Lovage \textit{(Levisticum officinale} W.D.J. Koch) Roots: A Source of Bioactive Compounds Towards a Circular Economy

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Abstract: Lovage \textit{(Levisticum officinale W.D.J. Koch)} is an aromatic plant from the Apiaceae (Umbelliferae) family used as a condiment in several regions of Europe and also described to have medicinal properties. While the aerial parts are used as foods, the roots are generally discarded. In the past, lovage roots were used in folk medicine for their diuretic, carminative, and spasmolytic properties. Therefore, the exploitation of this undervalued part of the plant can be a source of valuable bioactive compounds for food and/or pharmaceutical industries. Thus, in this study, extracts of different polarity were prepared and studied regarding their chemical composition and bioactive properties. To the best of our knowledge, the composition in phenolic compounds and the volatile profile of the \textit{n}-hexane extract are reported for the first time. A total of 24 compounds were identified by GC-MS in the \textit{n}-hexane extract, evidencing a high relative abundance of phthalides. A total of eight phenolic compounds were identified in lovage root extracts (decoction and hydroethanolic extract), with vanillic acid being the major compound. Regarding antioxidant activity, also reported for the first time, decoction and hydroethanolic extract exhibited a high antioxidant capacity in thiobarbituric acid reactive substances (TBARS) \(179 \pm 11 \mu g/mL\) and in oxidative hemolysis (OxHLIA) assays \(510 \pm 6 \mu g/mL\), respectively. \textit{n}-Hexane extract showed relevant anti-proliferative activity against all tumor cell lines tested \(\text{GI}_{50}, 48-69 \mu g/mL\), despite inhibiting also the growth of a non-tumoral hepatocyte cell line, however, presenting a significantly higher \(\text{GI}_{50}\) value \(147 \mu g/mL\). This study revealed that lovage root, an agri-food residue, can be a source of valuable bioactive compounds also presenting biological properties that deserve being explored, which could lead to a circular economy for food and/or the pharmaceutical industry.

Keywords: \textit{Levisticum officinale} roots; phenolic compounds; phthalides; bioactivity; agri-food waste; circular economy

1. Introduction

Since antiquity, numerous aromatic plants and spices have been used worldwide in folk medicine, in addition to their common usage for food purposes. More recently, the demand for novel biologically active substances and the need for studies that support the empiric use of different plants in folk medicine, has increased the interest and encouraged the study of several of these plants.
So far, several studies have shown that plants in the Apiaceae family have different phytochemical compounds and secondary metabolites of interest in their composition, such as terpenoids, saponins, flavonoids, tannins, phenolic acids, polyacetylenes, and steroids. These compounds have a potential source of natural bioactive and agrochemical compounds [1,2].

*Levisticum officinale* W.J.D. Koch, with the common name lovage, is a tall perennial aromatic plant that belongs to the Apiaceae family, being cultivated in numerous European countries. Among the numerous species of aromatic herbs used in culinary recipes, lovage was once much recognized, being extensively used either by the industry as well as by households in soups, stews, and meat dishes [3]. Currently, besides being used by the food industry, particularly for condiment production, lovage is increasingly used by renowned culinary chefs. However, only the leaves of lovage are used as a condiment, hereupon the roots of this plant go unnoticed, becoming a by-product that is frequently discarded. Nevertheless, lovage roots have been described to display different medicinal properties, and in particular, they are described as possessing diuretic, carminative, and spasmyloytic activity [4]. Moreover, in several different European countries, they have been considered to have a well-established use as a comminuted herbal substance for oral use in minor urinary complaints and inflammation [5]. Therefore, the chemical composition of lovage roots is worth being explored, as this agri-food by-product can be a valuable source of bioactive compounds and/or extracts to be exploited by the food and/or pharmaceutical industries.

So far, only a few studies are available on the chemical composition of lovage roots; those focusing mainly on the composition of the essential oil obtained by hydrodistillation [6,7] or by CO₂ supercritical extraction [8]. Recent studies have shown that the essential oil extracted from this plant is characterized by the presence of phthalides [9], which have been reported to exist in higher amounts in the roots compared to the aerial parts [7]. Phthalides have been described in other plant species used in folk medicine such as *Angelica sinensis* (known in Chinese medicine as Danggui) and have been associated with several different biological properties, such as anti-inflammatory [10,11], antioxidant [12], antitumor [13,14], and protective effects against neurological disorders [15,16].

Concerning other bioactives, such as phenolic compounds, which have been raising high interest for their beneficial health effects and possible use as food additives [17,18], as far as the literature consulted, no studies have been performed up until now. Therefore, to address this gap, this work aimed at the chemical characterization of lovage roots’ extracts prepared with solvents of different polarities. To this goal, the composition in phenolic compounds was determined for three different extracts (hydroethanolic and aqueous root extracts and hydroethanolic extract prepared with the plant residue obtained after hexane extraction). Moreover, the composition in volatile and apolar compounds of the essential oil extracted by hydrodistillation and that of the hexane extract was also evaluated. Besides the chemical characterization, a comprehensive evaluation of the bioactive properties of lovage roots’ extracts was also performed, comprising the evaluation of antioxidant activity measured by several different assays, and antimicrobial activity and cytotoxicity against tumoral and non-tumoral cell lines.

2. Materials and Methods

2.1. Samples

Dried roots of *L. officinale* were bought in October 2018 from a specialized herbal shop in Spain (Biomanantial, Madrid) and were produced by the company Pinisan (Madrid, Spain). After identification, a specimen voucher was deposited in the herbarium of the School of Agriculture, Polytechnic Institute of Bragança (Portugal). The roots were weighed, lyophilized (FreeZone 4.5, Labconco, MO, USA), ground to a fine powder (20 mesh), and kept at −20 °C until analysis.
2.2. Preparation of Extracts

Four different extracts were prepared, namely, a decoction, a hydroethanolic, n-hexane, and a hydroethanolic extract prepared from the residue of the n-hexane extract. The decoction was prepared by boiling 3 g of the lyophilized roots with 300 mL of deionized water (TGI Pure Water Systems, Greenville, SC, USA) for 5 min and was allowed to stand at room temperature for another 5 min and then filtered through Whatman filter paper No. 4. The obtained solution was lyophilized.

