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Anti-phytopathogenic activities and chemical composition of *Ulex europaeus* L. extracts

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Abstract

Ulex europaeus L. (Fabaceae) is a plant species introduced in Chile and is known as one of the most invasive plants in the world, whose biological potential has been scarcely explored. The objectives of this study were to perform a chemical characterization and to analyze the anti-phytopathogenic activity of *U. europaeus* L. extracts for their potential application in agriculture. GC-MS analysis identified high amounts of terpenoids in three organic extracts (hexane, ethyl acetate, ethanol). The most abundant compounds were β-amyrin, lupeol, dl α-tocopherol, sitosterol and neophytadiene; all of these are known to possess antimicrobial properties. The ethanolic extract of *U. europaeus* had the highest antioxidant activity and phenolic content. The activity against phytopathogens was evaluated on bacteria (*Pectobacterium carotovorum* NCPPB 312, *Pseudomonas syringae* NCPPB 281, *Rhizobium radiobacter* C58C1), fungi (*Botrytis cinerea*, *Gibberella fujikuroi* IMI 58289) and one oomycete (*Phytophthora cinnamomi*). The ethanolic extract produced the highest growth inhibition of the bacteria (100±3.4%; 90±3.4% and 100±4.8%, respectively), whereas the ethyl acetate extract produced the highest inhibition of the fungi (65±1.4% and 100±0.1%, respectively) and the oomycete (100±1%). These results open new alternatives to use the invasive plant *U. europaeus* L. in agri-food areas for the control of phytopathogens.

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Introduction

Invasive plants are a major problem for the Chilean flora with numerous species threatening the maintenance of biodiversity, function of natural ecosystems and cause productive losses. For example, *Ulex europaeus* L. (commonly known as gorse) is a species of the Fabaceae family that was introduced in Chile in the nineteenth century to contain livestock and to feed animals (Norambuena et al. 2000). However, *U. europaeus* is highly invasive in many parts of the world (Altamirano et al., 2016) and it is widely distributed in Southern and Central Chile, competing for land used in agriculture, silviculture and forestry (Norambuena et al. 2000; Altamirano et al., 2016). Studies that contribute to the search for interesting biological properties in the pharmacological, agricultural and industrial areas could change the negative concept that is attributed to an invasive plant whose new uses (added value) could mitigate its cost of management and removal.

The Fabaceae family has been characterized by the presence of terpenic compounds flavonoids and alkaloids that has been associated with antidiabetic, anti-inflammatory and antimicrobial activities (Dzoyem et al., 2014; Wink, 2003). In particular, *U. europaeus* has been shown to contain important defensive secondary metabolites, especially alkaloids such as anagrin, cytisine, *N*-methylcytisine and lupanine that are related to defence mechanism against herbivores, insects and microorganisms, and may also play a role in allelopathic interrelations (Máximo and Lourenço, 2000; Máximo et al., 2006; Hornoy et al., 2012).

Ulex species are rich in phenolic compounds, such as isoflavones and pterocarpan. Some of these compounds are phytoalexins that have insecticidal or cytotoxic effects (Máximo et al., 2002). Chalcone glucosides and five flavonol glucosides (the 7-, the 4'-glucoside and the 3,7-diglucoside of quercetine and two glucosides of 3,7,4'-trihydroxyflavone) have been reported in gorse flowers (Harborne, 1962). Four novel complex isoflavones ulexones A, B, C and D, genistein and 5-O-methylgenistein were isolated from root bark of *U. europaeus* (Russel et al., 1990).

The non-saponifiable fraction of *U. europaeus* extracts has a high content of terpenic compounds such as β-amyrin, lupeol and β-sitosterol. In addition, Soyasapogenol C was identified from the ethanolic extract (McLean and Thompson, 1963). Some of these compounds have antibacterial and antifungal activities (Corrêa et al., 2014). However, the effects of *U. europaeus* extracts on plant pathogens has not been addressed.

The aim of this study was to investigate the potential of *U. europaeus* applicable in the agricultural sector and to promote added value, by identifying the ability of its extracts to control the growth of important phytopathogens.

This study characterized the organic extracts of *U. europaeus* from Southern Chile and evaluated their potential inhibitory effect on the growth of phytopathogenic bacteria, fungi and an oomycete because these phytopathogens infect crops of high economic importance, such as tomato, avocado and berries as well as ornamental plants (Xin and He, 2013; Dehgahi et al., 2015).

