm	
		H. litoralis	Resinous exudate	MIC 1.25 µg	(Urzúa *et al*., 2012)
		H. chrysanthemifolius		MIC 2.5 µg	
		H. scrobiculatus			
		H. baylahuen	Decoction	Total inhibition	(Lazo, 1990)
			Ethanolic extract		
			Ethyl acetate extract		
			Dichloromethane extract	45% inhibition at 1000 µg/mL	(Brodkiewicz *et al*., 2017)
			Methanolic extract		
Bacillus subtilis	8	*H. uncinatus*	Resinous exudate	MIC 63 µg/mL	(Urzúa *et al*., 2006)
				Inhibition zone 11-13 mm	(Urzúa *et al*., 1995)
		H. foliosus	Methanolic solution of resinous extract	MIC 63 µg/mL	(Urzúa & Mendoza, 2001)
				MIC 250 µg/mL	
			Resinous exudate	MIC 2.5 µg/mL	(Urzúa *et al*., 2003)
		H. diplopappus			(Urzúa *et al*., 1995)
		H. anthylloides			
		H. schumannii		Inhibition zone 9-10 mm	
		H. pusillus			
		H. velutinus			
		H. illinitus		Inhibition zone 11-13 mm	
		H. multifolius		Inhibition zone 11-13 mm	(Urzúa *et al*., 1995)
			Ethanolic extract	Inhibition zone 7.8 mm	(Padilla *et al*., 2021)
			Infusion	No inhibition	
		H. taeda	Ethanolic extract	Inhibition zone 12.6 mm	
			Infusion	Inhibition zone 8.5 mm	
		H. rigidus	Ethanolic extract	Inhibition zone 11-20 mm	(Morales *et al*., 2003)
			Chloroform extract	Inhibition zone 6-10 mm	
		H. baylahuen	Decoction	Total inhibition	(Lazo, 1990)
			Ethanolic extract		
			Ethyl acetate extract		
		H. litoralis	Resinous exudate	MIC 0.625 µg	
		H. chrysanthemifolius			
		H. scrobiculatus		MIC 1.25 µg	
Escherichia coli	8	*H. uncinatus*	Resinous exudate	No inhibition	(Urzúa *et al*., 2006)
					(Urzúa & Mendoza, 2001)
		H. foliosus		Inhibition zone 9-10 mm inhibition	(Urzúa *et al*., 2003)
		H. diplopappus			(Urzúa *et al*., 1995)
		H. velutinus			
		H. illinitus		No inhibition	
		H. anthylloides		Inhibition zone 9-10 mm	
		H. schumannii			
		H. cuneifolius (H. pusillus)			
		H. multifolius	Ethanolic extract	No inhibition	(Elgueta *et al*., 2021)
			Infusion		
		H. taeda	Ethanolic extract		
			Infusion		
		H. rigidus	Ethanolic extract		(Morales *et al*., 2003)
			Chloroform extract		
		H. baylahuen	Decoction		(Lazo, 1990)
			Ethanolic extract		
			Ethyl acetate extract		
		H. litoralis	Resinous exudate		(Urzúa *et al*., 2012)
		H. chrysanthemifolius			
		H. scrobiculatus			

