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Chemical and bioactive characterization of the aromatic plant *Levisticum officinale* W.D.J. Koch: a comprehensive study

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Aromatic plants have been used since antiquity as seasoning ingredients to impart unique flavours to foods, and also as folk medicines, and are currently used as a source of several compounds of interest for different industries. Lovage (*Levisticum officinale* W.J.D. Koch) is an aromatic plant from the Apiaceae family with a strong flavour that has long been used in culinary products, in traditional medicine and by the food industry. Despite its significance and relevance, apart from its composition in volatile compounds, there is a scarcity of information about this plant species. To the best of our knowledge, this study documents for the first time the nutritional value and composition in fatty acids, organic acids and tocopherols of the edible aerial part of lovage, evidencing a low caloric value, a predominance of polyunsaturated fatty acids, mainly α-linolenic acid, oxalic acid as the most abundant organic acid and α-tocopherol as the most abundant vitamin E isoform. The essential oil was mainly characterised by the presence of monoterpenes, showing also a high abundance of phthalides. In addition, a total of 7 phenolic compounds were identified in the decoction and hydroethanolic extracts, which showed interesting antioxidant properties and bacteriostatic activity, particularly against Gram-positive bacteria. Only the decoction showed cytotoxicity against a tumoral cell line (HepG2).

1. Introduction

Since ancient times, several aromatic plants and spices have been used worldwide for culinary purposes aiming to modify, enhance or improve the flavour of foods. Besides their significance in gastronomy, several of them are also recognized for their empirical use in folk medicine due to their beneficial health effects. In fact, beyond their nutritional value, several of these plants are known to have many phytochemicals, including polyphenols and terpenoid compounds, which can significantly contribute to their biological activity.[1,2] Currently, aromatic plants are also considered as a valuable source of natural products, many of them being secondary metabolites that have diverse applications in the chemical, pharmaceutical, cosmetic and food industries.[3] Among such products, essential oils have long been used by the food industry primarily as flavouring agents.[4] However, because several essential oils have a Generally Recognized as Safe (GRAS) status attributed by the Food and Drug Administration (FDA), presently, the possibility of their utilization as natural preservatives to extend the shelf-life of foods has been attracting the attention of the food industry.[5] In fact, the addition of synthetic additives in foods is increasingly raising concerns in consumers about eventual harmful effects. In this regard, several studies focusing on different aromatic plants have suggested the use of essential oils from these plants, or their constituents, as promising alternatives to synthetic preservatives.[4,5]

Among the several species of aromatic plants used in culinary products, *Levisticum officinale* W.J.D. Koch, also known as lovage, was once much recognized, being considerably used by the condiment's industry[6] and by households in soups, stews, meat dishes, *etc*. Nevertheless, with the exception of quite a few culinary chefs, nowadays, the use of lovage in gastronomy is most probably unknown to the vast majority of people. *L. officinale* is a plant native to south-western Asia and Southern Europe, being classified as a perennial dicotyledonous plant belonging to the Apiaceae family. As several other plants of this family, lovage is mainly used for its aromatic properties. Due to its strong taste like celery combined with

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parsley with a scent of aniseed and curry, it reminds us of some commercial condiments, thus being also commonly designated as "Maggi plant" in several countries.[7] Consumers who know this plant frequently grow it in private gardens and use it as a natural substitute of commercial condiments or stocks. Besides their significance in culinary products, *L. officinale* has also been used as a medicinal plant due to its carminative, spasmolytic and diuretic effects, being approved by the German Commission E for lower urinary tract infections.[8] Moreover, the decoction extract prepared from the aerial parts of the plant has been described as an antiseptic for wounds.[9] Despite the significance of this aromatic plant, so far only some studies concerning the volatile composition of the essential oil extracted from lovage grown in different countries are available in the literature. To the best of our knowledge, we are unaware of any previous studies regarding the nutritional value, composition in bioactive compounds and biological properties of lovage. Therefore, this study aims at addressing this gap, contributing towards expanding the knowledge on this plant species.

2. Materials and methods

2.1. Samples

Edible aerial parts (leaves and stems) of *L. officinale* were bought in October 2018 from a local supermarket in Porto, Portugal. The leaves and stems were weighed, lyophilized (FreeZone 4.5, Labconco, MO, USA), powdered (20 mesh) and stored at −20 °C for further analysis.

2.2. Preparation of extracts

Two types of extracts were prepared, namely a decoction and a hydroethanolic extract. To prepare the decoction, 3 g of lyophilized leaves and stems were extracted with 300 mL of deionized water by boiling for 5 min. After standing at room temperature for another 5 min, the mixture was filtered through Whatman filter paper no. 4 and then subjected to freeze-drying (FreeZone 4.5, Labconco, USA).

For the hydroethanolic extract, 3 g of the lyophilized leaves and stems were extracted with 90 mL of ethanol : water (80 : 20, v/v) under stirring at room temperature, for 1 hour. The mixture was filtered through Whatman paper filter no. 4 and the residue was re-extracted by repeating the procedure. After gathering the two filtrates, ethanol was eliminated under vacuum at 40 °C by using a rotary evaporator (Buchi R-2010). The obtained solution was frozen and further lyophilized.

2.3. Chemical parameters

2.3.1. Macronutrients and energetic value. The lyophilized leaves and stems were analysed for moisture, ash and macronutrients according to the AOAC methods.[10] Briefly, the crude protein was evaluated by the macro-Kjeldahl method ($N \times 6.25$) using an automatic distillation and titration unit (model Pro-Nitro-A, JP Selecta, Barcelona), the crude fat was determined by extraction with petroleum ether using Soxhlet apparatus, the ash content was determined by incineration at 550 ± 15 °C, and total carbohydrates were determined by calculating the difference. Energy was calculated according to the Atwater system: energy (kcal per 100 g) = $4 \times (g_{\text{proteins}} + g_{\text{carbohydrates}}) + 9 \times (g_{\text{fat}})$.

2.3.2. Free sugars. The content of free sugars was determined in the lyophilized sample and in both extracts. For the lyophilized sample, the procedure was performed as previously described[11] while for the extracts 30 mg of each extract was dissolved in 2 mL of distilled water, filtered through a 0.22 μm disposable LC filter disk and directly injected on a chromatographic system consisting of a high performance liquid chromatograph coupled with a refraction index detector (Knauer, Smartline system 1000, Berlin, Germany) operating under the conditions described by Barros *et al.*[11] Compounds were identified by comparison with standards and quantification was achieved using melizitose (25 mg mL^{-1}) as an internal standard. The obtained data were handled using the Clarity 2.4 software (DataApex, Prague, Czech Republic) and the results were expressed in g per 100 g of fresh weight (fw) or in mg g^{-1} extract, for the lyophilized aerial parts or the extracts, respectively.

