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HPLC Analysis of Polyphenolic Compounds in *Lysimachia nummularia* L. and Comparative Determination of Antioxidant Capacity

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Citation: Suciu, F.; Stoicescu, I.; Lupu, E.C.; Musuc, A.M.; Popescu, A.; Mititelu, M.; Roșca, A.C.; Dumitrescu, D.-E.; Badea, F.C.; Caraiane, A.; et al. HPLC Analysis of Polyphenolic Compounds in *Lysimachia nummularia* L. and Comparative Determination of Antioxidant Capacity. *Appl. Sci.* **2023**, *13*, 2159. https://doi.org/10.3390/app13042159

Academic Editor: Alessandra Biancolillo

Received: 21 January 2023
Revised: 2 February 2023
Accepted: 7 February 2023
Published: 8 February 2023

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Abstract: *Lysimachia nummularia* L. is a perennial herbaceous plant rich in bioactive compounds, which can be utilized for medicinal purposes. The present work aims to analyze the phenolic compounds from different parts of the *Lysimachia nummularia* L. plant using the HPLC technique: *Lysimachiae radix* (the root part), *Lysimachiae herba* (the aerial part), *Lysimachiae flores* (flowers). In order to determine the phenolic compounds, extraction from the three categories of vegetable products was performed with ethanol 70% (*v/v*) using three extraction methods: (i) Soxhlet extraction, (ii) maceration and (iii) ultrasonic-assisted extraction. The content of polyphenols was determined by the Folin–Ciocalteu method, and the antioxidant activity was evaluated by the DPPH, ABTS, FRAP and CUPRAC methods. The antioxidant activity was correlated with the content of phenolic compounds in the analyzed extracts. The following phenolic compounds were separated, identified and quantified: 3-*O*-methylgallic, gallic, ferulic, caffeic, chlorogenic, p-coumaric acids and *trans*-resveratrol. According to the experimental data, the highest content of total polyphenols was observed in the hydroethanolic extract from *Lysimachiae flores* (22.10 ± 1.48 mg gallic acid/g), which also presented remarkable antioxidant activity.

Keywords: *Lysimachia*; polyphenols; antioxidant activity; *trans*-resveratrol; 3-*O*-methylgallic acid; bioactive compounds

1. Introduction

Deciphering the existing links between oro-dental diseases and those that evolve at a distance from the oral cavity is very important. The understanding of these phenomena opens new perspectives in identifying the most effective means of treatment both for oro-dental affections and for those at a distance from this area.

Thus, clinical studies have revealed that periodontal disease is correlated with atherosclerosis and cardiovascular diseases, with statistics showing that there is a high risk of acute myocardial infarction in patients with periodontitis [1,2]. The common element in the etiopathogenesis of these diseases is oxidative stress, as well as the consecutive production of large amounts of reactive oxygen species (ROS), which are the basis of functional changes and systemic inflammatory processes.

Lysimachia nummularia L. is part of the *Primulaceae* family, *Myrsinaceaae* subkingdom, and is a perennial herbaceous plant (common name: coin plant) that has proven to be a valuable source of bioactive compounds with therapeutic potential, including phenolic compounds [3,4].

The literature mentions the use of plants belonging to the *Primula* family in traditional medicine for various ailments, without specifying exactly the bioactive compounds responsible for the amelioration or healing effects on the disease. Thus, the use of these plants is recommended in different illnesses, such as stomatitis, gingivitis, periodontitis, rheumatic pain, gastro-intestinal diseases [5], hepato-biliary stones, urination [6], nephritis edema, damp jaundice [6–8], swelling and edema [9] and viral infections with hepatitis E virus [10], due to their anthelmintic [5], diuretic, detoxifying [6], analgesic [11], antibacterial, antitumor [12,13] and antioxidant [6,14,15] effects.

According to the specialized literature, the antioxidant properties of the species *Lysimachia nummularia* L. are attributed to secondary metabolites, namely phenolic compounds, especially flavonic glycosides [5,9] and triterpenic saponins [11,16], which were detected in the chemical composition of species, as well as flavonoids [5,8,17] and other phenolic compounds, with the exception of caffeic acid derivatives, ferulic acid, chlorogenic acid, p-coumaric acid [18] and gallic acid [19].

Polyphenols are organic compounds that have intense antioxidant activity; therefore, they are used to combat oxidative stress, to detoxify the body, to reduce degenerative phenomena, to prevent tumor processes, neuroprotection, etc. [20,21]. Although they can be obtained synthetically, polyphenolic compounds obtained from plants have higher bioavailability and better activity.

Phenolic compounds represent the largest group of natural polyphenols. They are mainly found in the leaves, seeds, stems, fruits, bark, flowers and other parts of plants. Specialists discuss their anticancer potential via inhibiting the activity of enzymes involved in the neovascularization process. Phenolic compounds are associated with estrogen secretion in the female body, prevent infections, have antiallergic properties and are characterized by hepatoprotective, choleretic and antiulcer properties [22,23].

The antioxidant activity of various plant products that belong to the species *Lysimachia nummularia* L. has also been investigated by in vitro methods, namely with 1,1-diphenyl-2-picrylhydrazyl (DPPH) [5]; with 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) from the aerial parts, flowers and roots [5,9]; the DPPH and ferric reducing antioxidant power assay (FRAP) from the aerial parts of *Lysimachia verticillaris* [19]; and the DPPH and Trolox equivalent antioxidant capacity (TEAC) assay in the aerial parts of *Lysimachia nummularia* L. [18].