The hydroethanolic extract was obtained by extracting 3 g of the lyophilized roots with 90 mL of ethanol/water (80:20, v/v) under constant magnetic stirring at room temperature for 1 h. After filtering by a Whatman paper filter No. 4, the residue was re-extracted, the two filtrates obtained were gathered, and the ethanol was removed under vacuum by using a rotary evaporator (Buchi R-2010) set at 40 °C. The obtained solution was frozen and further lyophilized.

For the preparation of the hexane extract, 3 g of the root were stirred with 90 mL of hexane during 1 h at room temperature. The preparation was paper filtered, and the residue re-extracted by repeating the procedure. Subsequently, the n-hexane was evaporated using a rotary evaporator at 40 °C to obtain the extract.

A hydroethanolic extract was further prepared with the plant residue resulting from n-hexane extraction, using a procedure similar to the described for the preparation of the hydroethanolic extract.

2.3. Chemical Parameters

2.3.1. Phenolic Compounds

Phenolic compounds were analyzed in the following extracts: decoction, hydroethanolic, and hydroethanolic extract prepared from the hexane residue. Before analysis, the first was re-dissolved in water while the others were re-dissolved in ethanol/water (80:20, v/v), to a concentration of 5 mg/mL, and filtered through a 0.22 µm disposable LC filter disk. The compounds were evaluated using a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA) equipped with a quaternary pump and a diode array detector coupled in-series to an electrospray ionization mass spectrometry detector (LC-DAD-ESI/MSn) operating under the conditions [19].

The compound identification was made by comparing the data obtained concerning retention time, UV-VIS, and mass spectra (in full scan mode covering the mass range from 100 to 1500 m/z). The fragmentation patterns of the sample compounds were compared with those obtained from the available standards and/or reported data from the literature. For quantification purposes, calibration curves were constructed based on the UV-VIS signal of the commercial standards (Extrasynthese, Genay, France). The results were expressed in mg/g extract.

2.3.2. Volatile Compounds

The essential oil of the lovage roots was extracted by hydrodistillation using a Clevenger system. After distillation, the essential oil was recovered by adding 1 mL of HPLC grade n-hexane, due to its low yield. After the addition of anhydrous sodium sulphate to remove any water that could be present, the diluted oil was analyzed by GC-MS. Additionally, the n-hexane extract was also analyzed. Analyses were performed in a GC-2010 Plus (Shimadzu) gas chromatograph equipped with an AOC-20iPlus (Shimadzu, Kyoto, Japan) automatic injector, a mass spectrometry detector, and a SH-RXi-5ms column (30 m × 0.25 mm × 0.25 µm; Shimadzu, Kyoto, Japan), operating under the conditions previously described [20].

For compound identification, the obtained spectra was compared with those from the NIST17 mass spectral library (considering a similarity > 90%) and by comparing the linear retention index (LRI), which were determined based on the retention times obtained for a mixture of n-alkanes (C8–C40, ref. 40147-U, Supelco). When possible, comparisons were also performed with available data from literature and with spectra and retention time of commercial standard compounds.
The different compounds were quantified as a relative percentage of total volatiles using relative peak area values obtained from the total ion current (TIC) values.

2.4. Bioactive Properties

2.4.1. Antioxidant Activity

The four prepared extracts were evaluated for their antioxidant properties using different in vitro assays, including 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 inhibition of oxidative hemolysis (OxHLIA) as described previously [19,21]. Briefly, the reduction of the DPPH radical was determined by measuring the absorption at 515 nm; the radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: % RSA = [(A_DPPH − A_S)/A_DPPH] × 100, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_DPPH is the absorbance of the DPPH solution. Reducing power was calculated using the equation: (Abs_sample extract − Abs_control) and the absorbance was measured at 690 nm. β-Carotene bleaching inhibition was calculated using the following equation: inhibition ratio (%) = [(A − B)/A] × 100%, where A and B were the absorbance of the control and the sample extract, respectively. For the OxHLIA assay, the results were expressed as delayed time of hemolysis (Δt), which was calculated according to the equation: Δt (min) = Ht50 (sample extract) − Ht50 (control), where Ht50 is the time (min) corresponding to 50% hemolysis, graphically obtained from the hemolysis curve of each antioxidant sample concentration.

The hydroethanolic, n-hexane, and hydroethanolic extract prepared from the residue of hexane were re-dissolved in ethanol/water (80:20, v/v) and the decoction was re-dissolved in water. All extracts were re-dissolved at a final concentration of 5 mg/mL and further diluted in the range of 5–0.0391 mg/mL to perform the different in vitro assays.

The results of the assays were expressed as EC50, corresponding to the extract concentrations providing 50% of antioxidant activity, with the exception for the reducing power assay for which EC50 corresponds to 0.5 of absorbance at 690 nm. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard.

2.4.2. Hepatotoxicity and Cytotoxic Activity

Hepatotoxicity was evaluated by the Sulforhodamine B (Sigma-Aldrich, St. Louis, MO, USA) assay [22], using a primary cell culture (PLP2) prepared from a porcine liver and different concentrations of the hydroethanolic or decoction extracts, ranging from 400 to 6.5 µg/mL. The assay was not performed for the n-hexane and hydroethanolic extract from n-hexane residue due to incompatibilities with the culture reaction media. The anti-proliferative capacity of the two extracts was also evaluated by the same method but using four human tumor 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). The cell growth inhibition was calculated according to the equation: [(Abs_sample extract and cells − 0.05)/(Abs_control − 0.05) × 100]. In both cases, ellipticine (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control, and the results were expressed in GI50 values (µg/mL), corresponding to the extract concentration that provides 50% of cell growth inhibition.

