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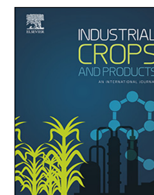
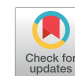


# Phenolic composition and biological activities of the *in vitro* cultured endangered *Eryngium viviparum* J. Gay



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ABSTRACT

*Eryngium viviparum* is an endangered species that requires management efforts based on complementary *ex situ* conservation strategies, such as *in vitro* culture. This study was carried out to evaluate the phenolic composition and the antioxidant, cytotoxic, and antimicrobial properties of *E. viviparum* aerial and root parts obtained by this micropropagation technique. The HPLC-DAD-ESI/$MS^n$ analysis showed that phenolic compounds were more abundant in the root (102 ± 4 mg $g^{-1}$ extract) than in the aerial part (40.6 ± 0.8 mg $g^{-1}$ extract) of the plant. The major compound was *trans* rosmarinic acid, followed by *trans* 3-*O*-caffeoylquinic acid. The root extract also showed higher antioxidant activity, with a result close to that of trolox in the thiobarbituric acid reactive substances (TBARS) formation inhibition assay, and a moderate toxicity to lung (NCI-H460), breast (MCF-7) and liver (HepG2) tumour cells. It was also more effective than ketoconazole against *Penicillium ochrochloron*. In turn, the aerial part extract inhibited *Salmonella typhimurium* more effectively than ampicillin. This study highlights *E. viviparum* as an unexplored source of bioactive compounds with potential application in the food, pharmaceutical, and other industrial sectors. Consequently, it promotes the interest of conserving this endangered species.

## 1. Introduction

*Eryngium viviparum* J. Gay (Fam. Apiaceae) is an endemic plant from the northwest of the Iberian Peninsula and France. Its current population is dramatically small and faces a high risk of extinction, being included in the International Union for Conservation of Nature's red list of threatened species since 2011 (Aguiar, 2003; Lansdown, 2011). To promote their protection, usual *in situ* conservation strategies have been implemented, such as management of wild populations and protection of natural habitats (Bañares et al., 2004). Currently, in Spain and Brittany (France), only policies based on *in situ* conservation are commonly used (Rascle et al., 2018; Romero et al., 2004). However, in some critical situations, it is necessary to resort to *ex situ* strategies such as *in vitro* plant tissue culture.

Micropropagation is an *in vitro* culture technique that allows the large-scale multiplication of plant species from excised plant parts within a short span of time, as well as for the storage and preservation of germplasm. The obtained *in vitro*-cultured plants (clones of the original mother plant) can be used in plant reintroduction programs and for research purposes, without damaging the existing populations (Ayuso et al., 2019; Engelmann, 2011; Sarasan et al., 2006). Plant tissue culture is also used to stimulate the synthesis and accumulation of secondary metabolites in the plant tissues, including alkaloids, terpenes, and phenolic compounds (Atanasov et al., 2015; Dias et al., 2016).

Several *Eryngium* species have been used in traditional medicine for the treatment of various human physiological disorders due to their bioactive chemical constituents. Aerial and root parts are used in the treatment of skin disorders and kidney stones, and to regulate arterial pressure (Wang et al., 2012). Infusions of these parts are also used to treat tapeworms and pinworms, digestive problems, and headache, and as a diuretic (Erdem et al., 2015; Vukic et al., 2018). Current research with plants of this genus has confirmed the presence of phytochemicals with pharmacological activities, specifically flavonoids and phenolic acids (Erdem et al., 2015; Wang et al., 2012), which have been reported as one of the main groups of phytochemicals with antioxidant,

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antimicrobial, and cytotoxic properties (Belkaid et al., 2006; Gugliucci and Bastos, 2009; Petersen and Simmonds, 2003).

Nowadays, the interest in natural and bio-based ingredients has increased due to the high market demand from the consumer and the food industry, among other sectors. However, the discovery of new bioactive compounds and the use of more sustainable sources at an industrial scale face some challenges related to the rational exploitation of natural resources and biodiversity (Atanasov et al., 2015; Lavergne et al., 2005; Li and Vederas, 2009). Although *E. viviparum* is a threatened species, the conditions for its germination and *in vitro* culture have already been established in recent studies (Ayuso et al., 2019, 2017); nevertheless, to the best of authors' knowledge, the phenolic profile and bioactive properties of this plant remain unknown. Therefore, this work was carried out to determine the composition in phenolic compounds and the antioxidant, antimicrobial, and cytotoxic activities of *E. viviparum* aerial and root parts obtained by *in vitro* culture.