The presence of phenolic acids such as *trans*-resveratrol or 3-*O*-methylgallic acid has not been mentioned in the specialized literature.

This study aims to investigate different plant products obtained from the *Lysimachia nummularia* L. species in order to determine the total polyphenol content (TPC), as well as the identification, separation and quantitative determination of phenolic compounds at the level of the component parts of the species. The content of polyphenolic compounds from different parts of the plant is correlated with the antioxidant activity.

The novelty of this work consists in establishing an optimal method of extracting phenolic compounds from the analyzed vegetable products. The objective is to determine the antioxidant capacity of the extractive solutions obtained by several methods, and to identify, separate and quantify new phenolic compounds not mentioned in the specialized literature until this moment. The aim of the study is to explore their therapeutic potential in the future, by making standardized extracts. In order to capitalize on the therapeutic potential, clinical studies are carried out to follow the assessment of toxicity, as well as specific analyses to determine the presence of various contaminants that may come from the environment. This represents mandatory preliminary research for the safe administration of bio-compounds isolated from different natural sources [24–27].

Oxidative stress is an essential link in the etiopathogenesis of many diseases, including those in the oro-dental cavity. Since the antioxidant properties of the identified phenolic compounds are known, there are prospects for their use in pharmaceutical products intended for the treatment of oro-dental and degenerative diseases. In the specialized literature, it is stated that there are over 200 diseases whose etiopathogenic mechanism includes oxidative stress; among those with a higher incidence potential, we mention periodontal disease and various forms of oropharyngeal neoplasia [28].

2. Materials and Methods

2.1. Plant Material

The material used in the present research was represented by the *Lysimachia nummularia* L. plant, harvested in July 2021, from the edge of the Taul-Brazi Lake in the Roșia Montană area (46°18′09″ N; 23°08′10,84″ E), Romania (Figure 1).

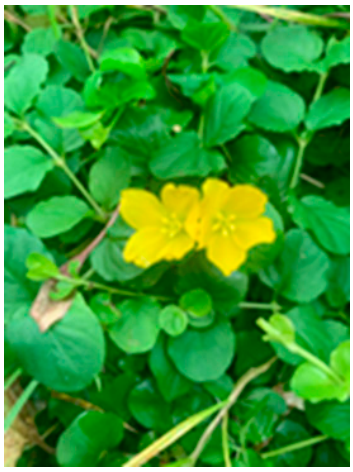


Figure 1. *Lysimachia nummularia* L.

Several specimens of the whole harvested species are included in the *Exiccata* collection of the Discipline of Pharmacognosy at the Faculty of Pharmacy, "Ovidius" University Constanţa. Moreover, the identification of the plant was performed with the help of the Herbarium of the Discipline of Pharmacognosy, and the identification voucher was submitted to the Department of Analysis and Quality Control of Medicines of the Faculty of Pharmacy, "Ovidius" University Constanța.

2.2. Extraction Methods

The freshly harvested products were separated into 3 fractions (*radix*, *herba* and *flores*) and cleaned and dried in a closed oven (BIOBASE BOV-T30C, Jinan, China) at 60 °C for 24 h. Then, each sample was ground and kept in the dark and in a cool place (+4 °C) in a sealed glass container until further analysis.

Maceration: the maceration extraction method was carried out as follows [29]: 10 g of plant was added to 100 mL ethanol 70% (*v/v*) (Merck, Darmstadt, Germany), and the final

solution was stirred using a magnetic stirrer (model IKA C-MAG HS 7, Sigma-Aldrich, Taufkirchen, Germany) at 30 °C, 150 RPM, for 72 h. The maceration extraction was also carried out with ethanol 50% (*v/v*) (Merck, Germany) to evaluate the optimal alcohol concentration for extraction.

Ultrasonic-assisted extraction (UAE): the sample extraction process was performed with the help of an ultrasonic bath (ULT AG 440, Radevormwald, Germany) (frequency of 35 kHz and power density of 180 W) as follows: 10 g of each plant sample was mixed with 100 mL ethanol 70% (*v/v*) and then kept in the ultrasonic bath at 45 °C for 72 h.

Soxhlet extraction method: to obtain the extracts, 10 g of each dry sample was taken and inserted into the extraction thimble, after which it was sealed with a cotton plug. The extraction cartridge loaded with the sample of interest was inserted into the Soxhlet extractor. Then, 10 g of each sample was extracted using 100 mL ethanol 70% (*v/v*) for 2 h.

After cooling, all the extracts obtained by the 3 extraction methods were filtered using Whatman No. 1 filter paper. Subsequently, the samples were kept at +4 °C, in the dark, until further analyses.

For each set of conditions, three independent extracts were prepared and analyzed. The results were expressed as mean ± SD (standard deviation).

2.3. Total Polyphenol Content Analysis

The total polyphenolic content (TPC) of the extracts was determined by the spectrophotometric method [30], with some modifications.