2.3.3. Organic acids. Organic acids were determined in the lyophilized sample and in both extracts. The lyophilized sample was extracted using a methodology previously described and optimized,[11] while the extracts (10 mg) were re-dissolved in 1 mL of metaphosphoric acid (4.5%), filtered (0.22 μm) and directly analysed in the chromatographic system. The analysis was performed using an ultra-fast liquid chromatography system coupled with a diode-array detector (Shimadzu Corporation, Japan) as previously described.[11] Compounds were identified and quantified by comparison of the retention time, spectra and peak area recorded at 245 nm for ascorbic acid and at 215 nm for the remaining compounds with those of commercial standards (oxalic, quinic, malic, ascorbic, citric and fumaric acids; Sigma-Aldrich, St Louis, USA). The results were processed using the software LabSolutions Multi LC-PDA (Shimadzu Corporation, Kyoto, Japan), and expressed in g per 100 g fw or in mg g^{-1} extract, for the lyophilized aerial parts or the extracts, respectively.

2.3.4. Fatty acids. Fatty acid methyl esters (FAME) were prepared as previously described,[12] and determined by gas–liquid chromatography with flame ionization detection, using a DANI model GC 1000 instrument. Separation was achieved on a Zebron–Kame column (30 m × 0.25 mm ID × 0.20 μm, Phenomenex, Lisbon, Portugal) operating under the following oven temperature program: initial temperature of 100 °C, held for 2 min, increase at 10 °C min^{-1} to 140 °C, followed by a 3 °C min^{-1} ramp to 190 °C, a 30 °C min^{-1} ramp to 260 °C and held for 2 min. The carrier gas (hydrogen) flow-rate was 1.1 mL min^{-1}, measured at 100 °C. The injection split ratio was 1 : 50, with injector and detector temperatures being set at 250 °C and 260 °C, respectively. Fatty acid identification and quantification (Clarity DataApex 4.0 Software, Prague, Czech Republic) were performed by comparing the relative retention times of FAME peaks from samples with standards (standard mixture

47885-U, Sigma, St Louis, USA) and the results were expressed as the relative percentage of each fatty acid.

2.3.5. Tocopherols. Tocopherols were determined in the lyophilized sample using a HPLC system coupled to a fluorescence detector (FP-2020, Jasco, Easton, USA) as previously described.[13] Compounds were identified by comparison with tocopherol standards (α-, β-, γ-, and δ-isoforms, Sigma, St Louis, MO, USA) and quantification was performed by the internal standard (IS) method using tocol (Matreya, Pleasant Gap, USA) as the IS. The results were processed using the Clarity 2.4 software and expressed in mg per 100 g fw.

2.3.6. Volatile compounds. Leaves and stems were subjected to essential oil extraction by hydrodistillation in Clevenger apparatus. The essential oil was separated from water and recovered directly without adding any solvent. After drying by adding anhydrous sodium sulphate, the oil was diluted in HPLC grade *n*-hexane and analysed in a GC-2010 Plus (Shimadzu) gas chromatography system with an AOC-20iPlus automatic injector and a mass spectrometry detector. Separation was achieved on an SH-RXi-5 ms column (30 m × 0.25 mm × 0.25 μm; Shimadzu, USA) operating under the following conditions: initial oven temperature of 40 °C for 4 min, raised at 3 °C min^{-1} to 175 °C, then at 15 °C min^{-1} to 300 °C and held for 10 min. Helium was used as the carrier gas adjusted to a linear velocity of 30 cm s^{-1}. The injected sample was 1 μL, with a split ratio of 1 : 10. The injector temperature was set at 260 °C, with a transfer line at 280 °C and an ion source at 220 °C. The ionization energy was 70 eV and a scan range of 35–500 u with a scan time of 0.3 s was used.

Identification of compounds was based on the NIST17 mass spectral library and by determining the linear retention index (LRI) based on the retention times obtained for a mixture of *n*-alkanes (C8–C40, ref. 40147-U, Supelco) analyzed under identical conditions. When possible, comparisons were also performed with commercial standard compounds and with published data.

Quantification was performed and the results were expressed as the relative percentage of total volatiles using relative peak area values obtained directly from the total ion current (TIC) values.

2.3.7. Phenolic compounds. Phenolic compounds were analysed in both hydroethanolic and decoction extracts after they were re-dissolved in ethanol/water (80 : 20, v/v) and water, respectively, to a concentration of 5 mg mL^{-1} and filtered (0.22 μm). The compounds were evaluated using a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, USA) equipped with a quaternary pump and a diode array coupled in-series to an electrospray ionization mass spectrometry detector (LC-DAD-ESI/MS^n) operating under the conditions described by Bessada *et al.*[14] The identification of compounds was performed by comparison of data regarding retention time, UV-VIS spectra, mass spectra (full scan mode from *m*/*z* 100 to 1500) and fragmentation patterns of the sample compounds with those obtained from the available standards and/or reported data from the literature. For quantification, calibration curves constructed based on the UV-vis signal of the following standards, chlorogenic acid, apigenine-6-*C*-glucoside and quercetin-3-*O*-glucoside (Extrasynthese, Genay, France), were used. The results were expressed in mg g^{-1} extract.

2.4. Bioactive properties

2.4.1. Antioxidant activity. Antioxidant properties were evaluated following different *in vitro* assays, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, reducing power, β-carotene bleaching inhibition, lipid peroxidation inhibition by evaluating thiobarbituric acid reactive substances (TBARS), and antihaemolytic activity evaluating the inhibition of oxidative hemolysis (OxHLIA). The hydroethanolic and decoction crude extracts were re-dissolved in ethanol : water (80 : 20, v/v) and water, respectively, at a final concentration of 5 mg mL^{-1} and further diluted at different concentrations (in the range of 5–0.0391 mg mL^{-1}) to perform the different *in vitro* assays, as previously described.[15]

The results of the assays were expressed as EC_{50}, corresponding to the extract concentrations providing 50% of antioxidant activity, with the exception for the reducing power assay for which EC_{50} corresponds to 0.5 of absorbance at 690 nm. Trolox (Sigma-Aldrich, St Louis, USA) was used as a standard.