Materials and Methods

Chemical characterization of U. europaeus L. extracts

Plant material and organic extraction

Samples of *U. europaeus* were collected from Colaco (41°45'48.80"S, 73°21'44.2"W), Región de los Lagos, Chile. Fresh leaves and branches were collected, washed under running tap water and then immediately transported to the laboratory to obtain the extracts.

The samples were air-dried and crushed. The vegetative material was extracted using maceration with three organic solvents of different polarity: hexane, ethyl acetate and ethanol, sequentially. The extracts were dried on sodium sulphate. The solvent was evaporated using a rotatory evaporator and stored in dark glass bottles (sealed amber vials) at 4 °C until the chemical analysis and biological activities were performed. The extracts obtained with the three solvents (hereafter S1, S2 and S3 for hexane, ethyl acetate and ethanol, respectively) were evaluated for chemical composition, antioxidant activity and antimicrobial activity for plant pathogenic bacteria, fungi and an oomycete.

The percentage yield of the extracts was determined based on the dry weight (d.w.) using Equation 1):

$$Yield(\%) = \frac{Weight\ extract(g)}{Weight\ of\ dried\ milled\ plant(g)} \times 100 \quad (1)$$

Gas chromatography/mass spectrometry

The samples were analyzed using a Gas Chromatograph Thermo Scientific trace 1310 with an ISQ/LTQ quadruple mass spectrometer in splitless mode (5 min). Helium was used as the carrier gas at constant flow (1.0 mL/min). Separation was conducted in a 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness Rtx-5MS column (Restek; USA). The oven was programmed as follows: initial temperature of the column

at 100 °C (1 min hold) followed by ramping up at 20 °C/min until 160 °C (6 min hold), a second ramping up of 3 °C/min until 238 °C (2 min hold) and a final ramp up to 290 °C with a post ramp period of 15 min. The parameters of the detector were: an ion mass/charge ratio of 50:500, an electron impact ionization source temperature of 230 °C and an interface temperature of 250 °C, in scan mode.

The samples were analyzed by matching with specimens in the National Institute of Standard and Technologies (NIST) MS spectral library (2014).

Antioxidant activity as Trolox equivalent antioxidant content

The antioxidant content was evaluated by measuring the scavenging activity of the examined extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals as described (Carvajal et al., 2016), with modifications to adapt the screen for 96-well plates. A Thermo Scientific Multiskan GO 96-well plate spectrophotometer (Waltham, MA, USA) was used for analysis of the sample. A stock solution of essential oil was prepared in methanol at a concentration of 1 mg/mL. Methanol (90 µL), each sample solution (150 µL) and DPPH (60 µL, Sigma-Aldrich) in methanol (0.5 mM)—resulting in a final concentration of 0.1 mM DPPH—were added in a 96-well plate. Methanolic extract solution without DPPH but with additional methanol instead (60 µL) was used as the blank sample. The mixtures were incubated at 25 °C for 30 min and the absorbance was measured at 517 nm. The antioxidant content was determined using a standard curve (0–0.05 mM) of Trolox (Sigma-Aldrich; St Louis, MO, USA) and each solution was treated under equivalent conditions to the extracts. The average of three values was obtained, expressed as micro moles of Trolox equivalent antioxidant content (TEAC) per gram of extract using the regression equation, obtained from the calibration curve (Equation 2):

$$y = -0.0145x+0.9035 \ (R^2 = 0.9917) \quad (2)$$

where R2 is the coefficient of determination.

All extracts were analyzed in triplicate and results are expressed as average ± SD.

Ferric reducing antioxidant potential assay

The ferric reducing power of plant extracts was measured as described (Jara et al., 2017) with modifications to adapt to 96-well plate screening assay. This method was based on the reduction at low pH of a colorless ferric complex (Fe^{3+}-tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donating antioxidants. The reduction was monitored by measuring the change of absorbance at 620 nm on a spectrophotometer (Multiskan FC 96-well plate photometer; Thermo Scientific; Waltham, MA, USA). The ferric reducing antioxidant potential (FRAP) reagent was prepared daily by mixing 10 volumes of 300 mM acetate buffer at pH 3.6 with 1 volume of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. A standard curve was prepared using various concentrations of Trolox (0–1,000 µM). All solutions were used on the day of preparation. Sample solutions (10 µL) were added to freshly prepared FRAP reagent (300 µL). The reaction mixtures were incubated for 30 min at 37°C in an oven. Then, the absorbance of the samples was measured at 620 nm. A mixture of methanolic sample solution (10 µL) with 300 mM acetate buffer, pH 3.6 (300 µL) was used as blank for each sample. The difference between sample absorbance and blank absorbance was calculated and used to calculate the FRAP value. In this assay, the reducing capacity of the plant extracts tested was calculated with reference to the reaction signal given by a Trolox solution. FRAP values were expressed in micro moles Trolox per gram of sample. Additional dilutions were done if the absorbance value measured was outside the linear range of the standard curve. All measurements were performed in triplicate. The average of three values was obtained, expressed as µmol of Trolox per g of extract using the regression equation, obtained from the calibration curve (Equation 3):