Polyphenols contained in plant extracts react with Folin–Ciocalteu reagent. The resulted blue complex can be further quantified using visible-light spectrophotometry. A blue chromophore composed of a phosphotungstic–phosphomolybdenum complex is obtained from the reaction. The maximum absorption of the chromophores depends on the concentration of phenolic compounds and the alkaline solution [31].

Here, 0.5 mL of each extract was added to 2.5 mL Folin–Ciocalteu reagent solution (0.2 M) (from Merck, Germany). The solutions were left to rest for around 5 min and then 2 mL of 20% *w/v* sodium carbonate (from Merck, Germany) was added.

The blank solution was prepared under the same conditions but without extract. After 30 min of incubation at room temperature, the absorbance at 725 nm and 20 °C was read. To quantify the amount of phenolic compounds, the calibration curve of gallic acid was obtained (Chromadex, Wesel, Germany). The results were expressed as mg GAE/g sample dry plant material.

2.4. Antioxidant Activity Assays

2.4.1. DPPH Radical Scavenging Capacity Method

The DPPH method is one of the simplest and fastest methods for the analysis of antioxidant activity with multiple applications. This antioxidant assay is based on electron transfer.

The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is a stable and commercially available organic nitrogen radical that has an absorption maximum in the UV–Vis range at 515–517 nm (violet).

The analysis was carried out as follows [32]: To 1 mL extract of several different concentrations (between 0.5 and 2.5 mg/mL), 0.1 mL methanol was firstly added, and then 2.9 mL 9×10^{-5} M DPPH solution in methanol was added. The samples were homogenized with the help of a vortex and kept for 20 min in the dark.

The decrease in absorbance was measured using the UV–Vis Spectrophotometer V 630 Jasco; (Jasco, Heckmondwike, United Kingdom) model at 517 nm.

Trolox was used as a reference standard antioxidant compound, and for the drawing of the calibration line, 5 standard solutions were prepared by dilution in methanol with concentrations in the range of 0.2–1 µg/mL. The absorbance was measured for each standard solution against a blank of methanol.

The percentage of DPPH remaining was calculated using the following equation (Equation (1)):

$$IC\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \cdot 10 \quad (1)$$

where A_{blank} is the absorbance of the blank (methanol–DPPH, methanol solution) and A_{sample} is the absorbance of the sample mixed with 2.9 mL DPPH solution.

The degree of inhibition or radical scavenging capacity was expressed as IC_{50} in μg/mL. IC_{50} represents the amount of antioxidant needed to reduce the initial DPPH concentration by 50% or the extract concentration that inhibits DPPH activity by 50%.

2.4.2. ABTS Radical Scavenging Capacity Method

The $ABTS^{\bullet+}$ assay is based on the scavenging ability of antioxidants to the cationic radical ABTS.

The method for determining the antioxidant activity is based on the generation of the ABTS radical (2,2-azinobis-(3-ethyl benzthiazoline-6-sulfonic acid), obtained by oxidizing the ammonium salt of ABTS ($ABTS^{2-}$) with potassium persulfate for 12–16 h in the dark, which has the maximum absorption in the UV–Vis range at 734 nm (intense blue–violet color).

The ABTS cationic radical was produced by preparing a 7 mM ABTS stock solution, to which 2.45 mM potassium persulfate solution was added. The mixture was incubated at room temperature for 16 h, in the dark [33].

The solution containing the generated ABTS cation radicals was diluted with 10 mM phosphate buffer solution, pH 7.4, until the absorbance of the solution became 0.70 ± 0.02 at 734 nm. Then, 0.1 mL of each extract sample was added to 2.9 mL of diluted ABTS solution. The mixture was shaken and then incubated at room temperature for 10 min, in the dark.

The decrease in absorbance was measured after 15 min at 734 nm.

Trolox was used as a reference antioxidant standard compound, and for the drawing of the calibration line, 5 standard solutions were prepared by dilution in methanol with concentrations in the range of 0.2–1 μg/mL, and the value of absorbance in nm was measured for each standard solution against a blank of methanol.

The percentage of ABTS remaining was calculated with Equation (1), where A_{blank} is the absorbance for the blank (methanol–ABTS, methanol solution) and A_{sample} is the absorbance for the sample mixed with 2.9 mL of ABTS solution.

The degree of inhibition (radical scavenging capacity) was expressed as IC_{50} in μg/mL, and the antioxidant capacity of the studied samples or radical scavenging capacity was expressed as IC_{50} in μg/mL.

2.4.3. Ferric Reducing/Antioxidant Power (FRAP) Method

The FRAP method consists in the reduction, in acidic medium, of the ferric 2,4,6-tripyridyl-s triazine complex $[Fe(III)\text{-}(TPTZ)^2]^{3+}$ to the ferrous complex $[Fe(II)\text{-}(TPTZ)^2]^{2+}$ of intense blue color in the presence of the antioxidant species. Thus, the change in absorbance obtained at 593 nm for antioxidant samples is compared with the absorbance of solutions of a known concentration of ferrous ions.

The FRAP method, although it only indirectly reflects the antioxidant activity, is a simple and cheap method for the determination of antioxidant activity [34] and was performed according to the method described by Benzie, with some modifications [35].

To determine the antioxidant activity using the FRAP method, methanol (Sigma-Aldrich, Taufkirchen, Germany) was used to dilute the extracts. The other reagents used were iron (III) chloride, sodium acetate, 2,4,6-tris(2-pyridyl)-1,3,5-triazine (Sigma-Aldrich, Germany) and hydrochloric acid (Sigma-Aldrich, Germany).