## 2. Material and methods

### 2.1. Plant material and in vitro culture

Samples of *E. viviparum* were obtained from *in vitro* seed germination as formerly described by Ayuso et al. (2017). Briefly, the seedlings were cultured in MS basal medium, prepared as described by Murashige and Skoog (1962), supplemented with 0.1 mg $L^{-1}$ indole-3-butyric acid, 3% sucrose (*w/v*), and solidified with 1% agar (*w/v*) at pH 5.8. Cultures were incubated at 24 ± 1 °C with photoperiod (16/8; light/darkness). *E. viviparum* grows as a basal rosette and form shoot cluster on *in vitro* culture. Shoots clusters were divided into single shoots every 5 weeks and subculture to a fresh medium (Ayuso et al., 2019). The aerial parts and roots from fifth to eight subculture were collected and then freeze-dried (Telstar Cryodos, Telstar Industrial SL, Terrassa, Spain), reduced to powder, and stored at −20 °C.

### 2.2. Preparation of hydroethanolic extracts

Samples (~1 g) underwent to solid-liquid extraction twice with 80% ethanol (30 mL) for 1 h at 150 rpm and room temperature, as described by Bessada et al. (2016). After filtration though Whatman paper No. 4, ethanol was separated under pressure in a rotary evaporator (Büchi R-210, Flawil, Switzerland) and the aqueous phase was freeze-dried.

### 2.3. Analysis of phenolic compounds

The extracts were re-dissolved in 20 % ethanol at 5 mg $mL^{-1}$ and filtered through 0.22 μm syringe filters. The HPLC-DAD-ESI/$MS^n$ equipment and chromatographic conditions were formerly described by Bessada et al. (2016), as well as the identification and quantification procedures. Seven-level calibration curves (25–800 μg $mL^{-1}$ for quercetin-3-*O*-glucoside and 2.5–80 μg $mL^{-1}$ for the other standards were constructed for the standards rosmarinic acid y = –652,903 + 191291x; $r^2$ = 0.999; LOD = 0.15 μg $mL^{-1}$; LOQ = 0.68 μg $mL^{-1}$, chlorogenic acid y = –161,172 + 168823x; $r^2$ = 0.999; LOD = 0.20 μg $mL^{-1}$; LOQ = 0.68 μg $mL^{-1}$, caffeic acid y = 406,369 + 388345x; $r^2$ = 0.994; LOD = 0.78 μg $mL^{-1}$; LOQ = 1.97 μg $mL^{-1}$, ferulic acid y = –185,462 + 633126x; $r^2$ = 0.999; LOD = 0.20 μg $mL^{-1}$; LOQ = 1.01 μg $mL^{-1}$), and quercetin-3-*O*-glucoside y = –160,173 + 34843x, $r^2$ = 0.999; LOD = 0.21 μg $mL^{-1}$; LOQ = 0.71 μg $mL^{-1}$) and used in quantification. The results were expressed as mg/g dry extract.

### 2.4. Evaluation of biological activities

#### 2.4.1. Antioxidant activity

Two *in vitro* assays were performed to measure the extracts capacity to inhibit the formation of thiobarbituric acid reactive substances (TBARS) and the oxidative haemolysis (OxHLIA) as described by Lockowandt et al. (2019), using porcine brain cells and sheep erythrocytes as oxidizable subtracts, respectively. Trolox was the positive control. The results were given as $IC_{50}$ values (μg $mL^{-1}$) (Δ*t* of 30 min and 60 min were selected for the OxHLIA).

#### 2.4.2. Cytotoxic activity

The extracts (50–400 μg $mL^{-1}$ in ultrapure water) were tested against NCI-H460 (non-small cell lung carcinoma), MCF-7 (breast adenocarcinoma), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) human tumour cells by the sulforhodamine B assay (Guimarães et al., 2013a). A non-tumour PLP2 cell line (porcine liver primary cells) was also tested to evaluate possible hepatotoxicity. Ellipticine was the positive control. The results were given as $GI_{50}$ values (μg $mL^{-1}$).