$$y = 1821.7x + 10.664 \ (R^2 = 0.9967) \quad (3)$$

Determination of total phenolic content

The amount of total phenolic compounds in fruits was determined using the Folin-Ciocalteu (FC) method as described (Jara et al., 2017) with some modifications. Sample solution (15 µL) was placed in a 96-well plate and mixed with distilled water (15 µL) and 0.2 N FC reagent (150 µL). After 5 min, 3% sodium carbonate solution (120 µL) was added. The mixtures were incubated in the dark at room temperature for 2 hr. A mixture of distilled water (15 µL), methanolic sample solution (15 µL), HCl 0.2 N (150 µL) and 3% sodium carbonate solution (120 µL) was used as blanks for each sample. The absorbance of the solution was measured at 690 nm on a spectrophotometer (Thermo Scientific Multiskan FC 96-well plate photometer; Waltham, MA, USA) and the results were

expressed in gallic acid equivalents (GAE; mg/g extract) using a gallic acid (0–200 mg/L) standard curve. Additional dilutions were carried out if the absorbance value measured was over the linear range of the standard curve. All measurements were replicated three times. Data were expressed as milligrams of gallic acid equivalents per gram of extract using the regression equation obtained from the calibration curve (Equation 4):

$$y = 0.0023x + 0.0419 \ (R^2 = 0.9986) \quad (4)$$

Antimicrobial assays

In vitro antibacterial activity assays

Liquid-dilution methods were used to evaluate the antibacterial effect of each of the extracts on *Pectobacterium carotovorum* (NCPPB 312), *Rhizobium radiobacter* (formerly *Agrobacterium tumefaciens*) (C58C1) and *Pseudomonas syringae* (NCPPB 281). The bacteria were grown in sterile tubes with 10 mL of Müller-Hinton (MH) broth and incubated at 27°C for 12 hr. The antibacterial activity of the extracts was evaluated by the determination of growth of microorganisms based on measuring turbidity at 595nm at different extract concentrations (Carvajal et al., 2016). All assays were carried out in sterile 96-well microplates with a final volume of 200 µL containing MH broth inoculated with 1 µL aliquots of bacterial suspension (1×10^5–1×10^6 colony forming units/mL, initial culture) in the presence of different concentrations of extract (312.5 µg/mL, 625 µg/mL, 1,250 µg/mL and 2,500 µg/mL) based on the broth microdilution assay. MH broth was used as the negative control (C[−]) and MH broth with streptomycin (McManus et al., 2002) was used as a positive control (C[+]). The plates were incubated for 6 hr at 27 °C. Bacterial growth was monitored by measuring the turbidity at 595 nm every hour using a microplate reader. For each microorganism, all tests were performed based on 10 repetitions. Bacterial growth was indicated as the arithmetic mean expressed as the percentage of the control growth in the absence of extract. The lowest concentration of extract preventing growth was used to determine the minimal inhibitory concentration (MIC).