First, 30 mL of FRAP reagent was prepared using a buffer solution of sodium acetate in water with pH 3.6, and then 0.3257 g of $FeCl_3$ was dissolved in double-distilled water and brought to 100 mL. Next, 0.3124 g of 2,4,6-tripyridyl-triazine (TPTZ) was dissolved under heating at approximately 50 °C in 40 μmol/mL HCl solution. Then, 25 mL of sodium acetate buffer solution was mixed with 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3$

solution. The resulting solution was diluted with 2 volumes of double-distilled water and then incubated for 30 min at 37 °C.

Then, 0.1 mL of extract sample (properly diluted with methanol) and 2.9 mL of FRAP reagent were mixed. The mixture was kept at room temperature for 2 h in the dark, and the absorbance was read at 593 nm.

Trolox was used as a reference standard antioxidant compound, and for the drawing of the calibration line, 5 standard solutions were prepared by dilution in methanol with concentrations in the range of 0.2–1 µmol/mL, and the absorbance was measured for each standard solution against a blank of methanol.

The antioxidant capacity of the studied samples or FRAP radical scavenging capacity was expressed in µmol Trolox equivalent (TE)/100 mL.

2.4.4. CUPRAC Assay

The CUPRAC method assesses the color of a copper complex with neocupreine (2,9-dimethyl-1,10-phenanthroline). The reduction of the copper ion (II) to the copper iron (I) determines the color change from light green to reddish orange.

The CUPRAC reagent was obtained from 1 mL of 7.5 M non-cupric solution with 1 mL solution of 10 mM copper chloride and 1 mL of ammonium acetate buffer pH = 6.8.

The samples were prepared by adding 3 mL of CUPRAC reagent with 1.1 mL of solution containing 0.005 mL of extract.

The blank was obtained from 3 mL CUPRAC reagent and 1.1 mL purified water. The standard was prepared from 3 mL CUPRAC reagent and 1.1 mL solution containing 11.4, 22.8, 34.2 and 45.6 µg of Trolox, respectively.

The antioxidant capacity of the investigated samples was calculated using the standard curve, utilizing the standard Trolox/100 mL extract, and the absorbance increase was recorded at 450 nm (equation of the calibration curve is $y = 0.184x + 0.0127$; $R^2 = 0.9878$).

The calibration curve was plotted using concentrations ranging between 11.4 and 45.6 g/L Trolox standard.

2.5. Determination of Phenolic Compounds Using HPLC-DAD Method

Phenolic compounds were analyzed using HPLC-UV on a reverse-phase Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm), using a gradient program with two solvent systems (mobile phase A: 0.1% acetic acid, mobile phase B: acetonitrile) [36]. The elution gradient was achieved from high polarity and low pH to low polarity and high pH. Gradient: 0–5 min, 5–10% B; 5–7.5 min, 10–20% B; 7.5–10 min, 20% B; 10–12 min, 20–5% B; 12–13 min, 5% B. The injection volume was 10 µL and the temperature of the column was regulated in a column oven at 30 °C. A flow rate of 1 mL/min was used and the detection was performed at 216, 310 and 324 nm. In order to increase the repeatability, an internal standard (IS) technique was applied to the analysis [37].

The standard mixture of the 7 phenolic compounds was used as an internal standard to demonstrate the presence of these compounds in the ethanolic extracts obtained from each component part of the species.

All determinations were performed in triplicate. The results were expressed as mean ± SD (standard deviation).

All reagents used were for analytical use. Ethanol 70% (*v/v*), (Merck, Germany) was used for the extraction of the plant material. Acetonitrile and acetic acid (Lab-Scan, Munich, Germany) for HPLC were employed for the preparation of the mobile phase. A Milli-Q water system was used for the preparation of deionized water, which was used throughout. The prepared samples were filtered with a 0.45 mm Minisart-plus membrane filter (Teknokroma, Barcelona, Spain, CEE). Reference compounds of 3-*O*-methylgallic and ferulic acid were obtained from ChromaDex, Germany, and gallic, caffeic, chlorogenic and p-coumaric acids and *trans*-resveratrol were from Merck, Germany.

2.6. Statistical Analysis

For the statistical analysis of differences between variables, Student's t-test and one-way ANOVA with Tukey's post-hoc test, which allows multiple comparisons between pairs of data, were used, using GraphPad Software 9, Inc., San Diego, CA, USA. The Tukey (honestly significant difference—HSD) post hoc test is a method based on the q statistic and is preferred when all group comparisons are to be made, two by two.

3. Results

3.1. Total Polyphenol Content

The content of total polyphenols obtained from the maceration extracts with ethanol 50% (*v/v*) was expressed as mg gallic acid equivalent (GAE) per gram of sample in dry weight (mg/g): 22.10 ± 1.48 mg GAE/g in *herba* extract, 16.75 ± 0.49 mg GAE/g in *flores* extract, 18.72 ± 0.71 mg GAE/g in *radix* extract (Figure 2). The equation of the calibration curve of the gallic acid was $y = 0.96\,x - 0.148$; $R^2 = 0.997$. The obtained results were lower than those obtained with extraction by maceration with ethanol 70% (*v/v*) (Figure 3), which is why maceration performed with the higher alcohol concentration was further used for the analysis.