The antihaemolytic activity was evaluated by the oxidative haemolysis inhibition assay (OxHLIA) as previously described by Takebayashi *et al.*,[16] with some modifications. Blood was collected from healthy sheep and centrifuged at 1000*g* for 5 min at 10 °C. After discarding the plasma and buffy coats, the erythrocytes were first washed with NaCl (150 mM) and then three times with phosphate-buffered saline (PBS, pH 7.4). The erythrocyte pellet was then resuspended in PBS to obtain a concentration of 2.8% (v/v). Using a flat bottom 48-well microplate, 200 μL of the erythrocyte solution were mixed with 400 μL of PBS solution (control), antioxidant extracts dissolved in PBS, or water (for complete haemolysis). Trolox was used as a positive control. After pre-incubation at 37 °C for 10 min with shaking, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 160 mM in PBS, 200 μL) was added to each well and the optical density was measured at 690 nm every 10 min over 400 min while the microplate was re-incubated.[16] The percentage of the erythrocyte population that remained intact (P) was calculated according to the equation: $P(\%) = (S_t - CH_0/S_0 - CH_0) \times 100$, where S_t and S_0 correspond to the optical density of the sample at t and 0 min, respectively, and CH_0 is the optical density of the complete haemolysis at 0 min. The results were expressed as delayed time of haemolysis (Δt), which was calculated according to the equation: Δt(min) = Ht_{50} (sample) − Ht_{50} (control), where Ht_{50} is the time (min) corresponding to 50% haemolysis, graphically obtained from the haemolysis curve of each antioxidant sample concentration. The Δt values were then correlated with the extract concentrations,[16] and from the correlation obtained, the extract concentration able to promote a Δt haemolysis delay was calculated. The results were given as IC_{50} values (μg mL^{-1}) at Δt 60 and 120 min, *i.e.*, the extract concen-

tration required to keep 50% of the erythrocyte population intact for 60 and 120 min.

2.4.2. Hepatotoxicity and cytotoxic activity. Hepatotoxicity was evaluated by the Sulforhodamine B (Sigma-Aldrich, St Louis, USA) assay as described by Abreu *et al.*,[17] using a primary cell culture (PLP2) prepared from a porcine liver sample and different concentrations of the hydroethanolic or decoction extracts, ranging from 400 μg mL^{-1} to 6.5 μg mL^{-1}. The anti-proliferative capacity of the extracts was also evaluated by the same method but using four human tumour cell lines (acquired from Leibniz-Institut DSMZ): MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma). In both the cases, ellipticine (Sigma-Aldrich, St Louis, USA) was used as the positive control and the results were expressed as GI_{50} values (μg mL^{-1}), corresponding to the extract concentration that provides 50% of cell growth inhibition.

2.4.3. Antimicrobial activity. The antibacterial activity was evaluated by the broth microdilution method coupled to the rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay as previously described.[18] The microorganisms used were clinical isolates and included three Gram-positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes* and *methicillin-resistant Staphylococcus aureus*) and five Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganela morganii*, *Proteus mirabilis* and *Pseudomonas aeruginosa*). The minimum inhibitory concentration (MIC) was determined through the colorimetric microbial viability based on the reduction of the INT dye (0.2 mg mL^{-1}). The minimum bactericidal concentration (MBC) was evaluated by plating the content of the microwells without coloration in the MIC assay. Different antibiotics were used as negative controls, namely ampicillin and imipenem for Gram-negative bacteria, and vancomycin and ampicillin for Gram-positive bacteria.

2.5. Statistical analysis

All the analyses were performed at least in triplicate with results being expressed as mean ± standard deviation (SD). SPSS Statistics software (IBM SPSS Statistics for Windows, v. 23.0.) was used to analyse the existence of differences among the two extracts by applying Student's *t*-test at a 5% confidence level.

3. Results and discussion

3.1. Chemical characterization of *L. officinale* leaves and stems

The results of the nutritional value, free sugars and organic acid composition obtained for the analysis of the aerial edible parts of lovage (leaves and stems) are shown in Table 1. Carbohydrates were the most abundant macronutrient, followed by proteins, with lipids being present in a minor amount. Comparing the obtained results with those reported for other commonly consumed species of the Apiaceae family, such as parsley (*Petroselinum crispum*), coriander (*Coriandrum sativum*) and celery (*Apium graveolens*), it can be seen that the values of the proximate composition are very similar, since all show a high amount of moisture, and carbohydrates and lipids as the major and minor groups of macronutrients.[19,20] However, considering the values calculated for dry mass, while parsley, coriander and lovage have identical contents of carbohydrate (51.5 g per 100 g, 47.1 g per 100 g and 53.4 g per 100 g, respectively) and protein (24.2 g per 100 g, 27.3 g per 100 g, and 28.4 g per 100 g, respectively), celery is a bit less similar as it presents a higher carbohydrate content (65.0 g per 100 g) and a lower protein content (15.0 g per 100 g) compared to lovage. As expected, due to the high moisture content, the energetic value calculated for the edible parts of *L. officinale* was considerably low (38 kcal per 100 g fw). When searching the USDA food composition database using the words "lovage" or "levisticum" no results were obtained. Additionally, from the literature consulted, no information was found regarding the nutritional composition of lovage, with data being reported for the first time in the present study.

Table 1 Nutritional value, energetic value, free sugars and organic acids of the edible aerial parts (leaves and stems) of *L. officinale* (mean ± SD, *n* = 3)

Nutritional value	
Moisture (%)	89.4 ± 0.1
Ash (g per 100 g fw)	1.52 ± 0.06
Proteins (g per 100 g fw)	3.01 ± 0.06
Lipids (g per 100 g fw)	0.37 ± 0.02
Carbohydrates (g per 100 g fw)	5.7 ± 0.2
Energy (kcal per 100 g fw)	38.0 ± 0.6
Free sugars (g per 100 g fw)	
Arabinose	0.048 ± 0.001
Glucose	0.090 ± 0.001
Sucrose	0.092 ± 0.003
Trehalose	0.073 ± 0.002
Total free sugars	0.30 ± 0.01
Organic acids (g per 100 g fw)	
Oxalic	0.462 ± 0.001
Quinic	0.37 ± 0.06
Malic	0.203 ± 0.001
Shikimic	0.012 ± 0.001
Citric	0.21 ± 0.04
Fumaric	0.003 ± 0.001
Total organic acids	1.26 ± 0.02

L. officinale presented a low content of free soluble sugars, with only 4 compounds being detected (Table 1). Among those, the predominant compounds were sucrose and glucose, followed by trehalose and smaller amounts of arabinose.