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		H. uncinatus	Resinous exudate	No inhibition	(Urzúa *et al*., 2006)
				Inhibition zone 11-13 mm	(Urzúa *et al*., 1995)
			Methanolic solution of resinous extract	No inhibition	(Urzúa & Mendoza, 2001)
		H. foliosus	Resinous exudate	Inhibition zone 11-13 mm	(Urzúa *et al*., 2003)
Proteus vulgaris	6	*H. illinitus*	Resinous exudate	No inhibition	(Urzúa *et al*., 1995)
		H. diplopappus		Inhibition zone 9-10 mm	
		H. anthylloides			
		H. schumannii			
		H. cuneifolius (H. pusillus)			
		H. velutinus		Inhibition zone 11-13 mm	
				Inhibition zone 9-10 mm	
		H. multifolius	Ethanolic extract	No inhibition	(Padilla *et al*., 2021)
			Infusion		
		H. taeda	Ethanolic extract		
			Infusion		
		H. litoralis	Resinous exudate		(Urzúa *et al*., 2012)
		H. chrysanthemifolius			
		H. scrobiculatus			
			Resinous exudate		(Urzúa *et al*., 2006)
		H. uncinatus	Methanolic solution of resinous extract	MIC 250 µg/mL	(Urzúa & Mendoza, 2001)
Bacillus cereus	5	*H. foliosus*	Resinous exudate	MIC 2.5 µg	(Urzúa *et al*., 2003)
		H. litoralis		MIC 0.32 µg	(Urzúa *et al*., 2012)
		H. chrysanthemifolius		MIC 0.32 µg	
		H. scrobiculatus		MIC 1.25 µg	
		H. multifolius	Ethanolic extract	Inhibition zone 8.6 mm	(Padilla *et al*., 2021)
			Infusion	No inhibition	
		H. taeda	Ethanolic extract	Inhibition zone 15.2 mm	
			Infusion	Inhibition zone 12.2 mm	
		H. taeda	Ethanolic extract	No antimicrobial activity was observed	(Padilla *et al*., 2021)
			Infusion		
		H. multifolius	Ethanolic extract		
			Infusion		
		H. diplopappus	Resinous exudate	Inhibition zone 9-10 mm	(Urzúa *et al*., 1995)
		H. anthylloides			
		H. illinitus			
Pseudomonas aeruginosa	4	*H. schumannii*	Resinous exudate	No inhibition	
		H. cuneifolius (H. pusillus)			
		H. velutinus			
		H. uncinatus			
		H. foliosus			
		H. rigidus	Ethanolic extract	No inhibition	(Morales *et al*., 2003)
			Chloroform extract		
		H. baylahuen	Dichloromethane extract	100% inhibition at 1000 µg/mL	(Brodkiewicz *et al*., 2017)
			Methanolic extract	40% inhibition 1000 µg/mL	
Bacillus coagulans	2	*H. uncinatus*	Methanolic solution of resinous extract	MIC 125 µg/mL	(Urzúa & Mendoza, 2001)
		H. foliosus		MIC 1000 µg/mL	
			Resinous exudate	MIC 5 µg/mL	(Urzúa *et al*., 2003)

Antifungal properties of *Haplopappus* extracts

The antifungal activity was evaluated in only five species, with inhibitory effects observed against *Acremonium falciforme* and *Fusarium oxysporum*, as shown in Table 3. Complete inhibition was reported for decoction extracts, while slight or partial inhibition was observed with ethanolic and ethyl acetate extracts of *H. baylahuen* against *A. falciforme*. Additionally, the essential oil of *H. baylahuen* exhibited antifungal activity against *F. oxysporum*, with an antifungal index ranging from 54% to 78%. The antifungal index was calculated using the formula: Antifungal Index = (1 – Da/Db) x 100%, where Da is the diameter of the zone of inhibition on the test plate, and Db is the diameter of the zone of inhibition on the control plate (Wang *et al*., 2005).

These *Happlopappus* extracts were also studied in *Candida albicans, Candida tropicalis,* and *Saccharomyces cerevisiae*. Alcoholic extracts, infusions, resinous exudates, chloroform extract, decoction, and ethyl acetate extracts were evaluated in *C. albicans* (Lazo, 1990; Urzúa *et al*., 1995; Morales *et al*., 2003; Padilla *et al*., 2021). Inhibition of *C. tropicalis* was tested with ethanolic extracts and infusions (Padilla *et al*., 2021), and *S. cerevisiae* inhibition was studied with resinous exudates (Urzúa *et al*., 1995). However, no significant effects were found.

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Table 3: Antifungal effect of extracts of *Haplopappus* species and the frequency with which this activity was reported for each fungal species. The frequency is given by the number of original research articles that describe inhibitory effects for these microbial species. This table only shows the fungal species studied in which inhibitory effects were reported in at least one article.