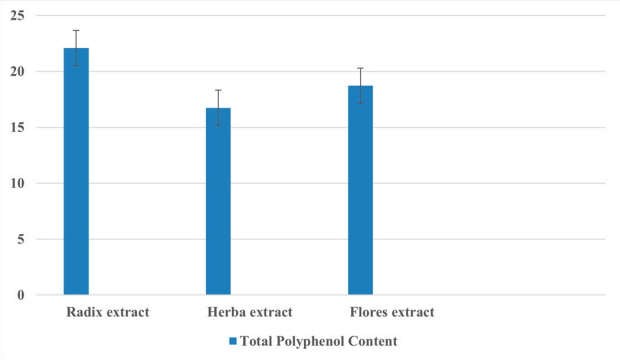


Figure 2. Total polyphenol content in the extracts with ethanol 50% (*v/v*) from *Lysimachia nummularia* L.

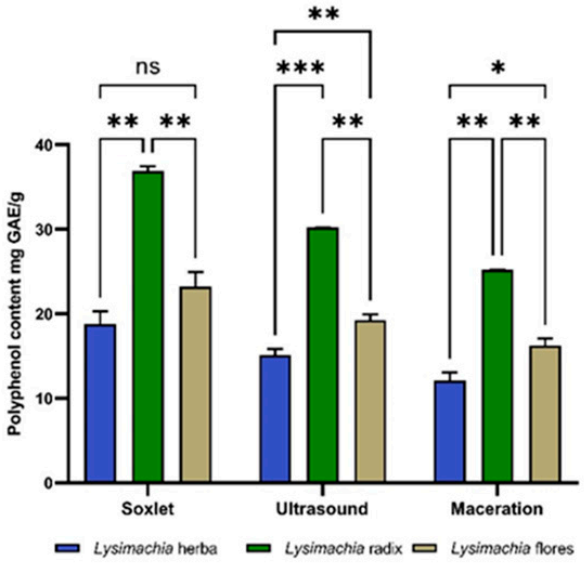


Figure 3. Comparative data of the content of total polyphenols obtained by the 3 extraction methods for *Lysimachia herba, radix* and *flores* extracts, with ethanol 70% (*v/v*). Results are indicated as mean ± SD of data ($n = 3$). Asterisks indicate statistical significance examined by ANOVA with Tukey post hoc test for multiple reciprocal comparison among the extracts (significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; not significant (ns): $p > 0.05$).

In Figure 3, a comparative representation of the total polyphenol content expressed as function of the three different extraction methods and for the three *Lysimachia herba*, *radix* and *flores* extracts is given.

3.2. Quantification of Phenolic Compounds Using HPLC-RP in the Extracts from Lysimachia nummularia L.

The seven phenolic compounds, gallic acid, chlorogenic acid, 3-*O*-methylgallic, caffeic p-coumaric, ferulic acid and *trans*-resveratrol, were separated, identified and quantitatively determined in the three extracts based on the calibration curves, the limit of detection (LOD) values, the limit of quantification (LOQ) and the retention times (Table 1).

Table 1. Calibration curves, LOD and LOQ for the determination of phenolic compounds by HPLC-DAD.

Analyte	Calibration Relationship	R^2	RSD (t_r)	RSD (Area)	LOD (μg/mL)	LOQ (μg/mL)
Gallic acid	y = 31.175x − 10.371	0.9994	0.182	2.182	1.457	4.415
3-*O*-Methylgallic acid	y = 22.558x + 17.797	0.9992	0.228	2.945	1.778	5.387
Chlorogenic acid	y = 8.231x − 7.331	0.9997	0.167	1.829	1.346	4.078
Caffeic acid	y = 12.006x − 4.727	0.9996	0.225	3.058	1.174	3.558
p-Coumaric acid	y = 30.371x + 3.228	0.9998	0.099	2.073	1.634	4.951
Ferulic acid	y = 6.001x + 0.991	0.9996	0.082	2.977	1.402	4.248
Trans-resveratrol	y = 0.993x − 0.877	0.9993	0.332	2.541	1.060	3.211

The phenolic acids were clearly noticeable at a wavelength of 216 nm for gallic acid, 3-*O*-methylgallic acid, 310 nm for p-coumaric acid and *trans*-resveratrol and 324 nm for chlorogenic, caffeic and ferulic acids. Both the retention time and UV spectrum were compared with the reference compound to identify the compounds. Results are expressed in mg/100 g of dry sample (Figures 4 and 5).