#### 2.4.3. Antimicrobial activity

The extracts (0.10–20 mg $mL^{-1}$ in water) were tested against *Bacillus cereus* (food isolate), *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (NCTC 7973), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 35030), and *Salmonella typhimurium* (ATCC 13311) by the microdilution and *p*-iodonitrotetrazolium violet methods (Heleno et al., 2013). *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839), and *Penicillium verrucosum* var. *cyclopium* (food isolate) were also tested. The Institute for Biological Research "Sinisa Stanković", Serbia, provided the microorganisms. Positive controls: streptomycin and ampicillin for the antibacterial activity; ketoconazole and bifonazole for the antifungal activity. The results were given as minimum inhibitory, bactericidal, and fungicidal concentrations (MIC, MBC, and MFC, respectively; mg $mL^{-1}$).

### 2.5. Statistical analysis

All experiments were performed in triplicate. Statistical tests were performed at a 5% significance level using SPSS Statistics (IBM SPSS Statistics for Windows, 22.0. Armonk, NY: IBM Corp.). Differences among samples were assessed by a Student's *t*-test (for the phenolic compounds content) or a one-way analysis of variance (ANOVA) (for the bioactivities).

## 3. Results and discussion

### 3.1. Phenolic composition

The phenolic profiles of the hydroethanolic extracts of *in vitro*-cultured *E. viviparum* aerial and root parts are shown in Fig. 1. Chromatographic data related to the compounds characterization process, tentative identities and contents are given in Table 1. Fourteen phenolic compounds were detected and grouped into phenolic acids (peaks 1–10) and flavonoids (peaks 11–14). Phenolic acids were the predominant compounds in both plant organs, with 38.3 ± 0.8 mg $g^{-1}$ and 102 ± 4 mg $g^{-1}$ of extract of aerial and root parts, respectively; while flavonoids were quantifiable just in the aerial part (2.24 ± 0.09 mg $g^{-1}$ extract).

Peak 9 was identified as *trans* rosmarinic acid based on its mass spectrum (Barros et al., 2013) with a pseudo molecular ion $[M\text{-}H]^-$ at *m/z* 359 and fragment ions at *m/z* 197, 179 and 161 (Table 1); its identity was set by comparison with the commercial standard. This phenolic acid was the major compound found in this endangered species, which corresponded to 71.4% and 78.7% of the total phenolic compounds quantified in aerial and root parts, respectively. Lower levels of rosmarinic acid than those found in the *E. viviparum* root have been reported in other Lamiaceae, such as rosemary (*Rosmarinus*

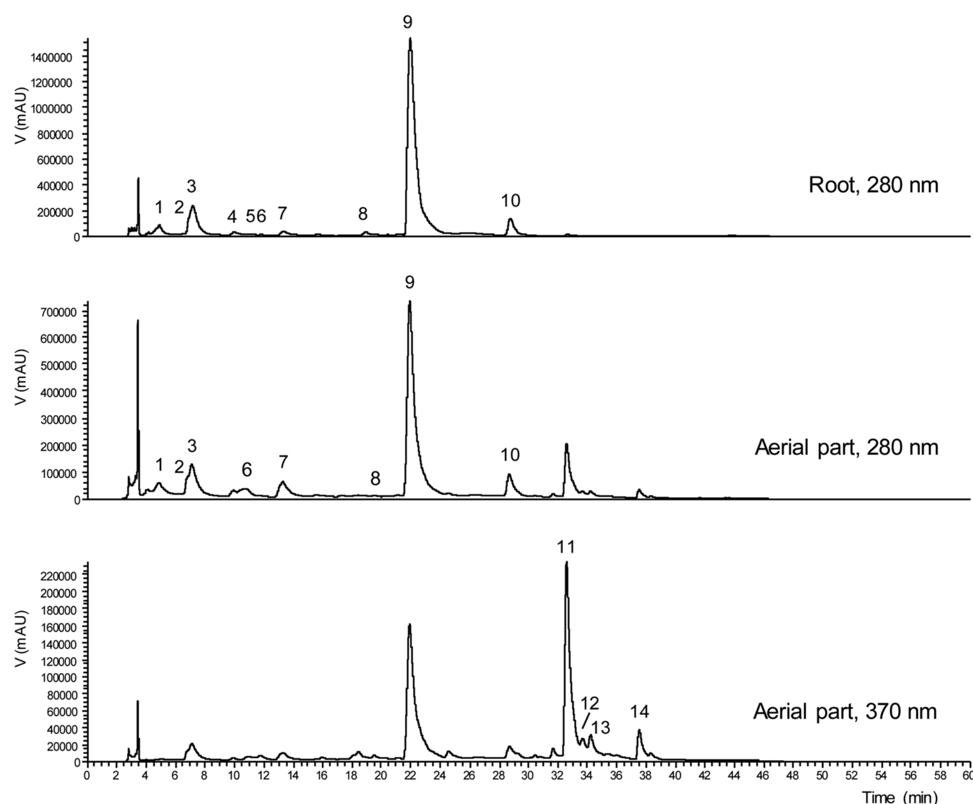


**Fig. 1.** HPLC phenolic profile of hydroethanolic extracts of *in vitro* cultured *E. viviparum* aerial and root parts. See Table 1 for peak identification.