Organic acids have been evaluated in previous studies regarding different vegetables as they are known to affect the organoleptic characteristics of vegetables, such as their flavour (mainly acidity) and appearance, besides playing important roles in plant metabolism and in microbial interactions.[21] Moreover, organic acids can also influence consumers' acceptability, being frequently used in the food industry as acidifying compounds. A total of six organic acids were identified in the edible aerial parts of *L. officinale* (Table 1), with oxalic acid being the predominant compound, followed by quinic, citric

and malic acids. Regarding oxalic acid, known to promote kidney stones when taken in high amounts, compared to other vegetables, lovage presents a moderate content of oxalic acid (0.46 g per 100 g fw) being lower than that found in parsley raw leaves (1.7 g per 100 g), but higher than those in celery (0.19 g per 100 g) and coriander (0.01 g per 100 g).[20]

The fatty acid profile is shown in Table 2, where it can be observed that a total of 19 fatty acids were identified, with polyunsaturated fatty acids (PUFA) representing the major group, followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). The edible aerial parts of lovage presented a predominant composition of unsaturated fatty acids, being particularly rich in omega-3 PUFA, mainly due to the high content of α-linolenic acid (48.7%). *L. officinale* leaves and stems also showed considerable amounts of linoleic (28.5%) and palmitic (10.0%) acids. To the best of our knowledge, this study reports for the first time the fatty acid profile of this vegetable species. Despite lipids being present in small amounts (Table 1), lovage presents an interesting fatty acid profile concerning omega-3 and also essential fatty acids. Several studies demonstrated that a deficient intake of these fatty acids can lead to various problems including dermatitis, immunosuppression, cardiac dysfunction, promotion of the development of some degenerative diseases and acceleration of the process of aging.[22] In this sense, the consumption of *L. officinale* can be a contribution to a healthy diet owing to its favourable fatty acid profile (Table 2).

Table 2 Composition of fatty acids (relative %) and tocopherols (mg per 100 g fw) of the edible aerial parts (leaves and stems) of *L. officinale* (mean ± SD, n = 3)

Fatty acids	Relative %
C8:0	0.067 ± 0.004
C10:0	0.076 ± 0.006
C12:0	0.117 ± 0.004
C14:0	0.63 ± 0.02
C15:0	0.237 ± 0.008
C16:0	10.0 ± 0.3
C16:1	1.05 ± 0.04
C17:0	0.162 ± 0.001
C18:0	1.44 ± 0.08
C18:1n9	1.83 ± 0.05
C18:2n6	28.5 ± 0.5
C18:3n3	48.7 ± 0.6
C20:0	0.388 ± 0.001
C20:1	0.043 ± 0.004
C20:2	1.38 ± 0.03
C20:2	0.063 ± 0.001
C21:0	0.44 ± 0.02
C20:3n3	1.03 ± 0.04
C23:0	3.84 ± 0.02
C24:0	0.067 ± 0.004
SFA	18.0 ± 0.2
MUFA	2.93 ± 0.09
PUFA	79.0 ± 0.1
ω-6	29.9 ± 0.3
ω-3	49.2 ± 0.41
Tocopherols (mg per 100 g fw)	
α-Tocopherol	0.773 ± 0.004
γ-Tocopherol	0.028 ± 0.001
Total tocopherols	0.80 ± 0.01

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; and PUFA: polyunsaturated fatty acids.

The results regarding vitamin E are also shown in Table 2. Vitamin E works *in vivo* as an antioxidant, preventing the spread of free radical damage caused by peroxyl radicals, particularly protecting PUFA. This prevention is possible because such radicals react 1000 times faster with vitamin E than with PUFA.[23] Only two isoforms of tocopherols (α- and γ-) were identified and quantified in the leaves of *L. officinale* (Table 2), with α-tocopherol being the predominant compound. The low content of tocopherols is most possibly related to the low amount of lipids in the studied sample.

Considering that aromatic plants are generally characterized by a strong taste and an intense aroma, they are used to aromatize and enhance the flavor of dishes and frequently allow the use of a reduced amount of sodium chloride, therefore imparting health benefits beyond their composition in bioactive compounds. Due to its intense and pungent organoleptic characteristics, which somehow reminds us of the flavor of some commercial seasonings, lovage extracts were once used as a raw material in the production of condiments to impart a seasoning-like flavor[6] and is still used by some consumers, in the plant form, as a healthier substitute of commercial seasonings. Therefore, the volatiles profile of the essential oil obtained by hydrodistillation of the aerial parts of the lovage sample grown in Portugal was studied, and is listed in Table 3. The GC-MS analysis allowed identification of 99.1% of the compounds, corresponding to a total of 44 identified compounds. Monoterpene hydrocarbons and oxygenated monoterpenes were the two main groups, each representing approximately 37% of the identified compounds, with phthalides also present in high amount (24.3%). α-Terpinyl acetate was found to be the major compound (33.6%), followed by *p*-cymene (20.5%), (*Z*)-ligustilide (22.2%), β-phellandrene (4.7%) and myrcene (4.2%). When comparing the obtained composition, in terms of most abundant compounds, with data previously published for the volatile composition of *L. officinale* grown in Iran,[9,24,25] Moldavia,[26] Lithuania,[27] and Estonia,[28] in general, the chemical profile of the Portuguese grown sample was considerably different. Despite the similarity to other oils that also presented α-terpinyl acetate as the major compound, a higher content has been previously reported in the aerial parts (52.4%),[24] leaves (55.8% and 49.7% to 70%),[27,28] and stems (49.1% to 69.0%)[27] of lovage. Other studies reported different major compounds, namely pentyl cyclohexa-1,3-diene (28.1%)[9] and γ-terpinene (14.5%).[25] Additionally, in the sample grown in Portugal, the second most abundant compound was found to be *p*-cymene (20.5%), which has not been identified or has been reported in minor amounts (ranging from 0.1% to 4.3%) in the previous studies. In the present work, different phthalides were detected, with the most abundant being (*Z*)-ligustilide (22.2%). This result is in good agreement with previous studies that reported also a considerable amount of this compound in the essential oil of lovage, ranging from 4.4% to 29.7%.[9,24,26–28] Phthalides are a relatively small group of