In vitro antifungal/anti-oomycete activity assays

A virulent *Botrytis cinerea* strain from naturally infected grape berries (Riggoti et al., 2002), and *Gibberella fujikuroi* strain IMI 58289 (Natural Products and Agrobiology Institute, CSIC, Santa Cruz de Tenerife, Canary Islands, Spain) were studied. The oomycetal *Phytophthora cinnamomi* strain was kindly provided by Instituto de Investigaciones Agropecuarias (INIA; La Platina, Santiago, Chile). The effect of organic extracts of *U. europaeus* on growth inhibition of these strains was performed using the agar-dilution technique (Alizadeh et al., 2007; Carvajal et al., 2018). Antifungal and anti-oomycetal activity assays were performed in microcultures by growing the strains in sterile Petri dishes (35 mm) at a final volume of 2 mL medium containing different extract concentrations. Clarified V8 (Campbell Soup; Camden, NJ, USA) medium was used for *P. cinnamomi*, whereas potato dextrose agar (PDA) medium was used for *B. cinerea* and *G. fujikuroi*. The negative control (C[−]) was represented by mycelial growth in the medium without extract, while the positive control (C[+]) control for *B. cinerea* and *G. fujikuroi* corresponded to growth in medium containing the commercial antifungal captan (CAS 133-06-2; Sigma-Aldrich; St Louis, MO, USA) and the control for *P. cinnamomi* was the commercial anti-oomycete metalaxil (CAS 57837-19-1; Sigma-Aldrich; St Louis, MO, USA). The medium in each slot was inoculated with a small block (4 mm) of clarified V8 or PDA medium containing fungal hyphae excised from the edge of an actively growing culture. The fungal growth was monitored at different times (24–72 hr). To determine the mycelium growth in the agar plate, the surface of the mycelium was measured using the Sigma Scan Pro 5 software (SPSS Science; Chicago, Ill, USA). The results were expressed as a percentage of growth inhibition. Each experiment was performed in triplicate.

Statistical analysis

Data of the anti-phytopathogenic properties of the extracts were evaluated using one-way analysis of variance to identify significant differences among the treatment and control groups. To establish significant differences, the means were compared using Tukey's honestly significant difference test ($p < 0.05$) using the SPSS 13 for Windows software (SPSS Inc; IBM Corp.; Armonk, NY, USA). All data were presented as the mean ± SD.

Results

Analysis of chemical composition of extracts

The identified compounds of the *U. europaeus* ethyl acetate, hexane and ethanol extracts are listed in Tables 1, 2 and 3, respectively. The highest number of compounds (22) were identified in the ethyl acetate extract, whereas 10 compounds were identified in the ethanol and hexane extracts.

Table 1 Chemical composition of hexane extract from *Ulex europaeus*

R.T. (min)	Hexane	Area (%)
17.360	*n*-Hexadecanoic acid	8.2
18.194	Hexadecanoic acid, ethyl ester	0.23
21.506	Phytol	0.37
22.799	Ethyl (9*Z*,11*E*)-octadecadienoate	0.08
22.989	Ethyl 9,12,15-octadecatrienoate	0.27
35.713	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.34
38.454	*dl*-α-Tocopherol	3.93
43.678	Sitosterol	4.6
44.440	*β*-Amyrin	48.1
45.399	Lupeol	23.45

R.T. = retention time

Table 2 Chemical composition of ethyl acetate extract from *Ulex europaeus*

R.T. (min)	Compound	Area (%)
8.218	3-Hydroxy-4-methoxybenzoic acid	3.77
9.681	Benzophenone	2.72
9.946	1-Hexadecanol, 2-methyl-	0.75
11.160	2-Dodecen-1-yl(-)succinic anhydride	0.82
11.874	Coniferyl alcohol	6.30
13.112	2,3,5,5,8a-Pentamethyl-4a,5,6,7,8,8a-hexahydro-4H-chromene	6.97
14.085	Neophytadiene	7.75
16.357	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	1.34
17.292	*n*-Hexadecanoic acid	1.75
18.190	Hexadecanoic acid, ethyl ester	2.16
13.309	*trans*-Sinapyl alcohol	0.89
21.149	9,12,15-Octadecatrienoic acid, methyl ester	0.82
21.465	phytol	5.64
22.799	Ethyl (9*Z*,11*E*)-octadecadienoate	0.46
22.993	Ethyl 9,12,15-octadecatrienoate	3.27
24.360	Phytol, acetate	0.81
26.441	*cis*-13-Eicosenoic acid	1.10
35.709	6a,12a-Dihydro-6H-[1,3]dioxolo[5,6][1]benzofuro[3,2-c]chromen-3-yl hexopyranoside	4.23
36.866	*dl*-α-Tocopherol	12.22
43.651	Sitosterol	11.71
44.396	*β*-Amyrin	10.91
45.375	Lupeol	5.06

R.T. = retention time

The chemical composition of *U. europaeus* L. extracts had a predominance of terpenic compounds, of which *β*-amyrin, lupeol, sitosterol and *dl*-*α*-tocopherol were the most abundant and present in all the extracts, independent of the extraction method. The hexane extract was particularly high in the terpenoid compounds, representing > 80% of total compounds, mainly based on the high contents of *β*-amyrin (48.1%) and lupeol (23.45%). In the ethyl acetate and ethanol extracts, terpenoids were also the most abundant but represented about 50% of the total compounds identified. The hexane and ethyl acetate extracts had substantial fractions of fatty acid derivative compounds (12.47% and 9.12%, respectively). In

addition, the content of phenolic compounds was relatively high (24.88%) in the ethyl acetate extract, while phenols were not identified in the other extracts. Finally, the ethanolic extract was high in 3-*O*-methyl-D-glucose, representing 53.34% of total compounds.