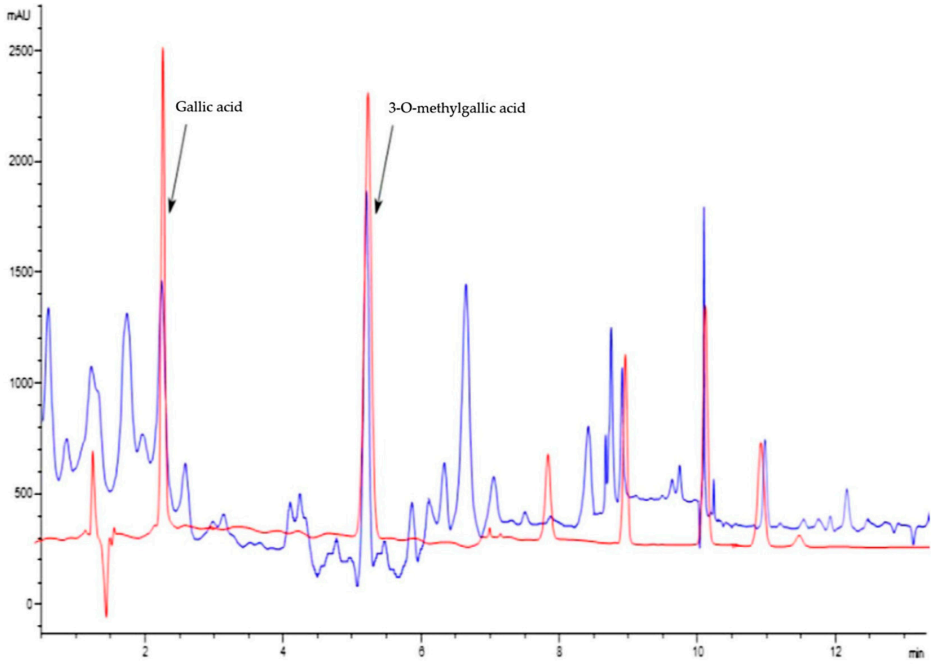


Figure 4. HPLC-DAD chromatograms using the developed method of mixed standards at 216 nm. Red line corresponds to the ethanolic solution of *Lysimachia nummularia herba* samples with standard solution, blue line corresponds to the ethanolic extracts without standard solution.

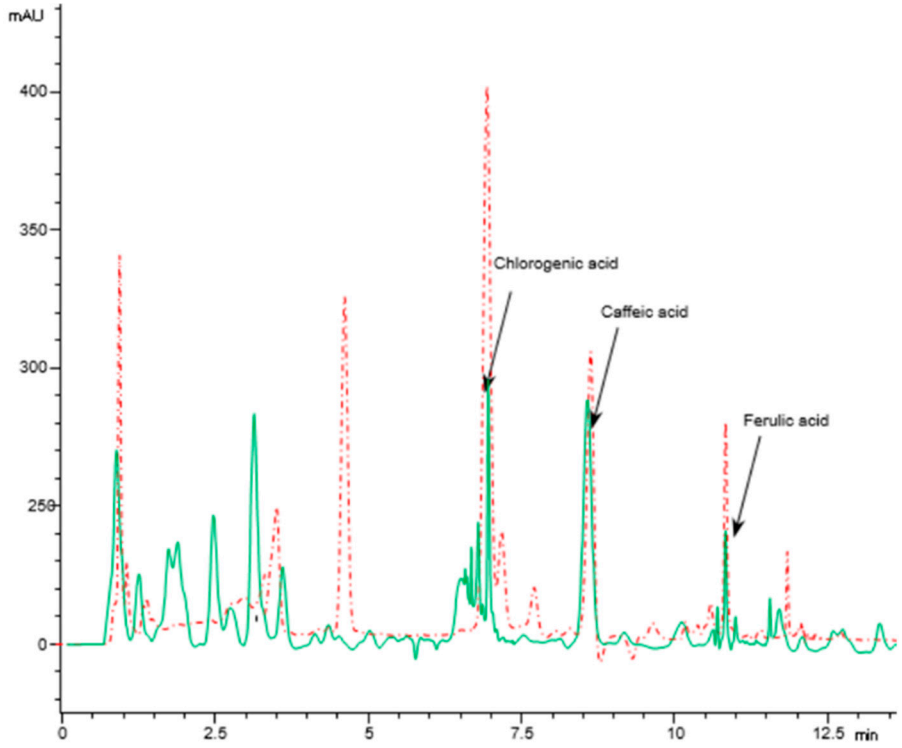


Figure 5. HPLC-DAD chromatograms using the developed method of mixed standards at 324 nm. Red line corresponds to the ethanolic solution of *Lysimachia nummularia herba* samples with the standard solution, green line corresponds to the ethanolic extracts without the standard solution.

Table 2 displays the experimental results obtained for the identification of the different phenolic compounds from the three extracts obtained from different parts of the *Lysimachia nummularia* L. plant (*radix, herba* and *flores*) and the graphic representations are presented in Figure 6. The results are expressed in mg/100 g of dry sample.

Table 2. Phenolic compounds in the extracts from *Lysimachia nummularia* L.

Extract	Gallic Acid	3-*O*-Methylgallic Acid	Caffeic Acid	Ferulic Acid	Chlorogenic Acid	p-Coumaric Acid	*trans*-Resveratrol
Radix	76.3392± 10.55	41.2526± 9.22	38.3477± 1.15	29.2526 ± 5.14	37.3024± 2.11	41.6825± 3.41	27.7784± 1.12
Flores	56.4867± 11.48	39.8613± 10.11	28.6386± 1.22	34.8613± 12.01	-	37.9556± 10.16	-
Herba	57.4983± 12.81	40.7341± 8.28	24.6112± 1.02	28.7341± 7.66	-	25.0828± 4.15	26.8829± 1.71

3.3. Antioxidant Activity

In the present research, the antioxidant activity of the extracts obtained by the three methods was assessed by in vitro methods: the DPPH, ABTS, FRAP and CUPRAC methods.