*officinalis* L.; 6.9 ± 0.2 mg $g^{-1}$ extract; Gonçalves et al. (2019), and 68.5 mg $g^{-1}$ extract; Ribeiro et al. (2016)) and spearmint (*Mentha spicata* L. 73.4 ± 0.8 mg $g^{-1}$ extract; Chrysargyris et al. (2019)).

Peak 1 ($[M-H]^-$ at *m/z* 197) was identified as 3-(3,4-dihydroxyphenyl)-lactic acid, also known as salvianic acid A or "Danshensu", based on its characteristic fragmentation pattern found in the literature (Zeng et al., 2006). Peak 4 ($[M-H]^-$ at *m/z* 179) was identified as caffeic acid according to its retention time and UV–vis and

**Table 1**
Phenolic compounds identified and levels quantified in the hydroethanolic extracts of *E. viviparum* root and aerial part. It is presented the retention time (Rt), the wavelengths of maximum absorption in the visible region ($\lambda_{max}$), mass spectral data ($M-H^-$), and relative abundances of fragment ions ($MS^2$).

| Peak | Rt (min) | $\lambda_{max}$ (nm) | $[M-H]^-$ (*m/z*) | $MS^2$ (*m/z*) | Tentative identification | Content (mg $g^{-1}$ extracts) | | Statistics[x] |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Root | Aerial part | *p*-value |
| 1 | 4.88 | 281 | 197 | 179(100), 135(5) | 3-(3,4-Dihydroxyphenyl)-lactic acid[1] | 2.41 ± 0.05 | 1.24 ± 0.05 | 0.002 |
| 2 | 6.86 | 326 | 353 | 191(100), 179(6), 161(5), 135(5) | *cis* 3-*O*-Caffeoylquinic acid[2] | 2.65 ± 0.03 | 1.25 ± 0.05 | 0.001 |
| 3 | 7.14 | 326 | 353 | 191(100), 179(6), 161(5), 135(5) | *trans* 3-*O*-Caffeoylquinic acid[2] | 10.2 ± 0.4 | 3.65 ± 0.09 | 0.002 |
| 4 | 9.99 | 324 | 179 | 135(100) | Caffeic acid[3] | 0.24 ± 0.01 | tr | – |
| 5 | 11.19 | 287/ sh331 | 357 | 313(100), 269(39), 203(41), 159(5), 109(5) | Prolithospermic acid (isomer)[1] | 1.35 ± 0.05 | nd | – |
| 6 | 11.89 | 293/ sh332 | 357 | 313(100), 269(39), 203(39), 159(5), 109(5) | Prolithospermic acid (isomer)[1] | 1.30 ± 0.06 | 1.48 ± 0.07 | 0.104 |
| 7 | 13.34 | 326 | 367 | 193(7), 191(100), 173(5), 134(5) | 5-*O*-Feruloylquinic acid[4] | 0.56 ± 0.02 | 0.73 ± 0.02 | 0.014 |
| 8 | 18.9 | 325 | 515 | 353(100), 191(5), 179(69), 161(5), 135(6) | 3,5-*O*-Dicaffeoylquinic acid[2] | 1.30 ± 0.06 | 0.28 ± 0.01 | 0.002 |
| 9 | 21.94 | 327 | 359 | 197(27), 179(36), 161(100), 135(5) | *trans* Rosmarinic acid[1] | 80 ± 3 | 29.0 ± 0.7 | 0.002 |
| 10 | 28.75 | 328 | 373 | 355(38), 197(13), 193(6), 179(100), 161(62), 135(65) | Feruloyl-hydrocaffeic acid[4] | 1.72 ± 0.08 | 0.73 ± 0.02 | 0.003 |
| 11 | 32.66 | 266/ sh347 | 475 | 299(100), 175(10) | Tectorigenin-*O*-glucuronide[5] | tr | 2.24 ± 0.09 | – |
| 12 | 33.61 | 266/ sh340 | 547 | 503(100), 461(5), 299(5) | Tectorigenin-malonyl-hexoside[5] | nd | tr | – |
| 13 | 34.21 | 266/ sh345 | 547 | 503(100), 461(5), 299(5) | Tectorigenin-malonyl-hexoside[5] | nd | tr | – |
| 14 | 37.51 | 344 | 517 | 457(5), 299(100), 285(5), 217(14) | *O*-Methylluteolin-*O*-(acetyl) glucoronide[5] | nd | tr | – |
| | | | | | Σ Phenolic acids | 102 ± 4 | 38 ± 0.8 | 0.002 |
| | | | | | Σ Flavonoids | – | 2.24 ± 0.09 | – |
| | | | | | Σ Phenolic compounds | 102 ± 4 | 40.6 ± 0.8 | 0.002 |