The yields of the hexane, ethyl acetate and ethanol extracts of *U. europaeus* L. were 0.99%, 1.50% and 2.25% (weight per weight), respectively.

Antioxidant activity and total phenolic content of extracts

The extracts of *U. europaeus* L were evaluated for their antioxidant properties based on the TEAC and FRAP tests and total phenolic content (FC). In general, the extracts had different TEAC, FRAP and FC values (Table 5), except for the FC values for hexane and ethyl acetate extracts (18.41 and 15.89 mg GAE/g extract, respectively). The ethanolic extract produced the highest TEAC, FRAP and FC values. The FRAP values had the same trend as the TEAC and FC values though there was less difference. The total phenolic compounds (FC) and antioxidant content (TEAC) were significantly higher in the ethanolic extract than in the others extracts, indicating that the polar extracts had higher antioxidant activity. It was likely that the phenolic compounds were strongly involved in the antioxidant activity of the extracts.

Table 3 Chemical composition of ethanol extract from *Ulex europaeus*

R.T. (min)	Ethanol	Area (%)
8.239	β-D-Glucopyranoside, methyl	0.52
10.127	3-*O*-Methyl-d-glucose	53.34
14.037	1-Heptatriacotanol	0.09
14.089	Neophytadiene	4.28
15.187	Ethanol, 2-(9-octadecenyloxy)-, (*Z*)-	1.04
21.465	Phytol	3.85
38.447	*dl*-α-Tocopherol	2.78
43.641	Sitosterol	9.10
44.402	*β*-Amyrin	16.91
45.365	Lupeol	6.14

R.T. = retention time

Antimicrobial analysis of extracts

The *U. europaeus* L. extracts were tested for the growth inhibition of phytopathogenic bacteria (*R. radiobacter*, *P. carotovorum*, *P. syringae*), fungi (*B. cinerea*, *G. fujikuroi)* and the oomycete *P. cinnamomi*.

For *R. radiobacter*, the highest inhibition was observed with the ethanolic extract, where approximately 90% growth inhibition was determined at a concentration of 1,250 µg/mL, while 100% growth inhibition was observed with a concentration ≥ 2,500 µg/mL (Fig. 1). The hexane extract produced about 50% growth inhibition within the range 625–10,000 µg/mL. Growth inhibition of *R. radiobacter* was < 20% when the ethyl acetate extract was used. In *P. syringae*, at low concentrations (625–1,250 µg/mL), the hexane extract produced the highest antibacterial effect (approximately 80%), while at a high concentration (10,000 µg/mL) the extracts obtained with hexane and ethanol produced the highest levels of growth inhibition (approximately 95%). The ethyl acetate extract was less effective for *P. syringae*, producing similar results to those obtained for *R. radiobacter*. However, for *P. carotovorum*, 100% growth inhibition was produced by the ethanolic extract at a concentration ≥ 2,500 µg/mL. The hexane and ethyl acetate extracts produced growth inhibition up to approximately 80% and 60%, respectively. These results showed that the ethanol extract produced the highest antibacterial activity, whereas the lowest growth inhibition was observed with the ethyl acetate extract. The half-maximal inhibitory concentration (IC_{50}) values of the *U. europaeus* extracts on phytopathogenic bacteria are shown in Table 4.

Table 4 Half-maximal inhibitory concentration (IC_{50}) of *Ulex europaeus* extracts on phytopathogens

Microorganism	Phytopathogen	Extract IC_{50} (µg/mL)		
		Hexane	Ethyl acetate	Ethanol
Bacteria	*P. syringae*	461	>10,000	545
	R. radiobacter	>10,000	>10,000	638
	P. carotovorum	>312.5	1,108	393
Fungi	*B. cinerea*	1,310	1,744	1,966
	G. fujikuroi	756	<350	1,000
Oomycete	*P. cinnamomi*	<350	<350	<350

Table 5 Antioxidant content (TEAC), ferric reducing antioxidant potential (FRAP) and total phenolic content (FC) (mean±SD) of *Ulex europaeus* extracts