The calibration curve equations obtained for the determination of the antioxidant activity for each used method are given in Table 3.

Table 3. Calibration curve relationships for the determination of antioxidant activity.

Methods	Calibration Curve Equation	R^2
DPPH assay	y = 75.562x + 3.569	0.9967
$ABTS^{\bullet+}$ assay	y = 87.731x + 6.488	0.9978
FRAP assay	y = 71.682x + 9.5777	0.9977
CUPRAC assay	y = 0.0148x + 0.0112	0.9733

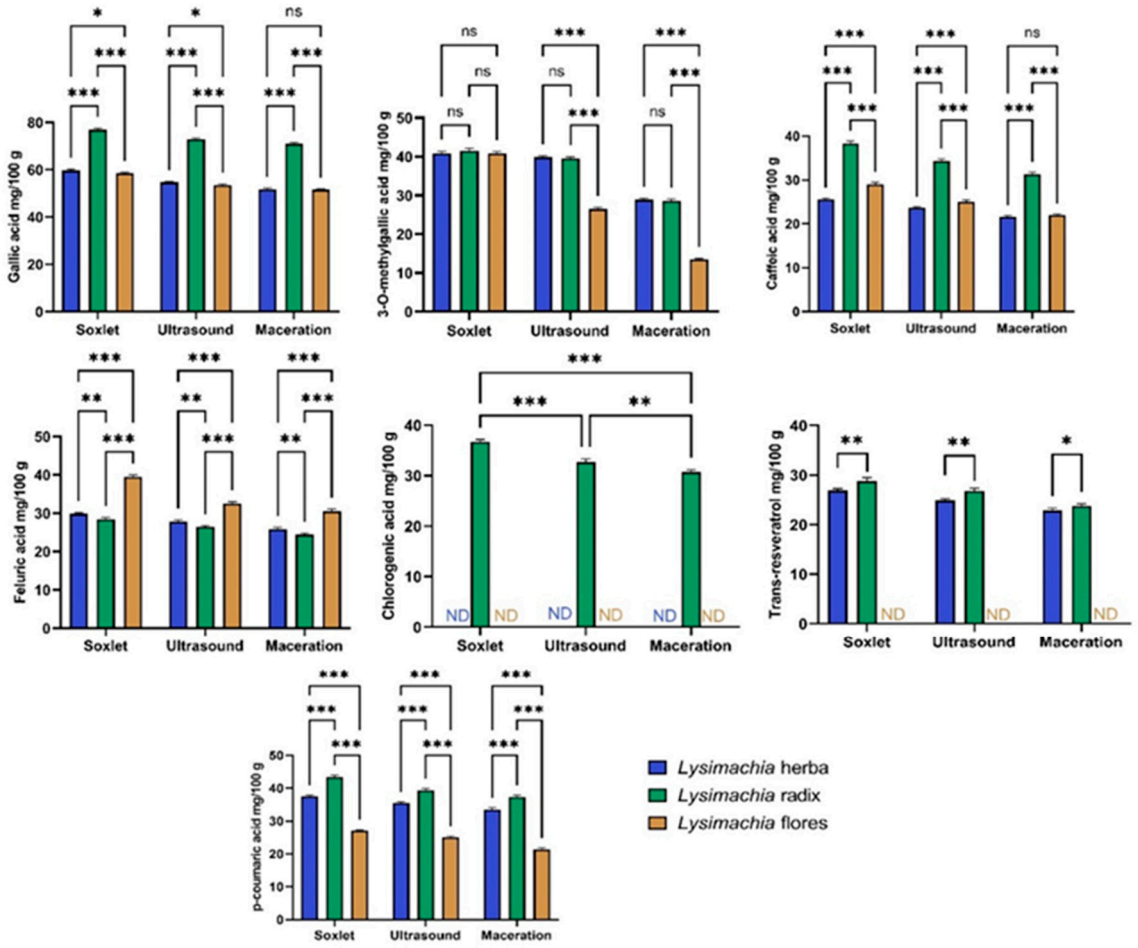


Figure 6. Gallic, chlorogenic, 3-*O*-methylgallic, p-coumaric, caffeic and ferulic acids and *trans*-resveratrol in *Lysimachiae nummularia L.* ethanolic 70% (*v/v*) extracts. The concentrations of phenolic compounds in the ethanolic extracts are expressed in mg/100 g dry sample. The results are presented as mean and error bars ($n = 5$). Asterisks indicate the statistical significance examined by Tukey test for multiple reciprocal comparison among the extracts (significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: $p > 0.05$).

The results regarding the antioxidant activity are summarized in Table 4, in which the data are expressed as the mean ± standard error of the mean ($n = 5$).

Table 4. Antioxidant activity results acquired for the tested *Lysimachia nummularia* L. samples.