Standards used in the quantification: ([1])- rosmarinic acid, ([2])- chlorogenic acid, ([3])- caffeic acid, ([4])- ferulic acid, and ([5])- quercetin-3-*O*-glucoside. tr: traces; nd: not detected.

[x] A *t*-student test was applied to evaluate statistically significant differences ($p < 0.05$) between samples.

mass characteristics in comparison with the commercial standard.

Peaks 2 and 3 ($[M\text{-}H]^-$ at *m/z* 353) were identified as *cis* and *trans* 3-*O*-caffeoylquinic acid, respectively (Zheng et al., 2017), yielding the base peak at *m/z* 191 and ions at *m/z* 179, 161 and 135. The *trans* isomer was the second most abundant quantified phenolic compounds, with aerial and root parts containing 3.65 ± 0.09 mg $g^{-1}$ and 10.2 ± 0.4 mg $g^{-1}$ of extract, respectively.

Both rosmarinic and caffeoylquinic acids were previously reported as the main phenolic acids found in *Eryngium* spp. (Kikowska et al., 2014; Vukic et al., 2018). Rosmarinic acid is a well-known antioxidant with several biological properties, including antibacterial, antiviral, anti-inflammatory, antidiabetic, and anticarcinogenic activities and neuroprotective and cardioprotective effects (Nabavi et al., 2015; Nunes et al., 2015). This compound has major structural features that may contribute to the *in vitro* activities, including a OH group, two phenol−OH groups, a C—C bond, an alkoxy group, and an ester moiety (Taguchi et al., 2017).

Peaks 5 and 6 ($[M\text{-}H]^-$ at *m/z* 357) were identified as prolithospermic acid isomers (Grzegorczyk-Karolak et al., 2018), a product of salvianolic acid B degradation often found in the Lamiaceae family (Fecka et al., 2007; Zhou et al., 2011). Peak 7 ($[M–H]^-$ at *m/z* 367) and peak 8 ($[M–H]^-$ at *m/z* 515) were assigned as 5-*O*-feruloylquinic acid and 3,5-*O*-dicaffeoylquinic acid, respectively (Guimarães et al., 2013b).

Regarding flavonoids, a glucuronide-conjugated of tectorigenin, tectorigenin-*O*-glucuronide ($[M\text{-}H]^-$ at *m/z* 475), was identified in both organs of *E. viviparum*, being particularly abundant in the aerial part (2.24 ± 0.09 mg $g^{-1}$ extract); only a trace amount was detected in the root extract. Tectorigenin was previously reported in flowers of *Pueraria thomsonii* Benth. and rhizomes of *Belamcanda chinensis* (L.) DC. (Wang et al., 2013). According to Applová et al. (2017), this isoflavone is a more potent antiplatelet compound than acetylsalicylic acid. It has also been shown to exert anti-proliferative effects on tumour cells (Thelen et al., 2005). To the best of the authors' knowledge, this is the first study reporting the presence of tectorigenin in *Eryngium* and Apiaceae spp.

Traces of tectorigenin-malonyl-hexoside (peaks 12 and 13) and *O*-methylluteolin-*O*-(acetyl)glucoronide (peak 14) were detected just in the aerial part of *E. viviparum*.