Table 3 Chemical composition of the essential oil extracted from the edible aerial parts (leaves and stems) of *L. officinale* by hydrodistillation (mean ± SD, n = 3)

Peak number	Compound	RT (min)	LRI[a]	LRI[b]	Relative[c] %
1	α-Thujene	13.93	926	924	0.094 ± 0.002
2	α-Pinene	14.25	932	932	0.93 ± 0.02
3	Camphene	15.00	946	946	0.135 ± 0.002
4	Sabinene	16.34	972	969	1.05 ± 0.02
5	β-Pinene	16.47	974	974	0.270 ± 0.007
6	Myrcene	17.32	991	988	4.20 ± 0.05
7	α-Phellandrene	17.93	1003	1000	1.34 ± 0.02
8	3-Carene	18.25	1009	1008	0.059 ± 0.002
9	α-Terpinene	18.59	1015	1014	0.234 ± 0.006
10	*p*-Cymene	19.01	1023	1020	20.53 ± 0.09
11	β-Phellandrene	19.21	1027	1025	4.65 ± 0.04
12	(*Z*)-β-Ocimene	19.75	1038	1032	0.224 ± 0.003
13	(*E*)-β-Ocimene	20.27	1048	1044	1.05 ± 0.02
14	γ-Terpinene	20.79	1058	1054	0.609 ± 0.005
15	Terpinolene	22.32	1087	1086	0.093 ± 0.001
16	Linalool	22.91	1099	1095	0.055 ± 0.001
17	Nonanal	23.10	1102	1100	0.053 ± 0.001
18	(*E*)-*p*-Menth-2-en-1-ol	23.91	1119	1117	0.43 ± 0.01
19	Allo-ocimene	24.40	1129	1128	0.659 ± 0.008
20	5-Pentyl-1,3-cyclohexadiene	25.83	1157	1156	0.181 ± 0.001
21	Terpinen-4-ol	26.79	1177	1174	0.107 ± 0.001
22	Cryptone	27.20	1185	1183	1.90 ± 0.02
23	α-Terpineol	27.41	1189	1186	0.036 ± 0.001
24	Carvone	29.97	1243	1239	0.051 ± 0.001
25	Geraniol	30.47	1254	1249	0.264 ± 0.006
26	α-Terpinyl acetate	35.01	1354	1346	33.6 ± 0.2
27	Geranyl acetate	36.27	1383	1379	0.031 ± 0.006
28	β-Elemene	36.72	1393	1389	0.997 ± 0.004
29	γ-Elemene	38.51	1436	1434	0.143 ± 0.002
30	(*E*)-β-Farnesene	39.40	1457	1454	0.02 ± 0.01
31	Germacrene D	40.55	1484	1480	0.037 ± 0.004
32	β-Selinene	40.75	1489	1489	0.081 ± 0.003
33	Kessane	42.47	1532	1529	0.024 ± 0.001
34	Elemol	43.24	1552	1548	0.057 ± 0.001
35	Germacrene B	43.65	1562	1559	0.061 ± 0.001
36	Spathulenol	44.41	1581	1577	0.02 ± 0.001
37	3-Butylphthalide	47.19	1655	1647	0.018 ± 0.001
38	Neointermedeol	47.54	1664	1658	0.412 ± 0.001
39	(*Z*)-Butylidenephthalide	47.98	1676	1671	0.031 ± 0.001
40	(*E*)-Butylidenephthalide	49.59	1721	1717	0.320 ± 0.011
41	(*Z*)-Ligustilide	50.10	1749	1736	22.2 ± 0.1
42	(*E*)-Ligustilide	51.49	1808	1796	1.67 ± 0.01
43	(*Z*)-Ternine	52.2	1849	1844	0.027 ± 0.003
44	Methyl hexadecanoate	53.17	1927	1921	0.096 ± 0.009
Total identified					99.1 ± 0.1
Monoterpene hydrocarbons					37.3 ± 0.3
Oxygen-containing monoterpenes					36.7 ± 0.3
Sesquiterpene hydrocarbons					0.62 ± 0.01
Oxygen-containing sesquiterpenes					0.102 ± 0.002
Phthalides					24.26 ± 0.01
Others					0.12 ± 0.01

[a] LRI, linear retention index determined on a SH-RXi-5ms fused silica column relative to a series of *n*-alkanes (C8–C40). [b] Linear retention index reported in the literature (Adams, 2017).[30] [c] Relative % is given as mean ± SD, n = 3.

natural compounds confined to various plant families and some genera of fungi. Among the different plant families for which studies have been performed to characterize these compounds, the Apiaceae family stands out.[29] Different biological properties, including antioxidant activity, antihyperglycemic activity, and analgesic and neurological effects, have been ascribed to these compounds, in particular to (*Z*)-ligustilide. Moreover, phthalides have been reported as bioactive constituents of different plant species used in traditional medicine, such as *Angelica sinensis* (Chinese name: Danggui), *Angelica acutiloba* (Japanese name: Toki), and *Ligusticum porteri* (Hispanic name: Oshá).[29]

3.2. Chemical characterization of *L. officinale* extracts

Considering that the leaves and stems of lovage are used in gastronomy for flavoring dishes, being frequently added in stews and in meat marinades, besides the chemical characterization of the aerial parts of lovage, they were also subjected to

Table 4 Free sugars (mg g^{-1} extract) and organic acids (mg g^{-1} extract) of *L. officinale* (mean ± SD, n = 3)

	Decoction	Hydroethanolic extract	Student's *t*-test *p*-Value
Free sugars			
Arabinose	60.9 ± 0.5	97 ± 2	<0.001
Glucose	9.7 ± 0.7	21 ± 1	<0.001
Sucrose	29.7 ± 0.5	48 ± 2	<0.001
Trehalose	26 ± 1	32 ± 1	<0.001
Total free sugars	127 ± 3	199 ± 1	<0.001
Organic acids			
Oxalic	82.8 ± 0.7	87 ± 1	<0.001
Quinic	83 ± 1	66 ± 2	<0.001
Malic	104 ± 2	25 ± 3	<0.001
Shikimic	3.2 ± 0.1	3.03 ± 0.02	<0.001
Citric	47 ± 2	nd	—
Fumaric	1.46 ± 0.02	0.46 ± 0.001	<0.001
Total organic acids	322 ± 1	181 ± 4	<0.001

nd – not detected.

two different extractions (decoction and hydroethanolic). These extracts, besides being intended for the bioactivity assays, were also evaluated regarding their composition in hydrophilic compounds, namely soluble sugars, organic acids and phenolic compounds.