Fungal species	Frequency per article	*Haplopappus* species	Type of extract	Results	References
Acremonium falciforme	1	*H. baylahuen*	Decoction	Total inhibition of development	(Lazo, 1990)
			Ethanolic extract	Low or partial inhibition of development	
			Ethyl acetate extract		
Fusarium oxysporum	1	*H. baylahuen*	Essential oil	Antifungal index between 54% to 78%	(Becerra *et al*., 2010)

Discussion

Traditionally, the term "bailahuén complex" is used in Chile to describe a mixture of *Haplopappus* spp. However, it remains unclear which species are included in this complex or whether all Chilean species are considered part of *bailahuén*. Despite the many different extraction methods reported in the literature, the infusion is the only *Haplopappus* extract recognized by the MINSAL as a traditional herbal medicine (MINSAL, 2010). *Bailahuén* infusions are traditionally used to treat liver ailments, abdominal colic, chronic indigestion (dyspepsia), urinary tract diseases (such as kidney stones), flu, colds, and even as an aphrodisiac for male impotence. Also, these infusions are applied externally to clean wounds and skin ulcers (MINSAL, 2010). Given this traditional external use, this review focuses on the evidence supporting the potential antimicrobial properties of *Haplopappus* spp., extracts.

The articles analyzed in this review demonstrated the antimicrobial activity of various extracts, with most studies evaluating the activity of resinous exudates and ethanolic extracts. Antibacterial activity was primarily observed in gram-positive bacteria, with *S. aureus* showing the highest susceptibility to diverse extracts and *Haplopappus* species. This species was followed by *B. subtilis, B. cereus,* and *B. coagulans*, which exhibited the most potent activities based on their MICs. However, comparing results across studies is challenging due to variations in how antimicrobial activity is reported. Some authors describe the activity qualitatively as inhibition, partial inhibition, or total inhibition (Lazo, 1990) or report MICs by mass (Urzúa *et al*., 2003; Urzúa *et al.,* 2012), while most studies present results as ZOI (mm) or MIC (µg/mL). In the case of antifungal activity, some studies calculated an antifungal index, comparing the extract's activity to ketoconazole, but without clearly describing the extract's specific effects (Becerra *et al*., 2010).

The minimum inhibitory concentration (MIC) serves as a parameter for describing the activity and potency of a given compound against a microorganism. Focusing on this parameter, we found that resinous exudates from *H. foliosus, H. litoralis*, and *H. chrysanthemifolius* exhibited the most potent inhibitory activities, with the lowest MICs of 0.32 µg and 2.5 µg/mL. Since MIC is based on concentration, it is typically expressed in units of µg/mL. The MIC of 2.5 µg/mL for *H. foliosus* resinous exudates against *B. subtilis* represents the most potent activity found in this review, followed by *B. coagulans* with an MIC of 5 µg/mL for the same exudate. Other MICs reported in this review ranged from 250 to 1000 µg/mL, with the latter often representing the highest tested concentration from which several two-fold dilutions are prepared (European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Diseases, 2003).

The antimicrobial potential of *Haplopappus* extracts can be better understood when their chemical composition is linked to known mechanisms of action. Flavonoids and other phenolic compounds, frequently identified in *Haplopappus* species, are reported to impair bacterial membrane integrity, increase permeability, and promote oxidative stress, which correlates with their activity against *Staphylococcus aureus* and *Bacillus spp.* (Jubair *et al.*, 2021). Terpenoids, including clerodane-type diterpenes described in *H. uncinatus*, are lipophilic molecules capable of intercalating into bacterial membranes, causing destabilization and leakage of intracellular contents, as observed against *B. subtilis* and *B. cereus* (Faini *et al.*, 2011). The identification of such metabolites in extracts that show low MICs highlights their potential as therapeutic candidates, particularly in the management of infections caused by clinically significant pathogens such as *S. aureus* and *B. cereus*.