### 3.2. *In vitro* biological activities

#### 3.2.1. Antioxidant activity

The results of the two antioxidant activity assays performed with the extracts of aerial and root parts of *E. viviparum* are presented in Table 2. For the OxHLIA assay, it is given the extract concentration required to protect 50% of the erythrocytes from the haemolysis induced by the hydrophilic free radical initiator AAPH for 30 and 60 min. In addition, $IC_{50}$ values are given at two Δ*t*, as natural extracts contain a wide range of antioxidant compounds capable of offering protection at different time periods (Lockowandt et al., 2019). For the TBARS assay, it is given the extract concentration required to provide 50% of antioxidant activity, in other words, the dose at which the extracts can avoid the formation of malondialdehyde through the H donation to lipid radicals. Hence, in both assays, the lower the $IC_{50}$, the higher the antioxidant activity of the tested extracts.

As observed in Table 2, the antioxidant activity of the root extract was higher than that of the aerial part of *E. viviparum*. In TBARS, the $IC_{50}$ value achieved with root extract (14.9 ± 0.3 μg $mL^{-1}$) was just 2.8-fold higher than that of trolox (5.4 ± 0.3 μg $mL^{-1}$), while the aerial part extract was 4.7-fold less effective than this synthetic antioxidant. However, it is interesting to note that trolox is a pure antioxidant compound, while plant extracts are complex mixtures of different phytochemicals with or without bioactive properties. In OxHLIA, 68 ± 3 μg $mL^{-1}$ and 141 ± 6 μg $mL^{-1}$ of root extract were needed to protect 50% of the erythrocyte population for 30 min and 60 min, respectively. This extract was 2.6% more effective than the one of the aerial part at both given Δ*t*. Comparing with trolox, it can also be concluded that the *E. viviparum* extracts were more effective at 60 min than at 30 min. These results can be linked to the higher content of bioactive phenolic compounds, such as rosmarinic and caffeoylquinic acids, detected in the root of the studied endangered plant (Table 1), since polyphenols have the capacity to donate $H^+$ and $e^-$ to free radicals, stabilizing them and giving rise to a quite stable phenolic radical (Heim et al., 2002).

To the best of authors' knowledge, this is the first study reporting the *in vitro* antioxidant activity of *E. viviparum* extracts, but there are reports with other *Eryngium* spp. including *E. serbicum* L. (Vukic et al., 2018), *E. bourgatii* (Cádiz-Gurrea et al., 2013), and *E. maritimum* (Mejri et al., 2017).

#### 3.2.2. Cytotoxic activity

The results of the cytotoxic activity of *E. viviparum* extracts against four human tumour cells and a non-tumour porcine liver cell culture are displayed in Table 2. These are given as $GI_{50}$ values, which correspond to the extract dose needed to inhibit 50% of cell growth. Hepatocellular carcinoma HepG2 was the most sensitive tumour cell line to both *E. viviparum* extracts ($GI_{50}$ = 239 μg $mL^{-1}$). The root extract was also cytotoxic to the cell lines NCI-H460 (non-small cell lung carcinoma) and MCF-7 (breast adenocarcinoma), with $GI_{50}$ values of 305 ± 10 μg $mL^{-1}$ and 350 ± 12 μg $mL^{-1}$, respectively. On the other hand, none of the extracts was cytotoxic to HeLa cells (cervical carcinoma) or hepatotoxic to the normal PLP2 cells at the tested concentrations ($GI_{50}$ > 400 μg $mL^{-1}$). Ellipticine, a potent antineoplastic agent used as positive control, revealed significantly higher cytotoxicity to the human tumour cells than *E. viviparum* extracts, but also to the non-tumour cell line. Once again, these results may be linked to the higher level of bioactive polyphenols detected in the root (Table 1).

Extracts or isolates from *Eryngium* spp. have been described to have cytotoxic activity against tumour cells (Wang et al., 2012). Monks et al.