Regarding the quantification of free sugars (Table 4), arabinose was the major sugar present followed by sucrose, trehalose and glucose, the main free sugars also present in the dry plant. The hydroethanolic extract presented higher amounts compared to the decoction extract ($p < 0.05$); this was expected due to the extraction solvent used.

Regarding organic acids, all the compounds identified in the plant were present in the decoction while citric acid was not detected in the hydroethanolic extract. In comparison with the hydroethanolic extract, the decoction allowed the extraction of a significantly higher amount of total organic acids ($p < 0.05$), the difference regarding the malic acid content being particularly noticeable, which was about 4× higher in the aqueous extract (Table 4).

The results of the phenolic compound analysis carried out on the two extracts are shown in Fig. 1 and Table 5. Chromatographic and spectral data obtained by HPLC-DAD-ESI/MS^n analysis, namely retention time, λ_{max} in the UV-vis region, molecular ions and main fragment ions observed in MS^2, are detailed in Table 5 and were used for the tentative identification of compounds and respective quantification. The compounds *trans* 5-*O*-caffeolylquinic acid (chlorogenic acid, peak 4), quercetin 3-*O*-rutinoside (peak 6) and kaempferol 3-*O*-rutinoside (peak 7) were positively identified according to their retention time, mass spectra and UV-Vis characteristics by comparison with commercial standards. Peaks 1, 2 and 3 were tentatively identified as 3-*O*-caffeolylquinic acid, 4-*O*-caffeolylquinic acid and *cis*-5-*O*-caffeolylquinic acid, taking into account the hierarchical key fragmentation pattern described by Clifford *et al.*[31] Peak 5 presented a pseudomolecular ion $[M - H]^-$ at m/z 423, being tentatively identified as maclurin-3-*C*-glucoside based on the UV spectrum and

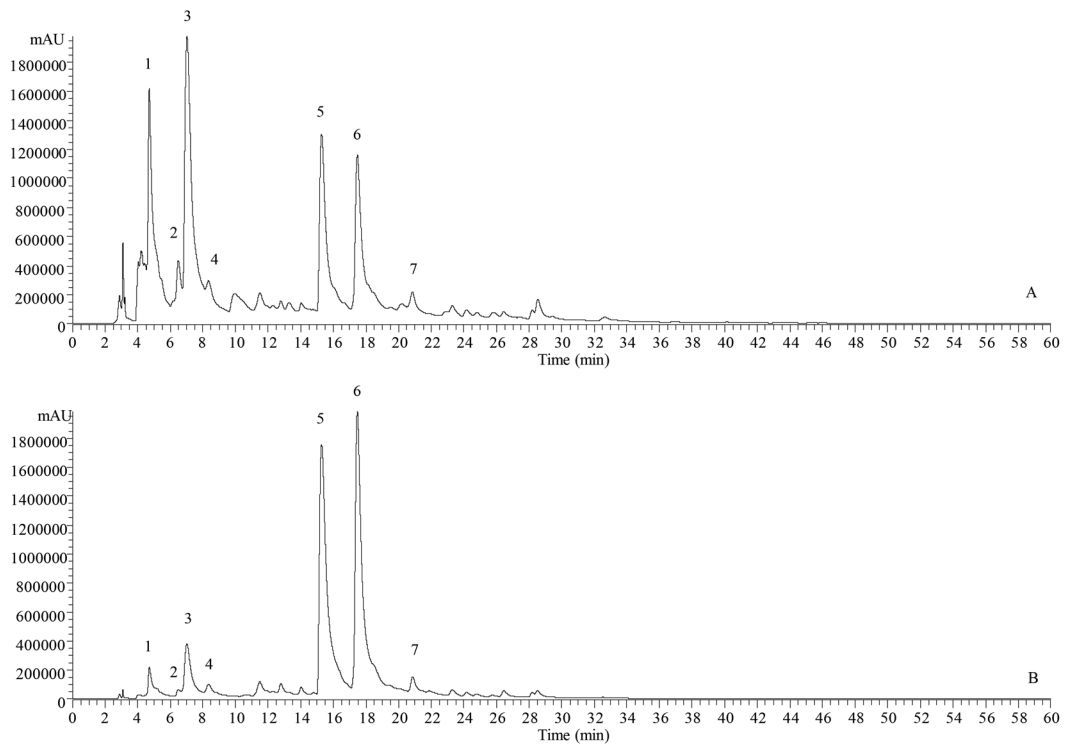

Fig. 1 Phenolic profile of leaves of *Levisticum officinale* recorded at 280 nm (A) and 370 nm (B). Peak numbers as defined in Table 5.

Table 5 Retention time (R_t), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification, and quantification (mg g^{-1} extract, mean ± SD) of phenolic compounds in the decoction and hydroalcoholic extracts of *L. officinale* (mean ± SD, n = 3)

Peak	R_t (min)	$\lambda_{max.}$ (nm)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Decoction	Hydroethanolic extract	Student's t-test p-Value	References
1	4.71	324	353	191(100), 179(52), 173(5), 161(3), 135(18)	3-*O*-Caffeoylquinic acid[a]	9.5 ± 0.1	17.2 ± 0.1	<0.001	Clifford *et al.*,[31]
2	6.50	324	353	191(18), 179(56), 173(100), 135(5)	4-*O*-Caffeoylquinic acid[a]	4.76 ± 0.06	5.78 ± 0.03	<0.001	Clifford *et al.*,[31]
3	7.03	326	353	191(100), 179(14), 161(3), 135(5)	*cis* 5-*O*-Caffeoylquinic acid[a]	7.2 ± 0.1	43 ± 1	<0.001	Clifford *et al.*,[31]
4	8.35	325	353	191(100), 179(12), 161(3), 135(3)	*trans* 5-*O*-Caffeoylquinic acid[a]	3.265 ± 0.01	4.8 ± 0.1	<0.001	Clifford *et al.*,[31]
5	15.28	234, 264, 314	423	303(100), 261(35), 243(22)	Maclurin-3-*C*-glucoside[b]	50.9 ± 0.9	79.3 ± 0.9	<0.001	Beelders *et al.*,[32,33]
6	17.48	352	609	301(100)	Quercetin-3-*O*-rutinoside[c]	36.71 ± 0.03	77 ± 1	<0.001	Spinola *et al.*, 2015[41]
7	20.87	348	593	285(100)	Kaempferol-3-*O*-rutinoside[c]	5.31 ± 0.06	6.3 ± 0.2	<0.001	Spinola *et al.*, 2015[41]
					TPA	24.7 ± 0.1	71 ± 1	<0.001	
					TF	93 ± 1	163 ± 2	<0.001	
					TPC	118 ± 1	233 ± 1	<0.001	

TPA: total phenolic acids, TF: total flavonoids, TPC: total phenolic compounds; nd: non detected; and nq: non-quantified; compounds were quantified using the following calibration curves: [a] Chlorogenic acid (y = 168 823x − 161 172, R^2 = 0.990). [b] Apigenine-6-*C*-glucoside (y = 107025x + 61 531, R^2 = 0.998). [c] Quercetin-3-*O*-glucoside (y = 34 843x − 160 173, R^2 = 0.999).