B. coagulans is a probiotic widely used in medicine and the food industry, with studies also reporting therapeutic effects on intestinal diseases through microbiota modulation (Mu & Cong, 2019). In contrast, *B. cereus* and *B. subtilis* are commonly associated with food poisoning and gastrointestinal symptoms such as nausea and vomiting (Jessberger *et al*., 2020). *B. cereus* is considered an environmental contaminant in clinical settings. It can cause severe wound and device-associated infections, such as those affecting the respiratory tract or resulting from contact lens use (Ehling-Schulz *et al*., 2019). It also expresses several virulence factors, including enterotoxins, beta-lactamases, and proteases (Kotiranta *et al*., 2000).

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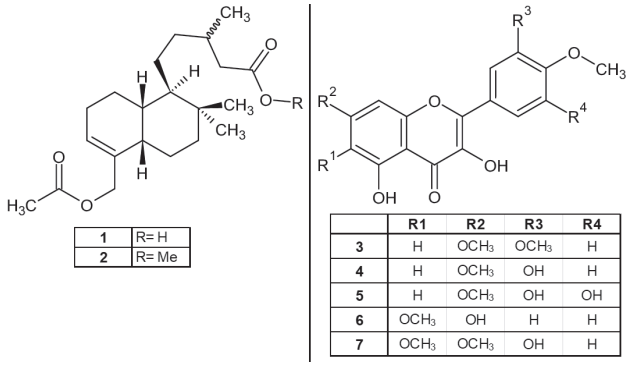
On the other hand, *S. aureus* is a major human pathogen responsible for respiratory and wound infections, commonly found in the normal human flora and the environment. The ability to express adhesins, along with the production of virulence factors such as α-hemolysin and protein A, contributes to its persistence in respiratory infections, causing severe tissue damage and poor clinical outcomes (Pivard *et al*., 2021). Furthermore, *S. aureus* is one of the most frequently isolated bacteria from complex wounds (Serra *et al*., 2015).

Given the clinical relevance of *S. aureus* and *B. cereus*, further investigation into the chemical constituents of the resinous exudates from *H. foliosus, H. litoralis,* and *H. chrysanthemifolius*, as well as their mechanisms of action, is warranted as a promising avenue for developing alternative treatments.

The chemical constituents of resinous exudates tested for antimicrobial activity, as shown in Figure 3, were identified in the resinous exudates of *H. uncinatus* (compounds 1, 2, and 7) (Urzúa *et al*., 2006), *H. litoralis* (compounds 3 and 4), *H. chrysanthemifolius* (compounds 4 and 5), and *H. scrobiculatus* (compounds 6 and 7) (Urzúa *et al*., 2012). Among these, the only compound exhibiting antimicrobial activity was compound 1, a bicyclic diterpenoid, which showed activity against *B. subtilis* (MIC 63 µg/mL), *B. cereus* (MIC 125 µg/mL), *M. luteus* (MIC 32 µg/mL), and *C. michiganensis* subsp. *michiganensis* (MIC 32 µg/mL) (Urzúa *et al*., 2006).

Clerodane is a family of bicyclic diterpenoid compounds found in various plant species, including those from the Lamiaceae, Verbenaceae, and Asteraceae families. Within the Asteraceae family, these compounds have been reported in several *genus*, including *Aster, Baccharis, Conyza, Haplopappus, Microglossa, Nannoglottis,* and *Pulicaria* (Li *et al*., 2016). Members of this compound family have shown promising antimicrobial activity and exhibit additional bioactivities, including antifeedant (Li *et al*., 2016), antitumoral (Hayashi *et al*., 2002), and anti-inflammatory effects in both *in vitro* and *in vivo* models (Feng *et al*., 2020).