2.4.3. Antimicrobial Activity

The antibacterial activity was evaluated using the broth microdilution method coupled to the rapid p-iodonitrotetrazolium chloride (INT) colorimetric assay [23]. The microorganisms used were clinical isolates, namely three Gram-positive bacteria (Enterococcus faecalis, Listeria monocytogenes,
and methicillin-resistant \textit{Staphylococcus aureus} and five Gram-negative bacteria (\textit{Escherichia coli}, \textit{Klebsiella pneumoniae}, \textit{Morganella morganii}, \textit{Proteus mirabilis}, and \textit{Pseudomonas aeruginosa}). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were evaluated, and different antibiotics were used as negative control (ampicillin and imipenem for Gram-negative bacteria, and vancomycin and ampicillin for Gram-positive bacteria) [23].

2.5. Statistical Analysis

All experiments were carried out in triplicate and the results were expressed as mean ± standard deviation (SD). SPSS Statistics software (IBM SPSS Statistics for Windows, v. 23.0) was used and the data were statistically analyzed by analysis of variance (ANOVA), followed by the Tukey test. The statistical significance level was set at $p < 0.05$. When we have less than three samples, the results were analyzed by $t$-Student test, as a form to determine the significant differences between two samples, with $p = 0.05$.

3. Results and Discussion

3.1. Chemical Characterization of \textit{L. officinale} Roots

3.1.1. Composition in Phenolic Compounds

The phenolic compounds profile of \textit{L. officinale} roots can be visualized in Figure 1. Data regarding retention time, $\lambda_{\text{max}}$ in the visible region, molecular ion and main fragment ions observed in MS\textsuperscript{2}, and phenolic compound identification and quantification, are presented in Table 1.

![Figure 1](image_url)

\textbf{Figure 1.} Phenolic profile obtained for the hydroethanolic extract of \textit{Levisticum officinale} root recorded at 280 nm (A) and 370 nm (B). Compounds are numbered as indicated in Table 1.
Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region (\(\lambda_{\text{max}}\)), mass spectral data, and tentative identification and quantification (mg/g extract) of phenolic compounds in *Levisticum officinale* roots.

| Peak | Rt (min) | \(\lambda_{\text{max}}\) (nm) | Molecular Ion [M\(–\)H]\(^+\) (m/z) | Tentative Identification | Hydroethanolic Decoction | Hydroethanolic Extract of Hexane Residue | References |
|------|---------|-----------------------------|---------------------------------|-------------------------|--------------------------|------------------------------------------|-----------|
| 1    | 4.69    | 192, 258                    | 341 179 (100)                  | Caffeic acid hexoside \(^1\) | nd                       | 0.44 ± 0.02                              | nd        |
| 2    | 7.04    | 286, 320                    | 167 123 (100)                  | Vanillic acid \(^2\)     | 1.51 ± 0.03 \(^b\)       | 19.7 ± 0.4 \(^a\)                        | 0.49 ± 0.01 \(^c\) |
| 3    | 14.07   | 201, 225                    | 533 515 (5), 371 (100), 353 (13), 209 (7), 191 (5), 179 (3) | di-Caffeoylglucaric acid \(^1\) | 0.056 ± 0.002 \(^b\)       | 0.6163 ± 0.0004 \(^a\)                   | 0.025 ± 0.003 \(^c\) |
| 4    | 15.44   | 200, 323                    | 561 367 (100), 193 (26), 191 (13), 173 (100), 129 (69), 111 (2) | Hydroferuloyl-feruloylquinic acid \(^3\) | 0.044 ± 0.002 \(^*\)       | 0.582 ± 0.006 \(^*\)                     | nd        |
| 5    | 16.79   | 307                         | 935 926 (10), 897 (10), 783 (12), 633 (100), 301 (48) | Galloyl-bis-HHDP-glucoside \(^4\) | 0.984 ± 0.001             | nd                                       | nd        |
| 6    | 17.20   | 324                         | 547 529 (8), 385 (100), 367 (27), 353 (21), 335 (3), 191 (7), 179 (3), 173 (3) | Caffeoyl-feruloylquinic acid \(^3\) | 0.171 ± 0.005 \(^b\)       | 1.62 ± 0.04 \(^*\)                       | 0.089 ± 0.002 \(^c\) |
| 7    | 20.61   | 196, 233, 280               | 361 343 (100), 325 (2), 199 (44), 181 (3) | Catalpol \(^5\)          | 0.126 ± 0.005 \(^b\)      | 0.66 ± 0.02 \(^*\)                       | 0.059 ± 0.002 \(^c\) |
| 8    | 22.81   | 234, 282, 322               | 251 233 (22), 207 (100), 193 (9), 179 (28), 175 (42), 153 (2) | Unknown                  | nd                        | nd                                       | nd        |
| 9    | 25.19   | 233, 281, 322               | 389 371 (100), 341 (25), 327 (7), 193 (38) | Methoxylic resinol \(^5\) | 0.171 ± 0.006 \(^b\)      | 0.639 ± 0.006 \(^*\)                     | 0.0484 ± 0.0005 \(^c\) |
|      |         |                             |                               | TPA                      | 1.78 ± 0.04 \(^b\)        | 23.1 ± 0.5 \(^a\)                       | 0.61 ± 0.01 \(^c\) |
|      |         |                             |                               | Other compounds          | 0.298 ± 0.001 \(^b\)      | 1.295 ± 0.01 \(^a\)                      | 0.108 ± 0.002 \(^c\) |