**Table 2**
Antioxidant and cytotoxic activities of *E. viviparum* extracts and positive controls.

| | Root | Aerial part | Positive control | Statistics[x] | |
|---|---|---|---|---|---|
| Antioxidant activity ($IC_{50}$, μg/mL) | | | Trolox | Homoscedasticity | *p*-value |
| TBARS | 14.9 ± 0.3 [b] | 25.6 ± 0.2 [a] | 5.4 ± 0.3 [c] | 0.896 | < 0.001 |
| OxHLIA, Δt = 30 min | 68 ± 3 [b] | 178 ± 3 [a] | 8.1 ± 0.3 [c] | 0.239 | < 0.001 |
| OxHLIA, Δt = 60 min | 141 ± 6 [b] | 371 ± 7 [a] | 20.6 ± 0.9 [c] | 0.358 | < 0.001 |
| Cytotoxic activity ($GI_{50}$, μg/mL) | | | Ellipticine | | |
| NCI-H460 (lung cancer) | 305 ± 10 | > 400 | 1.03 ± 0.09 | 0.119 | < 0.001 |
| MCF-7 (breast carcinoma) | 350 ± 12 | > 400 | 0.91 ± 0.04 | 0.117 | < 0.001 |
| HeLa (cervical carcinoma) | > 400 | > 400 | 1.91 ± 0.06 | – | – |
| HepG2 (hepatocellular carcinoma) | 239 ± 7 [a] | 239 ± 2 [a] | 1.1 ± 0.2 [b] | 0.144 | < 0.001 |
| Hepatotoxicity ($GI_{50}$, μg/mL) | | | | | |
| PLP2 (liver primary culture) | > 400 | > 400 | 3.2 ± 0.7 | – | – |

[x] Statistically significant differences ($p < 0.05$) between three or two variables were assessed by a one-way ANOVA, using the Tukey's HSD test for homoscedastic samples ($p > 0.05$), or by a Student's *t*-test, respectively.

**Table 3**
Antimicrobial activity of *E. viviparum* extracts and positive controls.

| | Root | | Aerial part | | Positive control | | | |
|---|---|---|---|---|---|---|---|---|
| Antibacterial activity | | | | | Streptomycin | | Ampicillin | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| *B. cereus* | 1 | 2 | 2 | 4 | 0.1 | 0.2 | 0.25 | 0.4 |
| *S. aureus* | 4 | 8 | 4 | 8 | 0.04 | 0.1 | 0.25 | 0.45 |
| *L. monocytogenes* | 2 | 4 | 2 | 4 | 0.2 | 0.3 | 0.4 | 0.5 |
| *E. coli* | 1 | 2 | 2 | 4 | 0.2 | 0.3 | 0.4 | 0.5 |
| *E. cloacae* | 2 | 4 | 2 | 4 | 0.2 | 0.3 | 0.25 | 0.5 |
| *S. typhimurium* | 2 | 4 | 0.5 | 1 | 0.2 | 0.3 | 0.75 | 1.2 |
| Antifungal activity | | | | | Ketoconazole | | Bifonazole | |
| | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| *A. fumigatus* | 1 | 2 | 8 | 16 | 0.25 | 0.5 | 0.15 | 0.2 |
| *A. ochraceus* | 1 | 2 | 4 | 8 | 0.2 | 0.5 | 0.1 | 0.2 |
| *A. niger* | 4 | 8 | 8 | 16 | 0.2 | 0.5 | 0.15 | 0.2 |
| *P. funiculosum* | 0.5 | 1 | 2 | 4 | 0.2 | 0.5 | 0.2 | 0.25 |
| *P. ochrochloron* | 1 | 2 | 4 | 8 | 2.5 | 3.5 | 0.2 | 0.25 |
| *P. verrucosum* | 0.5 | 1 | 2 | 4 | 0.2 | 0.3 | 0.1 | 0.2 |

MIC: minimal inhibitory concentration (mg $mL^{-1}$); MBC: minimal bactericidal concentration (mg $mL^{-1}$); MFC: minimum fungicidal concentration (mg $mL^{-1}$).

(2002) described *E. ebracteatum* leaf and root organic extracts and *E. nudicale* aerial part aqueous extracts as having cytotoxic effects to NCI-H460. Paşayeva et al. (2017) described *E. creticum* aqueous extracts and *E. billardieri* methanolic extracts as capable of inhibiting MCF-7 cell growth (Paşayeva et al., 2017). Furthermore, Yurdakök and Baydan (2013) also reported cytotoxicity of *E. kotschyi* and *E. maritimum* aqueous extracts from aerial and root parts against HepG2 cells (Yurdakök and Baydan, 2013).

### 3.2.3. *Antimicrobial activity*

The results of the antimicrobial activity of *E. viviparum* extracts are presented in Table 3. In general, the Gram-negative bacteria were more sensitive to the tested extracts. *Bacillus cereus* and *E. coli* were the most susceptible bacteria to the root extract (MIC = 1 mg $mL^{-1}$ and MBC = 2 mg $mL^{-1}$), which in general gave the best results. *Salmonella typhimurium* was the only microorganism to have greater sensitivity to the extract of aerial part and the one presenting the lowest MICs (0.5 mg $mL^{-1}$) and MBCs (1 mg $mL^{-1}$); interestingly, the MIC obtained for this enteric pathogen was lower than that of the positive control ampicillin (0.75 mg $mL^{-1}$). For *S. aureus* and L. *monocytogenes*, the MICs and MBCs obtained with the root extract were the same as those obtained with the aerial part.