Although the mechanism of action of clerodanes is not yet fully understood, Bhattacharya *et al*. investigated 16α-hydroxycleroda-3,13(14) Z-dien-15,16-olide, observing antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Neurospora crassa*. Alterations in the cell membrane permeability and an increase in reactive oxygen species (ROS) were identified, which may explain its greater efficacy against Gram-positive bacteria, characterized by the presence of a single plasma membrane (Bhattacharya *et al*., 2015).



	R1	R2	R3	R4
3	H	OCH_3	OCH_3	H
4	H	OCH_3	OH	H
5	H	OCH_3	OH	OH
6	OCH_3	OH	H	H
7	OCH_3	OCH_3	OH	H

Figure 3: Structure of compounds isolated from resinous exudate from *H. uncinatus, H. litoralis, H. chrysanthemifolius,* and *H. scrobiculatus.*

Plant extracts may exhibit activities against microorganisms beyond growth inhibition, such as the disruption or prevention of biofilm formation. An example of antibiofilm activity is the use of cranberry juice to treat or prevent UTI, based on compounds in the juice that inhibit bacterial adhesion and, consequently, biofilm formation (Reid *et al*., 2001). A widely used technique for determining bacterial adhesion is the crystal violet assay, which utilizes a dye that binds to negatively charged surface molecules and the polysaccharide matrix (Xu *et al*., 2016), enabling the quantification of biofilm biomass adhered to a surface.

The study by Elgueta *et al.* (2021) is the only one applying this technique to evaluate the effects of *H. baylahuen* extracts. This study found that the ethanolic extract of *H. baylahuen* reduced the adhesion of *Salmonella enterica* subsp. *enterica* to human intestinal cell cultures, suggesting that this extract may serve as a promising source of antibiofilm compounds.

Various studies have investigated plants with antimicrobial properties within the *Asteraceae* family, including those within the *Eupatorium* genus. Research has examined at least thirty *Eupatorium* species (Antonio *et al*., 2017). One such species is *E. salvium*, whose resinous exudate has been tested for its MIC against ten bacterial species. Notably, it exhibited activity against five gram-positive bacterial species, including *S. aureus* (MIC 250 µg/mL) and *M. luteus* (MIC 63 µg/mL). Still, it did not show activity against gram-negative bacteria, such as *E. coli*, as observed for *H. baylahuen* (Urzúa *et al*., 1998).

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Conclusion

This review analyzed the antimicrobial activity of various *Haplopappus* spp. extracts. *Haplopappus* species demonstrated higher activity against gram-positive bacteria, with little to no antimicrobial activity observed against gram-negative bacteria and fungi. While abundant evidence supports the antimicrobial efficacy of the extracts against *S. aureus*, there is insufficient evidence to confirm the efficacy of the traditionally used infusion. Instead, the most promising activity has been reported for resinous exudates and ethanolic extracts.

When comparing the reported MICs, the resinous exudates of *H. foliosus, H. litoralis,* and *H. chrysanthemifolius* exhibited the most potent inhibitory activity against *S. aureus* and *B. cereus*. Therefore, further research using standardized methodologies is required to strengthen the existing evidence. Additionally, studies on isolated compounds from these extracts, such as those from the clerodane family, are recommended as potential adjuvants in antimicrobial treatments.

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Authors contributions:

H Garrido-Vera: Formal analysis, investigation, and writing original draft; C Delgado-Gazzoni: writing - reviewing and editing; W Vera-Quezasa: Methodology and supervision; T Bahamondez-Cañas: Conceptualization, supervision and writing - reviewing and editing

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Supplementary material

Antibacterial effect of extracts of *Haplopappus* species and the frequency per article in which this activity was described for each bacterial species. The frequency is given by the number of original research articles that describe inhibitory effects for these microbial species. This table only shows the bacterial species studied in which inhibitory effects were reported in at least one article.