TPA—Total phenolic acids, nd—not detected; nq—not quantified. Calibration curves used: \(^1\)—caffeic acid (\(y = 388,345x + 406,369, R^2 = 0.998\)), \(^2\)—vanillic acid (\(y = 29,751x – 28,661, R^2 = 0.999\)), \(^3\)—ferulic acid (\(y = 633,126x – 185,462, R^2 = 0.999\)), \(^4\)—ellagic acid (\(y = 26,719x – 317,255, R^2 = 0.999\)), \(^5\)—protocatechic acid (\(y = 214,168x + 27,102, R^2 = 0.999\)). In each column different letters \((a, b, c)\) mean significant differences between the different extracts \((p < 0.05)\). * Means statistical differences obtained by a t-student teste.
Nine compounds were detected in the root samples, with eight being identified or tentatively identified and one of the analyzed molecules remaining unknown. Peak 1 ([M−H]− at m/z 341) released a fragment at m/z 179 [caffeic acid-H]− (−162 u, loss of a hexosyl residue) and therefore was tentatively identified as caffeic acid hexoside [24]. Vanillic acid (Peak 2, Figure 2) was positively identified according to its retention time characteristics, mass spectra, and UV-VIS compared to a commercial standard. Peak 3 ([M−H]− in m/z 533) was tentatively identified as di-caffeoyl-glucaric acid, taking into account the fragmentation pattern [25]. Similarly, Peak 4 presented a pseudomolecular ion at m/z 561 and was tentatively identified as hydroferuloyl feruloylquinic acid [25]. Peak 5 ([M−H]− at m/z 935) showed MS2 fragmentation ions at m/z 633 and 301, probably due to the loss of two HHDP (hexahydroxydiphenic) residues and a glucoside, consistent with a galloyl-bis-HHDP-glucoside [26]. Peak 6 showed a precursor ion at m/z 547 [M−H]−, the first loss being a water molecule ([M-18]− at m/z 529); the ion fragments in m/z 367 and 193 indicated the presence of a feruloylquinic and m/z 179 indicated a caffeoyl residue. Therefore, this compound was tentatively identified as caffeoyl-feruloylquinic acid based on the fragmentation pattern reported by [27]. Peak 7 was identified as belonging to the iridoid class. This compound had a deprotonated molecule at m/z 361 and was tentatively identified as catalpol (MW 362, Figure 2), a compound that has been previously detected in the Apiaceae family [28,29]. The compound in Peak 9 showed to be a lignan since it presented a pseudomolecular ion [M−H]− at m/z 389, releasing a fragment at m/z 341 ([M−H-48]−, loss of a methoxy residue, consistent with a lariciresinol. A similar compound with an identical fragmentation pattern has been described [30], so this compound was tentatively referred to as methoxylariciresinol. Nevertheless, it was not possible to achieve a possible identification for compound 8 ([M−H]− at m/z 251) and therefore, it remained unknown.

![Vanillic acid](image1.png)  
Vanillic acid

![Catalpol](image2.png)  
Catalpol

Figure 2. Chemical structures of some identified compounds.

From the four prepared extracts, only three were evaluated by HPLC-DAD-ESI/MSn, with the n-hexane extract being evaluated only by GC-MS due to its lower polarity, as it was not easily soluble in a polar solvent compatible with the conditions set for HPLC analysis. As it can be observed in Table 1, vanillic acid was the most abundant phenolic compound in all the extracts, being the decoctions as the extract that showed the highest amount of total phenolic compounds (24.3 ± 0.5 mg/g extract), mainly due to the high content of phenolic acids. This may be related to the higher polarity of the solvent used, as well as to the use of a higher extraction temperature.

In opposition, the extract prepared with the residue obtained from the n-hexane extraction showed the lowest abundance of phenolic compounds, presenting a statistical difference from the hydroethanolic extract. This can be related to experimental loss of compounds during the preparation of the extract and a lower extractability due to the fact that the vegetable cells were first embedded with an apolar solvent. Although in low amounts, all the extracts exhibited the presence of the iridoid compound catalpol, which has been described as possessing extensive pharmacological activity, and playing essential roles in the treatment of many diseases including kidney diseases [31], neurodegenerative diseases [32], and diabetes [33]. The presence of this compound thus may support the traditional use of lovage root as a diuretic in the treatment of minor urinary complaints. As far as we know, this is the first report on the phenolic compound composition of L. officinale roots.
3.1.2. Composition in Volatile Compounds

The composition of the essential oil extracted by hydrodistillation from *L. officinale* roots and that of the *n*-hexane extract (oily residue obtained after the evaporation of *n*-hexane) is presented in Table 2. Figure 3 shows a representative chromatogram of GC-MS analysis of the referred samples.

Table 2. Chemical composition of the essential oil extracted from the roots of *L. officinale* by hydrodistillation in a Clevenger system and with *n*-hexane (mean ± SD, n = 3).