The root extract also provided the best antifungal activity (Table 3), with MICs and MBCs between 0.5–4 mg $mL^{-1}$ and 1–8 mg $mL^{-1}$, respectively. *A. niger* and *A. fumigatus* were the most resistant fungi to the tested *E. viviparum* extracts. In turn, *P. funiculosum* and *P. verrucosum* var. *cyclopium* were the most susceptible to the tested extracts, being required 0.5 mg $mL^{-1}$ and 1 mg $mL^{-1}$ of root extract and 2 mg $mL^{-1}$ and 4 mg $mL^{-1}$ of aerial part extract to inhibit or kill these two microorganisms. Interestingly, the root extract was more effective than ketoconazole in inhibiting and killing *P. ochrochloron*. As mentioned above, caffeic acid derivatives have many biological actives, including antimicrobial effects (Nabavi et al., 2015; Nunes et al., 2015), and could be linked to the observed effect.

Extracts of *Eryngium* spp. have shown a broad antimicrobial activity against a number of bacteria and fungi (Erdem et al., 2015). *E. maritimum, E. planum* and *E. campestre* hydroethanolic extracts (Thiem et al., 2010) and the apolar fraction of a methanol-chloroform-water extract of *E. maritimum* (Meot-Duros et al., 2008) revealed antibacterial activity against *B. cereus* and *S. aureus* and antifungal activity against *A. niger*. Methanolic and chloroform extracts of *E. palmatum* leaves and roots also showed activity against *S. aureus* and *E. coli* (Marčetić et al., 2014). In addition, fractions of methanolic extracts obtained from *E. caeruleum* aerial parts were effective against *E. coli*, *S. typhimurium*, *A. fumigatus*, and *A. niger* (Sadiq et al., 2016). However, to the best of the authors' knowledge, this is the first report on *Eryngium* extracts with antibacterial activity against *Enterobacter* and *Listeria* strain and antifungal activity against *Penicillium* genus.

## 4. Conclusion

In this study, the *in vitro* cultured *E. viviparum* plant was characterized as an interesting source of phenolic acids, particularly rosmarinic and *trans* 3-*O*-caffeoylquinic acids, which were particularly abundant in the root. Flavonoids were quantified just in the aerial part. The root extract showed the highest antioxidant activity, especially in the TBARS assay, and cytotoxicity to the NCI-H460 (non-small cell lung carcinoma), MCF-7 (breast adenocarcinoma), and HepG2 (hepatocellular carcinoma) cell lines. On the other hand, none of the extracts was cytotoxic to non-tumour primary PLP2 cells up to the concentration of 400 μg/mL. In general, the root extract also provided better antimicrobial activity than the aerial part extract. *Bacillus cereus* and *E. coli* were the most susceptible bacteria to this extract, while *P. funiculosum* and *P. verrucosum* var. *cyclopium* were the most susceptible fungi. Interestingly, the root extract was more effective than ketoconazole against *P. ochrochloron*, whereas *S. typhimurium* was the only microorganism to have greater sensitivity to the extract of the aerial part, with a MIC lower than that of ampicillin. Therefore, this study promotes the interest of conserving *E. viviparum* given its content in phenolic acids with antioxidant and antimicrobial properties, which can be exploited by different industrial sectors interested in bio-based ingredients.

## CRediT authorship contribution statement

**Manuel Ayuso:** Methodology, Investigation, Formal analysis, Writing - original draft. **José Pinela:** Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Maria Inês Dias:** Methodology, Investigation. **Lillian Barros:** Conceptualization, Methodology, Formal analysis, Project administration, Writing - original draft, Writing - review & editing. **Marija Ivanov:** Methodology, Investigation. **Ricardo C. Calhelha:** Methodology. **Marina Soković:** Methodology, Investigation. **Pablo Ramil-Rego:** Funding acquisition, Project administration. **M. Esther Barreal:** Conceptualization, Investigation. **Pedro Pablo Gallego:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing. **Isabel C.F.R. Ferreira:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Project administration, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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