Bacterial specie	Frequency per article	*Haplopappus* species	Extract	Results	Reference
Staphylococcus aureus	9	*H. uncinatus*	Resinous exudate	No inhibition	(Urzúa *et al.*, 2006)
				Inhibition zone 11-13 mm	(Urzúa *et al.*, 1995)
		H. foliosus		MIC 1000 µg/mL	(Urzúa & Mendoza, 2001)
				MIC 500 µg/mL	
				MIC 2.5 µg	(Urzúa *et al.*, 2003)
		H. diplopappus		Inhibition zone 9-10 mm	(Urzúa *et al.*, 1995)
		H. anthylloides			
		H. schumannii			
		H. pusillus			
		H. velutinus			
		H. illinitus			
		H. multifolius		Inhibition zone 11-13 mm	
			Ethanolic extract	Inhibition zone 9.3 mm	(Padilla *et al.*, 2021)
			Infusion	Inhibition zone 12.4 mm	
		H. taeda	Ethanolic extract	Inhibition zone 7.9 mm	
			Infusion	Inhibition zone 7.2 mm	
		H. rigidus	Ethanolic extract	Inhibition zone 11-20 mm	(Morales *et al.*, 2003)
			Chloroform extract	Inhibition zone 6-10 mm	
		H. litoralis	Resinous exudate	MIC 1.25 µg	(Urzúa *et al.*, 2012)
		H. chrysanthemifolius		MIC 2.5 µg	
		H. scrobiculatus			
		H. baylahuen	Decoction	Total inhibition	(Lazo, 1990)
			Ethanolic extract		
			Ethyl acetate extract		
			Dichloromethane extract	45% inhibition at 1000 µg/mL	(Brodkiewicz *et al.*, 2017)
			Methanolic extract		
Bacillus subtilis	8	*H. uncinatus*	Resinous exudate	MIC 63 µg/mL	(Urzúa *et al.*, 2006)
		H. diplopappus			
		H. velutinus			
Escherichia coli	8	*H. illinitus*	Resinous exudate	No inhibition	(Urzúa *et al.*, 1995)
		H. anthylloides			
		H. schumannii		Inhibition zone 9-10 mm	
		H. cuneifolius (H. pusillus)			
		H. multifolius	Ethanolic extract	No inhibition	(Elgueta *et al.*, 2021)
			Infusion		
		H. taeda	Ethanolic extract		
			Infusion		
		H. rigidus	Ethanolic extract		(Morales *et al.*, 2003)
			Chloroform extract		
		H. baylahuen	Decoction		(Lazo, 1990)
			Ethanolic extract		
			Ethyl acetate extract		
		H. litoralis	Resinous exudate		(Urzúa *et al.*, 2012)
		H. chrysanthemifolius			
		H. scrobiculatus			
		H. diplopappus			
		H. velutinus			