| Compound                  | RT (min) | LRI a | LRI b | Relative % c |
|---------------------------|----------|-------|-------|--------------|
| Heptanal                  | 12.66    | 901   | 901   | 0.024 ± 0.005|
| α-Pinene                  | 14.25    | 932   | 932   | 0.091 ± 0.006|
| β-Pinene                  | 16.47    | 974   | 974   | 0.59 ± 0.04 | 0.097 ± 0.005|
| 2-Pentyl furane           | 17.31    | 991   | 984   | 0.073 ± 0.004|
| n-Octanal                 | 17.90    | 1002  | 998   | 0.046 ± 0.002|
| p-Cymene                  | 19.01    | 1025  | 1020  | 0.028 ± 0.003|
| β-Phellandrene            | 19.21    | 1027  | 1025  | 1.26 ± 0.008 | 0.79 ± 0.03|
| Linalool                  | 22.91    | 1099  | 1095  | 0.079 ± 0.001|
| Nonanal                   | 23.10    | 1102  | 1100  | 0.095 ± 0.007|
| β-Fenchol                 | 23.57    | 1112  | 1118  | 0.02 ± 0.01|
| α-Carpholene              | 24.19    | 1124  | 1122  | 0.015 ± 0.003|
| trans-Pinocarveol         | 24.84    | 1137  | 1135  | 0.07 ± 0.02|
| Menthol                   | 25.60    | 1153  | 1148  | 0.39 ± 0.02|
| 5-Pentacyclohexa-1,3-diene| 25.84    | 1154  | -     | 0.16 ± 0.01|
| Pentylbenzene             | 25.75    | 1156  | 1152  | 1.01 ± 0.06|
| Pinocarveol               | 26.04    | 1162  | 1160  | 0.009 ± 0.002|
| Menthan-3-one             | 26.12    | 1163  | 1158  | 0.28 ± 0.05|
| Menthol                   | 26.53    | 1172  | 1167  | 0.494 ± 0.005 | 0.04 ± 0.01|
| α-Terpineol               | 27.41    | 1189  | 1186  | 0.057 ± 0.002|
| Myrtenol + estragole      | 27.7     | 1195  | 1194  | 0.131 ± 0.005|
| n-Decanal                 | 28.11    | 1204  | 1201  | 0.033 ± 0.003|
| Pulegone                  | 29.75    | 1239  | 1233  | 0.073 ± 0.001|
| Carvone                   | 29.97    | 1243  | 1239  | 0.115 ± 0.002|
| p-Menth-1-en-7-al         | 31.41    | 1274  | 1269  | 0.075 ± 0.001|
| Anethole                  | 31.89    | 1284  | 1282 e | 0.34 ± 0.01 | 0.04 ± 0.01|
| p-Vinyl-allyl acetate     | 33.21    | 1313  | 1309  | 1.80 ± 0.01|
| α-Terpinyl acetate       | 34.83    | 1350  | 1346  | 0.13 ± 0.01|
| Valerofenol               | 35.07    | 1356  | 1359  | 0.96 ± 0.03 | 0.055 ± 0.004|
| Cyclosativene             | 35.69    | 1369  | 1369 k | 0.080 ± 0.007|
| α-Copaene                 | 36.04    | 1378  | 1374  | 0.066 ± 0.002|
| β-Elemene                 | 36.72    | 1393  | 1389  | 0.035 ± 0.002|
| Vanillin                  | 36.90    | 1397  | 1393  | 0.19 ± 0.02|
| α-Pompea                  | 37.55    | 1412  | 1407  | 0.097 ± 0.006|
| α-Guaiene                 | 38.23    | 1429  | 1431  | 0.109 ± 0.006|
| Aromadendrene             | 38.77    | 1442  | 1444  | 0.061 ± 0.003|
| β-Acoradiene              | 39.93    | 1469  | 1469  | 0.207 ± 0.009 | 0.04 ± 0.01|
| 10-epi-β-Acoradiene       | 40.24    | 1477  | 1474  | 0.37 ± 0.02|
| Ar-Curcumene              | 40.51    | 1483  | 1479  | 0.49 ± 0.002 | 0.07 ± 0.01|
| β-Selinene                | 40.75    | 1489  | 1489  | 0.091 ± 0.005|
| 4-epi-cis-Dihydro agarofurane | 40.87 | 1492  | 1499  | 0.228 ± 0.01|
| α-Zingiberene             | 41.04    | 1496  | 1493  | 0.40 ± 0.03|
| α-Murolene                | 41.31    | 1503  | 1500  | 0.28 ± 0.02|
| Cuparene                  | 41.55    | 1509  | 1504  | 1.07 ± 0.07 | 0.11 ± 0.01|
| β-Cadinene                | 42.22    | 1526  | 1522  | 0.84 ± 0.004 | 0.10 ± 0.01|
| Kessane                   | 42.47    | 1532  | 1529  | 2.1 ± 0.1 | 0.30 ± 0.01|
| α-Calacorene              | 43.01    | 1546  | 1544  | 0.149 ± 0.009|
| Elemicin                  | 43.44    | 1557  | 1555  | 0.060 ± 0.002|
| Spathulenol               | 44.41    | 1581  | 1577  | 6.3 ± 0.2 | 1.13 ± 0.03|
| Globulol                  | 44.71    | 1589  | 1590  | 0.96 ± 0.05|
| 6,6-Dimethyl-cyclooct-4-enone | 46.08 | 1625  | 1618  | 0.40 ± 0.01|
| 10-epi-β-Eudesmol         | 46.24    | 1629  | 1622  | 0.59 ± 0.02|
| 1-epi-Cubenol             | 46.35    | 1632  | 1627  | 0.41 ± 0.02|
| Hexahydro-3-butyolphthalide | 46.66 | 1640  | 1647  | 1.86 ± 0.08 | 0.46 ± 0.01|
| 3-Butyolphthalide         | 47.19    | 1655  | 1647  | 6.8 ± 0.3 | 1.72 ± 0.02|
| 5-Z-Butyldeneophthalide   | 47.98    | 1676  | 1671  | 29 ± 2 | 8.8 ± 0.2|
while only 24 were identified in the 2020 Resources the two samples in terms of the presence of monoterpenes, which are compounds frequently present under vacuum, due to their high volatility and low amount in the sample. Therefore, when comparing compounds extracted with a total of 66 di-chromatogram obtained by GC-MS for analysis of the hydrodistilled essential oil (Figure 3. a LRI, linear retention index determined on a DB-5 MS fused silica column relative to a series of n-alkanes (C8-C40). b Linear retention index reported in literature (Adams, 2017). c Relative % is given as mean ± SD, n = 3.