Extract	TEAC (µmol Trolox/g extract)	FRAP (µmol Trolox/g extract)	FC (mg GAE/g extract)
Hexane	16.14±0.91	443.78±17.78	18.41±3.66
Ethyl acetate	30.50±0.41	512.09±17.78	15.89±0.74
Ethanol	45.55±0.73	672.04±48.95	43.70±3.42

GAE = gallic acid equivalants

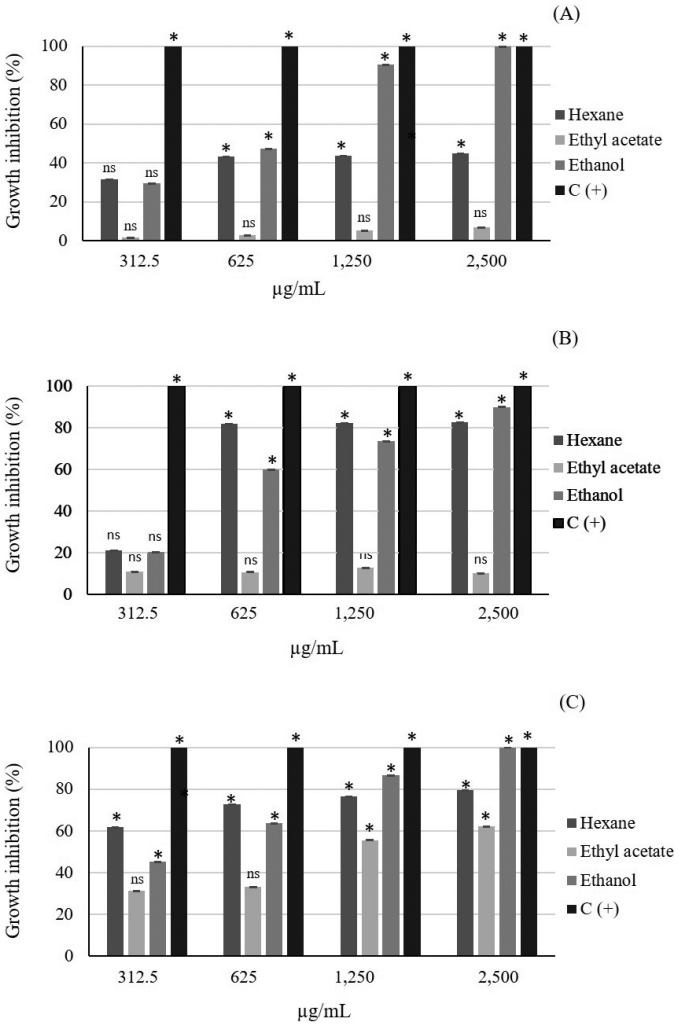


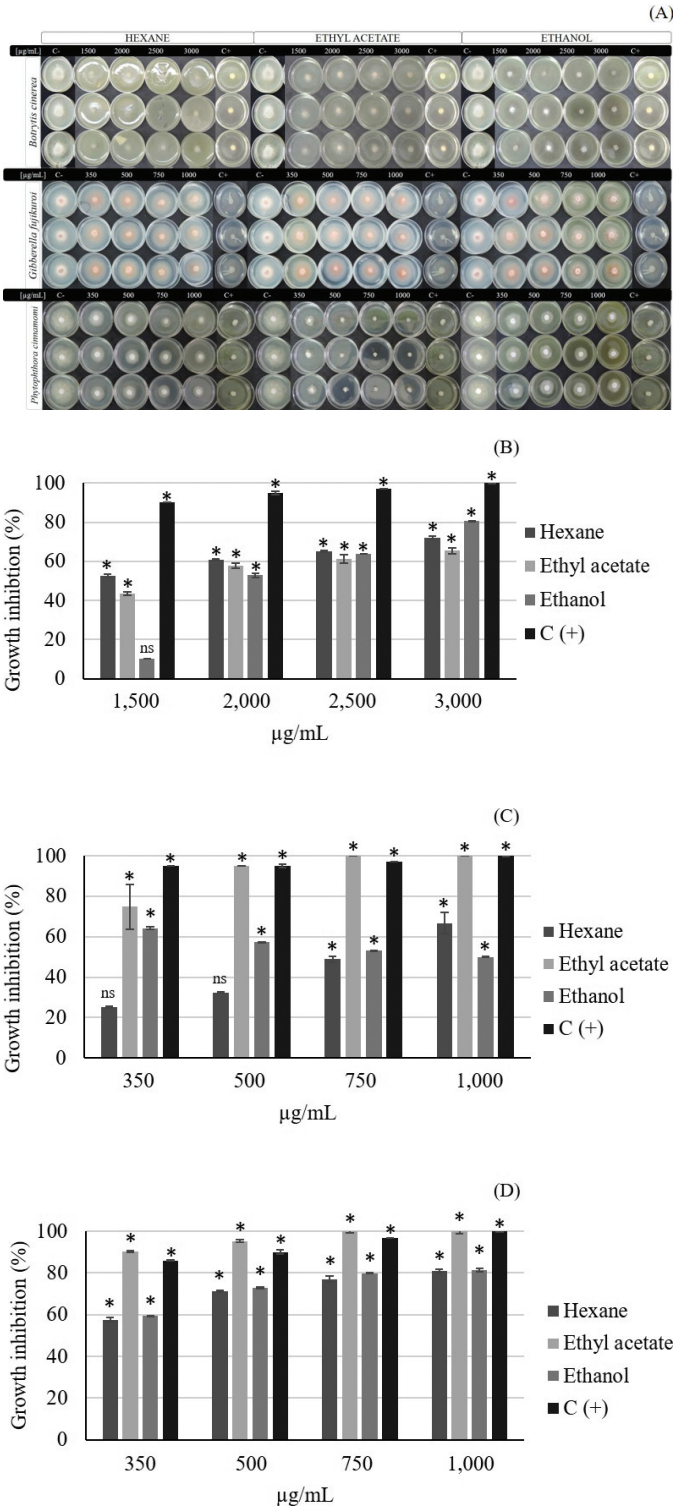
Fig. 1 Effect of extracts of *Ulex europaeus* from Southern Chile on growth of: (A) *R. radiobacter*; (B) *P. syringae*; (C) *P. carotovorum*, where results indicate percentage growth inhibition of bacterial growth measured at 6 hr of incubation, negative control (C[−]) represents bacteria growing in media without extract (100% of growth, data not shown), positive control (C[+]) corresponds to bacteria growing in media containing streptomycin, results are expressed as mean ± SD (n = 10), significant ($p < 0.05$) differences from their respective control (C[−]) treatment are marked with an asterisk and ns marked non-significant difference ($p > 0.05$).

The extracts produced different effects on the fungi and the oomycete compared to the bacteria (Fig. 2). For *B. cinerea*, all the extracts presented similar growth inhibition, except at the lower concentration (1,500 µg/mL), where the highest inhibition (approximately 50%) was observed with the hexane extract. For *G. fujikuroi* and *P. cinnamomi*, the highest inhibition was observed with the ethyl acetate extract, with 100% inhibition at a concentration ≥750 µg/mL. Notably, the ethyl extract produced higher *G. fujikuroi* and *P. cinnamomi* growth inhibition than the commercial