Escherichia coli (continuation)	8	*H. illinitus*	Resinous exudate	No inhibition	(Urzúa *et al.*, 1995)
		H. anthylloides		Inhibition zone 9-10 mm	
		H. schumannii			
		H. cuneifolius (H. pusillus)			
		H. multifolius	Ethanolic extract	No inhibition	(Elgueta *et al.*, 2021)
			Infusion		
		H. taeda	Ethanolic extract		
			Infusion		
		H. rigidus	Ethanolic extract		(Morales *et al.*, 2003)
			Chloroform extract		
		H. baylahuen	Decoction		(Lazo, 1990)
			Ethanolic extract		
			Ethyl acetate extract		
		H. litoralis	Resinous exudate		(Urzúa *et al.*, 2012)
		H. chrysanthemifolius			
		H. scrobiculatus			
Proteus vulgaris	6	*H. uncinatus*	Resinous exudate	No inhibition	(Urzúa *et al.*, 2006)
				Inhibition zone 11-13 mm	(Urzúa *et al.*, 1995)
			Methanolic solution of resinous extract	No inhibition	(Urzúa & Mendoza, 2001)
		H. foliosus	Resinous exudate		(Urzúa *et al.*, 2003)
				Inhibition zone 11-13 mm	(Urzúa *et al.*, 1995)
		H. illinitus	Resinous exudate	No inhibition	(Urzúa *et al.*, 1995)
		H. diplopappus		Inhibition zone 9-10 mm	
		H. anthylloides			
		H. schumannii			
		H. cuneifolius (H. pusillus)			
		H. velutinus		Inhibition zone 11-13 mm	
		H. multifolius		Inhibition zone 9-10 mm	
			Ethanolic extract	No inhibition	(Padilla *et al.*, 2021)
			Infusion		
		H. taeda	Ethanolic extract		
			Infusion		
		H. litoralis	Resinous exudate		(Urzúa *et al.*, 2012)
		H. chrysanthemifolius			
		H. scrobiculatus			
Bacillus cereus	5	*H. uncinatus*	Resinous exudate	MIC 250 µg/mL	(Urzúa *et al.*, 2006)
			Methanolic solution of resinous extract		(Urzúa & Mendoza, 2001)
		H. foliosus	Resinous exudate	MIC 2.5 µg	(Urzúa *et al.*, 2003)
		H. litoralis		MIC 0.32 µg	(Urzúa *et al.*, 2012)
		H. chrysanthemifolius		MIC 0.32 µg	
		H. scrobiculatus		MIC 1.25 µg	
		H. multifolius	Ethanolic extract	Inhibition zone 8.6 mm	(Padilla *et al.*, 2021)
			Infusion	No inhibition	
		H. taeda	Ethanolic extract	Inhibition zone 15.2 mm	
			Infusion	Inhibition zone 12.2 mm	

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Micrococcus luteus	5	*H. foliosus*	Resinous exudate	Inhibition zone 11-13 mm	(Urzúa *et al.*, 1995)
				MIC 2.5 µg	(Urzúa *et al.*, 2003)
			Methanolic solution of resinous extract	MIC 250 µg/mL	(Urzúa & Mendoza, 2001)
		H. uncinatus		MIC 500 µg/mL	
					(Urzúa *et al.*, 2006)
		H. multifolius	Resinous exudate	Inhibition zone 9-10 mm	(Urzúa *et al.*, 1995)
		H. illinitus		No inhibition	
		H. schumannii		Inhibition zone 9-10 mm	
		H. pusillus			
		H. velutinus			
		H. diplopappus		Inhibition zone 11-13 mm	
		H. anthylloides			
		H. litoralis		MIC 2.5 µg	(Urzúa *et al.*, 2012)
		H. chrysanthemifolius			
		H. scrobiculatus		MIC 1.25 µg	
Pseudomonas aeruginosa	4	*H. taeda*	Ethanolic extract	No antimicrobial activity was observed	(Padilla *et al.*, 2021)
			Infusion		
		H. multifolius	Ethanolic extract		
			Infusion		
		H. diplopappus	Resinous exudate	Inhibition zone 9-10 mm	(Urzúa *et al.*, 1995)
		H. anthylloides			
		H. illinitus			
		H. schumannii	Resinous exudate	No inhibition	(Urzúa *et al.*, 1995)
		H. cuneifolius (H. pusillus)			
		H. velutinus			
		H. uncinatus			
		H. foliosus			
		H. rigidus	Ethanolic extract	No inhibition	(Morales *et al.*, 2003)
			Chloroform extract		
		H. baylahuen	Dichloromethane extract	MIC 1000 µg/mL	(Brodkiewicz *et al.*, 2017)
			Methanolic extract	40% inhibition 1000 µg/mL	
Clavibacter michiganensis subsp michiganensis	3	*H. uncinatus*	Resinous exudate	MIC 63 µg/mL	(Urzúa *et al.*, 2006)
		H. foliosus		MIC 250 µg/mL	(Urzúa & Mendoza, 2001)
		H. litoralis		No antimicrobial activity was observed	(Urzúa *et al.*, 2012)
		H. chrysanthemifolius			
		H. scrobiculatus			
Bacillus coagulans	2	*H. uncinatus*	Methanolic solution of resinous extract	MIC 125 µg/mL	(Urzúa & Mendoza, 2001)
		H. foliosus		MIC 1000 µg/mL	
			Resinous exudate	MIC 5 µg/mL	(Urzúa *et al.*, 2003)
Enterococcus faecalis	2	*H. taeda*	Ethanolic extract	No antimicrobial activity was observed	(Padilla *et al.*, 2021)
			Infusion		
		H. multifolius	Ethanolic extract		
			Infusion		
		H. rigidus	Chloroform extract	Inhibition zone 6-10 mm	(Morales *et al.*, 2003)
			Ethanolic extract		
Salmonella enterica subsp enterica serovar	2	*H. taeda*	Ethanolic extract	No antimicrobial activity was observed	(Padilla *et al.*, 2021)
			Infusion		
		H. multifolius	Ethanolic extract		
			Infusion		
		H. baylahuen	Ethanolic extract	MIC 11 mg/mL	(Elgueta *et al.*, 2021)