| Compound                      | RT (min) | LRI a  | LRI b  | Relative % c |
|--------------------------------|----------|--------|--------|--------------|
| 56. E-Butylidenefthalide       | 49.59    | 1721   | 1717   | 8.3 ± 0.5    |
| 57. Neocnidilide               | 50.01    | 1745   | 1722   | 8.9 ± 0.7    |
| 58. Z-Ligustilide              | 50.1     | 1749   | 1736   | 8.5 ± 0.3    |
| 59. E-Ligustilide              | 51.49    | 1808   | 1796   | 1.87 ± 0.09  |
| 60. Z-Termine                  | 52.2     | 1849   | 1844   | 0.26 ± 0.03  |
| 61. n-Hexadecanol              | 52.59    | 1881   | 1874   | 0.146 ± 0.007|
| 62. Metil hexadecanoato        | 53.17    | 1927   | 1921   | 0.41 ± 0.05  |
| 63. Metil cis-6-octadecenoato  | 54.87    | 2097   | 2091   | 0.70 ± 0.1   |
| 64. Palmitic acid              | 55.62    | 1967   | -      | 4.2 ± 0.5    |
| 65. Linoleic acid              | 55.28    | 2149   | -      | 18 ± 1       |
| 66. α-Tocopherol               | 63.25    | 3166   | -      | 0.51 ± 0.06  |

|                        | Relative % |
|------------------------|------------|
| Total identified       | 88.4 ± 0.3 |
| Monoterpene hydrocarbons | 3.0 ± 0.2  |
| Oxygen-containing monoterpenes | 12.0 ± 0.3 |
| Sesquiterpene hydrocarbons | 5.3 ± 0.3  |
| Oxygen-containing sesquiterpenes | 3.4 ± 0.1  |
| Phthalides              | 52.2 ± 2.0 |
| Others                  | 12.5 ± 0.8 |
| α-Tocopherol            | 8.9 ± 0.4  |

Figure 3. Chromatogram obtained by GC-MS for analysis of the hydrodistilled essential oil (A) and n-hexane extract (B) from Levisticum officinale roots.

GC-MS analysis allowed the identification of 88.4–99.1% of the compounds, corresponding to a total of 66 different compounds, 60 of them being identified in the laboratory-hydrodistilled oil while only 24 were identified in the n-hexane extract. This result is probably do the fact that some compounds extracted with n-hexane could be eliminated during the step of removing n-hexane solvent under vacuum, due to their high volatility and low amount in the sample. Therefore, when comparing the two samples in terms of the presence of monoterpenes, which are compounds frequently present...
in many essential oil-bearing plants, it can be observed that several compounds that were present in minor amounts in the hydrodistilled sample (<1%) are no longer found in the n-hexane extract. Curiously, three compounds were only detected in the n-hexane extract, as shown in Table 2. To the best of our knowledge, the composition of the n-hexane extract obtained from lovage roots is herein described for the first time. In both the essential oil and n-hexane extract, the phthalide group was the major one, with a relative percentage of 52.2% in the former and a higher amount in the last (65.1%).

Phthalides, presenting the molecular form C₈H₆O₂, are a relatively small group of natural compounds confined to some plant families and some genera of fungi, among which the Apiaceae family stands out [34]. In terms of individual phthalide compounds, the two samples were very distinct, with the essential oil presenting (Z)-butylidenephthalide (29.0%) as the major phthalide while in the n-hexane extract (E)-ligustilide (25.7%) was the major compound, as shown in Figure 4, closely followed by its isomer (Z)-ligustilide (20.5%). A considerable amount (23.7%) of terpenes (including oxygenated and non-oxygenated mono and sesquiterpenes) was identified in the hydrodistilled oil, while only a few amounts (<3%) were found in the hexane extract. Compared to these results, the essential oil obtained from L. officinale leaves seems very distinct as it generally presents monoterpenes as the main constituent group followed by oxygenated monoterpenes, with papers reporting the composition of oils obtained from lovage grown in different countries frequently reporting α-terpinyl acetate as the main compound [4,6,35]. In opposition, the chemical composition of the roots revealed to have phthalides as the characteristic and main group of compounds. The obtained results are in good agreement with [6], that also reported phthalides as the major group of compounds in lovage root, but not with the results reported by [7], that found terpenes as the predominant compounds, namely Z-β-ocimene (28.1%), followed by α-terpinyl acetate (21.1%) and β-phellandrene (17.3%). Curiously, both these previous works regarded the composition of the essential oil obtained by hydrodistillation from roots of lovage plants grown in Iran [6,7]. However, the plants were grown in different regions of the country, namely Tehran, in the north [6] and the Hezar Mountain of Kerman province in the south [7]. Thus, the observed differences between those specimens and also with the composition of the herein studied sample submitted to Clevenger extraction, may be related to factors such as edaphoclimatic variations, which are known to affect plants’ chemical composition.

![Chemical structures of some identified compounds.](image)

**Figure 4.** Chemical structures of some identified compounds.

### 3.2. Bioactive Properties

#### 3.2.1. Antioxidant Activity

Up until now, several assays based on different mechanisms have been proposed in the literature for the evaluation of the antioxidant capacity of chemical compounds and natural products, such as foods and medicinal plants. In this study, five distinct methodologies were applied: scavenging of free DPPH radicals, reducing power, inhibition of β-carotene discoloration, TBARS, and OxHLIA. The obtained results are presented in Table 3.
Table 3. Antioxidant activity of extracts obtained from the roots of *L. officinale* (mean ± SD, n = 3).  