MS^2 fragmentation pattern as reported previously.[32–34] Quercetin-3-*O*-rutinoside was the most abundant flavonoid in both extracts, though it was present in a higher amount in the hydroethanolic extract. This is possibly related to the low solubility of this compound in aqueous solutions, which can also affect is bioavailability.[35] Still, quercetin-3-*O*-rutinoside, also known as rutin, has been shown to exhibit a wide range of biological activities mainly related to its antioxidant, antimicrobial, anti-inflammatory, anti-thrombogenic and anticancer activities.[36] Regarding the phenolic acid group, 3-*O*-caffeoylquinic and *cis*-5-*O*-caffeoylquinic acids were the main compounds in the decoction and hydroethanolic extracts, respectively. These compounds are frequently abundant in the human diet and have been linked to reduced risks of developing different chronic diseases mainly due to their antioxidant and anti-inflammatory properties.[37,38] The compound tentatively identified as maclurin-3-*C*-glucoside, a benzophenone derivative previously reported in honeybush,[34] was also present in considerable amounts in both extracts. Although no bioactive properties have been ascribed so far to this *C*-glycosylated compound, the aglycone maclurin has been reported to exhibit antioxidant activity, anti-cancer effects and suppression of enzymatic browning.[39,40]

Despite the similarity of the qualitative profiles in both types of extracts, significant differences were observed regarding the total amount of phenolic compounds and each individual compound ($p < 0.05$), with a higher extraction yield being achieved when using a mixture of ethanol : water as the extraction solvent. Previous data on the phenolic composition of methanolic extracts of *L. officinale* leaves have been recently reported by Złotek *et al.*[42] The authors reported the tentative identification of a total of 14 phenolic compounds, some being identical to the ones also reported in this work, such as 4-*O* and 5-*O*-caffeoylquinic acids and rutin. Nevertheless, other compounds described by Złotek *et al.*[42] such as apterin, caffeic, sinapic and *p*-coumaric acids were not found in the present work.

3.3. Bioactive properties of *L. officinale* hydroalcoholic and decoction extracts

The bioactive properties of plant extracts evaluated *in vitro* are known to be directly related to their chemical composition and concentration of bioactive compounds.[43] Although aromatic plants are generally consumed in small quantities, when ingested regularly they can be a potential source of beneficial compounds, thus playing a relevant role in the human diet. Therefore, this study also comprised the *in vitro* evaluation of different biological properties, namely antioxidant, cytotoxic and antimicrobial activities.

In this study, five different methods were applied to access the antioxidant properties of the two prepared extracts (Table 6), namely DPPH free radical uptake, reducing power, inhibition of β-carotene bleaching, inhibition of lipid peroxidation (TBARS) and inhibition of oxidative hemolysis (OxHLIA). In general, the hydroalcoholic extract showed superior activity since it presents the lowest EC_{50} values for most assays (with the exception of TBARS), which may be related to its higher concentration in phenolic compounds compared to that of the decoction.

In the OxHLIA assay, erythrocytes are subjected to hemolysis by the action of both hydrophilic and lipophilic radicals, therefore being considered an *in vitro* cell-based antioxidant assay. By observing the data presented in Table 6, it can be noticed that both extracts showed better results regarding antihemolytic activity in comparison with Trolox, a water-soluble analog of vitamin E widely used as an antioxidant in biochemi-

Table 6 Antioxidant, hepatotoxic and cytotoxic activities of extracts obtained from the edible aerial parts (leaves and stems) of *L. officinale* (mean ± SD, n = 3)

	Decoction	Hydroethanolic extract	Student's t-test p-Value
Antioxidant activity (EC_{50}, μg mL^{-1})			
DPPH[a]	124 ± 7	119 ± 3	0.335
Reducing power[b]	148 ± 1	66 ± 1	<0.001
β-Carotene bleaching inhibition[a]	609 ± 36	570 ± 7	<0.001
TBARS[a]	74 ± 5	87 ± 6	0.075
Antihaemolytic activity (IC_{50} values, μg mL^{-1})			
OxHLIA			
Δt = 60 min	29.4 ± 0.6	22.5 ± 0.6	<0.001
Δt = 120 min	54 ± 1	41 ± 1	<0.001
Cytotoxic activity (GI_{50}, μg mL^{-1})			
HeLa	>400	>400	—
NCI H460	>400	>400	—
MCF7	>400	>400	—
HepG2	>400	314 ± 6	—
Hepatotoxicity (GI_{50}, μg mL^{-1})			
PLP2	>400	>400	—

[a] EC_{50}: extract concentration corresponding to 50% of antioxidant activity. [b] EC_{50}: extract concentration corresponding to 0.5 of absorbance in the reducing power assay. Trolox EC_{50} values: 41 μg mL^{-1} (reducing power), 42 μg mL^{-1} (DPPH scavenging activity), 18 μg mL^{-1} (β-carotene bleaching inhibition), 23 μg mL^{-1} (TBARS inhibition), 19.6 μg mL^{-1} (OxHLIA Δt = 60 min) and 65.1 μg mL^{-1} (OxHLIA Δt = 120 min). GI_{50} values correspond to the sample concentration responsible for 50% inhibition of growth in tumor cells or in a primary culture of liver cells-PLP2. GI_{50} values for ellipticine (positive control): 1.2 μg mL^{-1} (MCF-7), 1.0 μg mL^{-1} (NCI-H460), 0.91 μg mL^{-1} (HeLa), 1.1 μg mL^{-1} (HepG2) and 2.3 μg mL^{-1} (PLP2).

cal assays, thus demonstrating very interesting antioxidant properties.