fungicide captan or the commercial anti-oomycete metalaxil, respectively. These results indicated that the *U. europaeus* ethyl acetate extract was most effective for antifungal or anti-oomycete use. The IC_{50} values (Table 4) indicated that *P. cinnamomi* (< 350 µg/mL for all extracts) was the most sensitive of the tested eukaryotic microorganisms to the *U. europaeus* extracts, whereas *B. cinerea* was the most resistant tested eukaryote to *U. europaeus* organic extracts.

Discussion

This study investigated the phytochemical characterization of extracts of *U. europaeus* L. from Southern Chile and their antimicrobial activities against common plant pathogens.

This study identified a high prevalence of terpenic compounds (*β*-amyrin, lupeol, *dl*-*α*-tocopherol, *β*-sitosterol) in the hexane, ethyl acetate and ethanol extracts. Terpenoids, such as phytol, *dl*-*α*-tocopherol, *β*-amyrin, lupeol and *β*-sitosterol, have been reported in *U. europaeus* leaf and stem extracts (McLean and Thomson, 1963; Osorio-Castiblanco et al., 2020). Terpenic compounds have been widely described in diverse plant species, playing a key role in plant-environmental interaction and in plant defense mechanisms (Dudareva et al., 2004; Velásquez et al., 2020a; Velásquez et al., 2020b). The growth inhibitory activity of the extracts may be attributed to the high content of terpenoids (80.5%, 54.1% and 43.1% in the hexane, ethyl acetate and ethanol extracts, respectively). The antimicrobial mechanism of action of terpenoids may have been related to the disruption of cell membrane due to