| Antioxidant Activity | Decoction | Hydroethanolic | Hexane | Hydroethanolic Extract of Hexane Residue | Positive Control |
|----------------------|-----------|----------------|--------|------------------------------------------|-----------------|
| DPPH <sup>a</sup>    | 101 ± 2<sup>c</sup> | 148 ± 5<sup>b</sup> | 469 ± 5<sup>a</sup> | 58 ± 1<sup>d</sup> | 42 ± 1 |
| Reducing power <sup>b</sup> | 153 ± 2<sup>b</sup> | 153 ± 2<sup>b</sup> | 1665 ± 64<sup>a</sup> | 114 ± 4<sup>b</sup> | 41 ± 1 |
| β-Carotene bleaching inhibition <sup>a</sup> | 59 ± 34<sup>b</sup> | 166 ± 6<sup>a</sup> | 188 ± 9<sup>a</sup> | 57 ± 4<sup>b</sup> | 18 ± 1 |
| TBARS<sup>a</sup> | 179 ± 11<sup>c</sup> | 510 ± 6<sup>b</sup> | 3252 ± 49<sup>a</sup> | 198 ± 14<sup>c</sup> | 23 ± 1 |
| OxHLIA (IC<sub>50</sub>; µg/mL) |  |
| ∆t = 60 min | 56.0 ± 0.8<sup>b</sup> | 41.4 ± 0.5<sup>c</sup> | nd | 218 ± 2<sup>a</sup> | 19.6 ± 0.1 |
| ∆t = 120 min | 100 ± 1<sup>b</sup> | 65.1 ± 0.7<sup>c</sup> | nd | 343 ± 5<sup>a</sup> | 65.1 ± 0.1 |

EC<sub>50</sub>: extract concentration corresponding to 50% of antioxidant activity or <sup>b</sup> 0.5 of absorbance in the reducing power assay. In each column different letters (c, b, a and d) mean significant differences between the different extracts (p < 0.05).

In general, significant differences were observed among the tested extracts, although for some assays similar results were obtained for the decoction and hydroethanolic extract prepared from the hexane residue. Surprisingly, this last extract was the one that presented better results in the DPPH assay. Nevertheless, this assay is based on the use of a chemical radical inexistent in living cells, thus generally being considered only as a screening assay.

Despite the hydroethanolic extract prepared from the hexane residue, also showing the best results in the reducing power and β-carotene bleaching inhibition assays, the results were not statistically different from those of the decoction extract.

Comparatively to the remaining assays, both TBARS and OxHLIA assays are considered as being more closely related to real living systems as they rely on the use of cells or tissues. Decoction was the extract that showed the best results in the TBARS assay while the hydroethanolic extract was the one performing better in the OxHLIA assay. Both the decoction and hydroethanolic extracts showed very promising antioxidant activity preventing the hemolysis of erythrocytes as they performed better in the OxHLIA at 60 min of activity (56.0 µg/mL and 41.4 µg/mL) when compared to the antioxidant Trolox used as positive control (65.1 µg/mL). In all assays, with the exception of OxHLIA that was not performed due to solubility incompatibility with the required culture media for the assay, the n-hexane extract was the one that performed worst. Regardless of previous papers reporting that some phthalides show a remarked in vitro antioxidant activity [36,37], in this study the n-hexane extract, containing different phthalides, evidenced a low antioxidant activity, as shown in Tables 2 and 3. This result can be possibly related to the fact that in those studies, different assays, based on different mechanisms, were used. Furthermore, despite some lipophilic compounds such as vitamin E being also considered to have high antioxidant activity, as shown in Table 3, this compound was found in low amounts (in terms of relative %).

To the best of our knowledge, this is the first study reporting the antioxidant activity of *L. officinale* roots, as previous studies focused only in lovage aerial parts [38].

3.2.2. Cytotoxic Activity

The results of cytotoxic activity against several cancer cell lines and pig hepatocytes are shown in Table 4. As it can be observed, only the hexane extract showed significant results against all tumor cell lines tested. However, it also exhibited toxicity against non-tumoral hepatocyte cells (PLP2 cell line), denoting that this extract has hepatotoxicity. However, it can be noticed that the GI<sub>50</sub> value for the PLP2 cell line is much higher (more than 2×) compared to the values obtained for the tumor cell lines, indicating a higher cytotoxic effect against tumoral cells, and therefore an interesting potential of the compounds in this extract. Considering that the assays were performed with extracts, that contain
several compounds, in further studies, it would be interesting to proceed with compound isolation and assay them individually in order to better understand the action and potential of this type of extract.

Table 4. Hepatotoxicity and cytotoxic activities of extracts obtained from the roots of *L. officinale* (mean ± SD, n = 3).

| Cytotoxic Activity (GI50, µg/mL) | Decoction | Hydroethanolic | Hexane | Hydroethanolic Extract of Hexane Residue | Positive Control |
|----------------------------------|-----------|----------------|--------|-----------------------------------------|-----------------|
|                                  |           |                |        |                                         | Ellipticine      |
| HeLa                             | >400      | >400           | 60 ± 2 | >400                                    | 0.9 ± 0.1       |
| NCI H460                         | >400      | >400           | 69 ± 3 | >400                                    | 1.03 ± 0.09     |
| MCF7                             | >400      | >400           | 48 ± 2 | >400                                    | 1.21 ± 0.02     |
| HepG2                            | >400      | >400           | 67 ± 4 | >400                                    | 1.10 ± 0.09     |

| Hepatotoxicity (GI50, µg/mL)     | PLP2      | >400           | 147 ± 5 | >400                                    | 2.3 ± 0.2       |

GI50 values correspond to the sample concentration responsible for 50% inhibition of growth in tumor cells or in a primary culture of liver cells-PLP2.

As mentioned before, there are studies suggesting that ligustilides have cytotoxic effects [39,40] which may explain the activity verified for n-hexane extracts, since the analyses by GC-MS confirmed the presence of these compounds (mainly (Z)- and (E)-ligustilide isomers) in this extract. The essential oil from *Levisticum officinale* plants demonstrated antitumor activity against HepG2 and MCF7 cells (at 98% and 95%) at a concentration of 100 µg/mL, showing poor activity at 50 µg/mL, and showing no activity at lower concentrations [38]. In the present study, it was not possible to evaluate the bioactive properties of the essential oil from the roots of lovage due to the very low yields obtained.