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Staphylococcus epidermidis	2	*H. taeda*	Ethanolic extract	Inhibition zone 6.8 mm	(Padilla *et al.*, 2021)
			Infusion	No inhibition	
		H. multifolius	Ethanolic extract	Inhibition zone 8.7 mm	
			Infusion	Inhibition zone 6.8 mm	
		H. illinitus	Resinous exudate	No inhibition	(Urzúa *et al.*, 1995)
		H. diplopappus		No antimicrobial activity was observed	
		H. anthylloides			
		H. schumannii		Inhibition zone 9-10 mm	
		H. cuneifolius (H. pusillus)			
		H. velutinus			
		H. uncinatus			
		H. foliosus			
Bacillus anthracis	1	*H. diplopappus*	Resinous exudate	Inhibition zone 9-10 mm	(Urzúa *et al.*, 1995)
		H. anthylloides			
		H. schumannii			
		H. cuneifolius (H. pusillus)			
		H. multifolius			
		H. illinitus			
		H. velutinus		Inhibition zone 11-13 mm	
		H. uncinatus			
		H. foliosus			
Bacillus pumilus	1	*H. schumannii*	Resinous exudate	Inhibition zone 9-10 mm	(Urzúa *et al.*, 1995)
		H. cuneifolius (H. pusillus)			
		H. multifolius			
		H. foliosus			
		H. diplopappus			
		H. anthylloides		Inhibition zone 11-13 mm	
		H. velutinus			
		H. uncinatus			
		H. illinitus			
Bordetella bronchiseptica	1	*H. anthylloides*	Resinous exudate	No inhibition	(Urzúa *et al.*, 1995)
		H. cuneifolius (H. pusillus)			
		H. foliosus			
		H. diplopappus			
		H. schumannii		Inhibition zone 9-10 mm	
		H. velutinus			
		H. uncinatus			
		H. multifolius	Resinous exudate	Inhibition zone 11-13 mm	(Urzúa *et al.*, 1995)
		H. illinitus			
Micrococcus flavus	1	*H. foliosus*	Resinous exudate	No inhibition	(Urzúa *et al.*, 1995)
		H. schumannii		Inhibition zone 9-10 mm	
		H. cuneifolius (H. pusillus)			
		H. velutinus			
		H. uncinatus			
		H. multifolius			
		H. illinitus			
		H. diplopappus		Inhibition zone 11-13 mm	
		H. anthylloides			
Streptococcus agalactiae	1	*H. multifolius*	Ethanolic extract	No antimicrobial activity was observed	(Padilla *et al.*, 2021)
			Infusion		
		H. taeda	Ethanolic extract	Inhibition zone 7.4 mm	
			Infusion	Inhibition zone 8.5 mm	

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Streptococcus pyogenes	1	*H. multifolius*	Ethanolic extract	No antimicrobial activity was observed	(Padilla *et al*., 2021)
			Infusion	Inhibition zone 10.9 mm	
		H. taeda	Ethanolic extract	Inhibition zone 7.4 mm	
			Infusion	Inhibition zone 12.1 mm	

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