Regarding the evaluation of *in vitro* cytotoxic activity in tumor cells, only the hydroalcoholic extract presented activity against HepG2 cells (Table 6). Bogucka-Kocka *et al.*[44] evaluated the cytotoxic potential of hydroethanolic extracts obtained from defatted fruits of *L. officinale* against seven leukaemia human cell lines and two normal cell lines (human T cells and human B cells) and found that the extract strongly affected two leukemic cell lines (C8166 and J45), being also highly significant in inducing apoptosis. Nevertheless, the authors also found that the extract caused a significant decrease of viable cells for the two normal cell lines (H9 and WICL). In the present study, the highest tested concentration of both extracts prepared from the leaves and stems of *L. officinale* did not inhibit the growth of porcine hepatic cells (PLP2), which demonstrates the absence of hepatotoxicity and also points to a low potential of causing damage in non-tumor cells.

Regarding the antibacterial activity results (Table 7), both extracts showed the capacity of inhibiting the growth of all tested microorganisms, with the exception of the hydroethanolic extract against *P. mirabilis* for which the highest tested concentration (20 mg mL^{-1}) did not exhibit any activity. Still, none

Table 7 Antimicrobial activity of the extracts obtained from the edible aerial parts (leaves and stems) of *L. officinale* (mean ± SD, n = 3)

	Decoction		Hydroethanolic extract		Ampicillin (20 mg mL^{-1})		Imipenem (1 mg mL^{-1})		Vancomycin (1 mg mL^{-1})	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
Escherichia coli	5	>20	10	>20	<0.15	<0.15	<0.0078	<0.0078	nt	nt
Klebsiella pneumoniae	5	>20	10	>20	10	20	<0.0078	<0.0078	nt	nt
Morganella morganii	5	>20	20	>20	20	>20	<0.0078	<0.0078	nt	nt
Proteus mirabilis	20	>20	>20	>20	<015	<0.15	<0.0078	<0.0078	nt	nt
Pseudomonas aeruginosa	20	>20	10	>20	>20	>20	0.5	1	nt	nt
Gram-positive bacteria										
Enterococcus faecalis	2.5	>20	10	>20	<0.15	<0.15	nt	nt	<0.0078	<0.0078
Listeria monocytogenes	2.5	>20	5	>20	<0.15	<0.15	<0.0078	<0.0078	nt	nt
MRSA	2.5	>20	10	>20	<0.15	<0.15	nt	nt	0.25	0.5

MRSA: methicillin resistant *Staphylococcus aureus*; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; and nt: not tested.

of the extracts, irrespective of the tested concentration, showed bactericidal activity against the assayed microorganisms. In general, the decoction extract exhibited the best results as it showed the lowest MIC values for all tested bacteria, with the exception of *P. aeruginosa*. Overall, the two extracts were more efficient against Gram-positive bacteria. This observation has been previously reported by several authors and is most possibly related to the fact that this group of microorganisms presents a less complex cell wall when compared to Gram-negative bacteria that have a selective outer membrane. Moreover, Gram-negative bacteria frequently present multidrug resistance pumps (efflux pumps), which may also explain the reduced antimicrobial activity observed *in vitro* for several extracts and plant-derived compounds against this type of bacteria.[45,46] In the present study, the extracts of lovage, in particular the decoction, presented interesting results as they were also able to inhibit the growth of different Gram-negative bacteria, although requiring higher MIC values compared to those of Gram-positive bacteria. Interestingly both extracts inhibited *P. aeruginosa*, with a lower MIC value being obtained for the hydroethanolic extract (Table 7). *P. aeruginosa* is an opportunistic pathogen frequently associated with nosocomial infections, and is being considered an emerging threat to public health due to its intrinsic resistance to several antibiotics. Moreover, infections caused by *P. aeruginosa* are becoming increasingly difficult to treat as the number of multidrug resistant strains is growing worldwide.[47,48] Since numerous strains have been reported as being resistant to several commonly used classes of antibiotics,[48] it has been suggested that medicinal plant extracts/compounds may provide a promising approach as adjuvant therapy, as several studies showed that the antibiotic activity can be potentiated when in combination with phytochemicals.[46,47,49] It should be noted that in the present study, the tested microorganisms were clinical isolates resistant to different antibiotics.

4. Conclusion

In this work, a comprehensive study on the nutritional, chemical and bioactive properties of the edible aerial parts (leaves and stems) of a Portuguese grown sample of *L. officinale* was performed. Similar to other aromatic plants, lovage is shown to be a low caloric food as it presents a high content of moisture. Regarding the composition of hydrophilic compounds in the leaves and stems of lovage, chromatographic analysis showed that sucrose and oxalic acid are the most abundant free sugar and organic acid, respectively. Moreover, a total of 7 phenolic compounds were detected and tentatively identified in the two types of extracts prepared (decoction and hydroethanolic extract), with 5-*O*-caffeoylquinic and 3-*O*-caffeoylquinic acid being the most abundant phenolic acids and quercetin-3-*O*-rutinoside and maclurin-3-*C*-glucoside the major flavonoids. Besides the presence of these bioactive compounds, lovage also showed an interesting fatty acid profile, as it is rich in polyunsaturated fatty acids, in particular having α-linolenic acid, an omega-3 essential fatty acid, as a major compound. In the lipidic fraction, two tocopherol isoforms, α and γ, were also detected. The aroma profile of the essential oil hydrodistilled from this aromatic plant showed that it was rich in monoterpenes, with α-terpinyl acetate being the most abundant volatile, followed by *p*-cymene. Interestingly, a high content of phthalides, which have been associated with health benefits, was also observed.

Regarding the bioactive potential of this plant, both extracts showed interesting properties, and it is worth mentioning that they showed better activity in the OxHLIA test when compared to the antioxidant used as the control (Trolox). The extracts were able to inhibit the growth of all tested bacteria, and were in general more active against Gram-positive bacteria. Regarding cytotoxicity against tumor cell lines, it was found that only the hydroethanolic extract showed activity against liver cancer cells. Moreover, both extracts did not show toxic effects against the q non-tumor liver cell line (PLP2), suggesting that they are safe for human use.

In brief, an extensive and detailed study was performed revealing that lovage has an interesting composition from the nutritional point of view being also a source of several bioactive compounds; therefore its inclusion as a seasoning/flavoring agent in different dishes should be promoted.

Conflicts of interest

All authors declare that they do not have any conflict of interest for publishing this research work.

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