3.3. Antimicrobial Activity

For determination of antimicrobial activity, samples of *L. officinale* roots were subjected to extractions with different solvents, as previously mentioned. Table 5 presents the results of the extracts against Gram-positive and Gram-negative clinical isolates.

Table 5. Antimicrobial activity of the extracts obtained from *L. officinale* roots (mean ± SD, n = 3).

|                                | Decoction | Hydroethanolic | Hexane | Hydroethanolic Extract of Hexane Residue | Ampicillin (20 mg/mL) | Imipenem (1 mg/mL) | Vancomycin (1 mg/mL) |
|--------------------------------|-----------|----------------|--------|-----------------------------------------|-----------------------|--------------------|---------------------|
|                                |           |                |        |                                         |                       |                    |                     |
|                                |           |                |        |                                         | MIC (µg/mL)           | MIC (µg/mL)        | MIC (µg/mL)         |
| **Gram-Negative Bacteria**     |           |                |        |                                         |                       |                    |                     |
| *Escherichia coli*             | >20       | >20            | 20     | >20                                     | 0.15                  | <0.0078            | <0.0078             |
|                                | >20       | >20            | 20     | >20                                     | 0.15                  | <0.0078            | <0.0078             |
|                                | >20       | >20            | >20    | >20                                     | 10                    | 0.0078             | 0.0078              |
|                                | >20       | >20            | >20    | >20                                     | >20                   | 0.0078             | 0.0078              |
|                                | >20       | >20            | >20    | >20                                     | >20                   | 0.0078             | 0.0078              |
|                                | >20       | >20            | >20    | >20                                     | >20                   | 0.0078             | 0.0078              |
| **Pseudomonas mirabilis**      | >20       | >20            | >20    | >20                                     | >20                   | 0.0078             | 0.0078              |
| **Pseudomonas aeruginosa**     | >20       | >20            | >20    | >20                                     | >20                   | 0.0078             | 0.0078              |
| **Gram-Positive Bacteria**     |           |                |        |                                         |                       |                    |                     |
| *Enterococcus faecalis*        | >20       | >20            | >20    | >20                                     | 0.15                  | <0.0078            | <0.0078             |
|                                | >20       | >20            | >20    | >20                                     | 0.15                  | <0.0078            | <0.0078             |
| **Listeria monocytogenes**     | >20       | >20            | >20    | >20                                     | 0.15                  | <0.0078            | <0.0078             |
| **MRSA**                       | >20       | >20            | >20    | >20                                     | 0.15                  | <0.0078            | <0.0078             |

MRSA: methicillin resistant *Staphylococcus aureus*; MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; nt: not tested.
Only the hydroethanolic extract of \( \text{n}-\)hexane residue was able to inhibit the growth of all the tested microorganisms, with the decoction showing the worst results in terms of the number of inhibited bacteria. In the assayed concentrations, none of the extracts showed bactericidal activity against the microorganisms. Overall, all the extracts were able to inhibit the growth of bacteria being more efficient against Gram-positive bacteria, which can be explained by the fact that this group of microorganisms has a less complex cell wall compared to Gram-negative bacteria. The lower MIC values against Gram-positive bacteria were obtained with the hydroethanolic extract. Finally, it should be noted that in this work, the assayed microorganisms were obtained from clinical isolates, which often have higher antibiotic resistance compared to commercial strains.

The antimicrobial activity of the hexane extract from the roots of another species from the same genus, namely \( L. \) persicum Freyn and Bornm, has been evaluated by [39]. The antimicrobial activity was evaluated by surface inoculation and disk diffusion assay, and the results were expressed as inhibition diameter (mm). Inhibition was observed against \( B. \) subtilis (19.7 mm), \( S. \) epidermidis (18.6 mm), \( S. \) aureus (16.5 mm), and \( E. \) coli (13.5 mm), thus also being higher for Gram-positive bacteria as in the present study.

In a previous study, the \( n \)-hexane extract of lovage root was found to significantly inhibit the growth of both \( M. \) smegmatis and \( M. \) bovis [40]. The authors concluded that the activity was most probably related to the compounds falcarindiol and \( \alpha \)-prethapsenol. Both compounds, the first being a polyacetylene and the second a sesquiterpene, were not identified in any of the studied extracts.

4. Conclusions

With this work, it was possible to characterize an agri-food by-product, namely lovage roots, and report for the first time data regarding its phenolic compound profile, the volatile composition of the \( n \)-hexane extract, and the in vitro biological activity of different extracts prepared from \( L. \) officinale roots. A total of 66 different compounds were identified in the essential oil and the \( n \)-hexane extract by GC-MS, with both types of extracts presenting a high percentage of phthalides. A total of eight phenolic compounds were, for the first time, identified in the decoction and hydroethanolic extracts, by HPLC-DAD-ESI-MS/MS, with the major compound being vanillic acid. In general, all the tested lovage roots’ extracts showed relevant antioxidant activity in the five tested methods, except in the \( n \)-hexane extract. All extracts were more efficient against Gram-positive bacteria. The hydroethanolic extract prepared from the \( n \)-hexane residue show bacteriostatic activity against all tested bacteria. Despite the low activity exhibited by the hexane extract regarding antioxidant and antimicrobial properties, this extract showed promising cytotoxic activity since it was capable of inhibiting the growth of all tested cancer lines.

Overall, this work allowed us to present for the first time data regarding the chemical composition and biological activities regarding an anatomical part of the lovage plant that is generally discarded and undervalued by the agri-food industry. Lovage roots were shown to have valuable bioactive compounds, such as phthalides and phenolic acids, as well the prepared extracts showing different biological properties. Therefore, this residue can be valorized as a source of bioactive compounds of possible interest for other industries, such as food or pharmaceutical, contributing to the goal of a circular economy.

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