their lipophilic properties. Terpenic compounds may alter the structures of the membrane through increasing its fluidity and permeability and thus creating disturbances across the respiration chain (Paduch et al., 2007). However, terpenes may have affected the bacteria and fungi differentially. Some terpenes are potent inhibitors of eukaryotic protein synthesis, interfering with peptidyl transferase activity or affecting *α*- and *β*-glucane synthesis (compounds of the fungal cell wall), affecting the fungal growth or inducing alterations in the hyphal morphology (Cundliffe et al., 1974; Onishi et al., 2000). In bacteria, it has been shown that terpene alcohols alter leakage of K^+, demonstrating an antibacterial effect by disturbing the cell membrane (Togashi et al., 2010). In the current study, the ethyl acetate extract had low growth inhibition on three Gram-negative bacteria, despite its high terpenoid content. Gram-positive bacteria are generally more susceptible to terpenes than Gram-negative strains (Paduch et al., 2007).

In contrast to the antibacterial effect, the ethyl acetate extract of *U. europaeus* produced the highest fungal/oomycetal growth inhibition. The ethyl acetate extract presented the most diverse composition, with terpenoids as the most prominent group, but also had a high content of phenolic compounds (24.9%). Phenolic compounds include a wide range of bioactive natural compounds with antibacterial and antifungal activity (Daayf et al., 2012). These compounds may act by disturbing the cell membrane, reducing the efflux pump activity or act as DNA gyrase inhibitors (Cámara et al., 2004; Khameneh et al., 2019). Some phenolic compounds with an antimicrobial effect are benzophenone and benzophenone derivatives that have antifungal properties (Sun et al., 2011). Coniferyl alcohol, a phenolic compound abundant in the ethyl acetate extract (6.3%), has been reported to inhibit fungal growth and melanization in the *Ascomycota* fungus *Verticillium longisporum* (König et al., 2014).

The ethanolic extract had the highest antibacterial activity. The ethanolic extract of *U. europaeus* contained high amounts of 3-*O*-methyl-D-glucose (53.3%), a compound that is commonly present in ethanolic extracts (Rani et al. 2013; Hussein et al., 2016; Nafiu and Ashafa, 2017). 3-*O*-methyl-D-glucose is a non-metabolized glucose that can be phosphorylated by

Fig. 2 Effect of extracts of *Ulex europaeus* from Southern Chile on growth of pathogenic fungi and an oomycete: (A) photographic record of testing mycelial growth inhibition in 12-wells system; (B) fungal/oomycetal growth inhibition at 72 h post-inoculation in presence of hexane, ethyl acetate and ethanolic extracts of *U. europaeus* on growth of *B. cinerea*; (C) growth of *G. fujikuroi*; (D) growth of *P. cinnamomi*, where results indicate percentage inhibition of fungal/oomycetal growth, negative control (C[−]) represents mycelial growth in media without extract (100% of growth, data not shown), positive control (C[+]) corresponds to growth in media containing captan (positive control for *B. cinerea* and *G. fujikuroi)* or metalaxil (positive control for *P. cinnamomi*), all values represent mean value of triplicate measurements ± SD, significant differences from their respective control (C[−]) treatment are marked with an asterisk ($p < 0.05$) and ns marked non-significant difference ($p > 0.05$).

hexokinase but not used as a sugar by plants (Cortès et al., 2003). Methyl glucosides are alkyl glycosides derived from sugars and can act as non-ionic surfactants with antibacterial properties (Matsumura et al., 1990).

Among the plant pathogens tested, the *U. europaeus* L. extracts had high anti-oomycete activity against *P. cinnamomi* (IC_{50} < 350 µg/mL), which may mean it has potential as an alternative for the control of this devastating plant pathogen that affects approximately 5,000 plant species, including many important plants in agriculture, silviculture, forestry and horticulture (Jung et al., 2013).

Although the cytotoxic effects of the individual compounds identified in the extracts of hexane, ethyl acetate and ethanol have been discussed, it is likely that there are some synergistic effects between the compounds, which will require further studies.

In conclusion, many of the compounds in the extracts of *U. europaeus* L. from Southern Chile reported in the current study (mainly terpenoids) have been described as metabolites with high antimicrobial activities. *U. europaeus* organic extracts showed growth inhibition of phytopathogenic bacteria, fungi and the oomycete *P. cinnamomi,* suggesting their potential application for the control of important plant pathogens.

The results of the current study indicated that it was possible to add value to *U. europaeus*, which is classified as an invasive species and that there is potential in exploring new uses of this plant for the development of novel, non-synthetic products for phytopathogen control.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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