Method of Determination	Extraction Method	Sample		
		Herba	***Radix***	***Flores***
DPPH assay IC_{50} in µg/mL	Soxhlet extraction	361.38 ± 0.54	531.99 ± 7.81	439.66 ± 2.92
	Maceration extraction	61.25 ± 0.34	17.58 ± 1.05	26.26 ± 1.62
	Ultrasound extraction	71.22 ± 0.24	22.15 ± 0.99	34.06 ± 0.48

Table 4. *Cont.*

Method of Determination	Extraction Method	Sample		
		Herba	***Radix***	***Flores***
ABTS$^{\bullet+}$ assay IC_{50} in µg/mL	Soxhlet extraction	15.14 ± 0.27	5.14 ± 0.26	9.54 ± 0.28
	Maceration extraction	19.46 ± 0.31	9.46 ± 0.31	17.46 ± 0.23
	Ultrasound extraction	16.58 ± 0.23	6.58 ± 0.22	12.18 ± 0.68
FRAP assay µmol TE */100 mL	Soxhlet extraction	163.18 ± 0.16	251.14 ± 0.17	197.06 ± 0.06
	Maceration extraction	143.18 ± 0.49	211.14 ± 0.26	177.06 ± 0.23
	Ultrasound extraction	152.38 ± 0.81	236.34 ± 0.63	182.66 ± 0.81
CUPRAC assay µmol TE */100 mL	Soxhlet extraction	146.58 ± 0.17	222.94 ± 0.21	181.66 ± 0.06
	Maceration extraction	126.58 ± 0.23	192.94 ± 0.55	151.66 ± 0.44
	Ultrasound extraction	133.38 ± 1.11	201.34 ± 0.63	166.34 ± 0.98

* TE—Trolox equivalent.

4. Discussion

All the analyzed parts of the *Lysimachia nummularia* L. plant contained notable amounts of polyphenol compounds. The results of the analysis of the total content of polyphenols are comparable to those reported in the literature. Hanganu et al. obtained the value of 35.512 ± 0.21 mg GAE/g at the level of the aerial part of the species *Lysimachia nummularia* L. [18].

The total polyphenol content from the extracts obtained by the three methods of extraction is higher than that previously obtained in extracts in ethanol 40% (*v/v*) by Felicia, S. et al. [38]. According to the presented results, the highest content of total polyphenols was found at the level of *Lysimachia radix* extracted in ethanol solvent 70% (*v/v*), obtained by Soxhlet extraction. These values are also higher compared to those obtained in a previous study for extracts carried out in solvent ethanol 40%, ethanol 96% and water [38]. In studies carried out by Toth et al. [5], the amount of polyphenols was between 3 and 4% in methanolic extracts, with the lowest value found in *Lysimachia nummularia herba*. The variations could be related to the pedoclimatic conditions and the harvest period, which could be correlated to the different phenological states of the plants.

The HPLC-DAD method was applied for the investigation of the phenolic-type compounds, with the quantitative analysis of seven components in *Lysimachia nummularia* L. plant extracts. Well-defined peaks for phenolic acid, in turn, with retention times as minutes for gallic acid (GA) (2.39), chlorogenic acid (ChA) (7.06), 3-*O*-methylgallic acid (3O-mGA) (5.35), p-coumaric acid (p-CA) (9.64), caffeic acid (CA) (8.33), ferulic acid (FA) (10.57) and *trans*-resveratrol (*trans*-RES) (11.02), appeared in the chromatograms (Figures 4 and 5). These compounds appear in different parts of the *Lysimachia nummularia* L. plant, in various ratios and combinations. The novelty of this study is represented by the identification and quantification of phenolic compounds by the HPLC method, namely gallic acid at the level of *Lysimachia radix*, the presence of 3-*O*-methylgallic acid and ferulic acid at the level of all the studied parts of the *Lysimachia nummularia* L. plant, caffeic acid at the level of *Lysimachia flores* and *trans*-resveratrol at the level of *Lysimachia radix* and *Lysimachia herba*—data not mentioned in the literature until now (Table 1).

Regarding the antioxidant activity of the extracts (Table 4) obtained by Soxhlet extraction, evaluated by the DPPH assay, it was observed that it was significantly higher compared to the other extraction methods. Moreover, the highest value of the antioxidant capacity was obtained at the level of *Lysimachia radix* in 70% ethanolic extracts by Soxhlet extraction (the IC_{50} value was 531.99 μg/mL in the DPPH assay) (Table 3). Among the three different parts of the *Lysimachia nummularia* L. plant, the *Lysimachia radix* proved to have the strongest DPPH scavenging capacity. In our study, in agreement with other researchers, it was noticed that there were correlations between the DPPH and $ABTS^{\cdot+}$ assays, as well as for the FRAP and CUPRAC methods. Otherwise, the variations among the antioxidant methods can be justified by their chemical backgrounds [39–41]. The highest scavenging activity in the *radix* part can be explained by its higher content of polyphenols. Phenolic acid compounds are well-known antioxidant compounds. In phenolic compounds, the existence of a 3-hydroxyl structure (caffeic acid, gallic acid) increases the antioxidant effect [42,43]. Various studies noticed a good linear correlation between the total phenolic content and the antioxidant activity of plants. The values that indicate that the antioxidant activity of the same plant species, reported in different studies in the literature, is also difficult to compare because there is a significant difference depending on the origin of the plant and the methods used to obtain the extracts. In general, the antioxidant activity is attributed to phenolic compounds.

Some research papers showed that the phenolic compounds, such as caffeic acid and ferulic acid, are related to hydroxycinnamic acids and have apoptotic and anti-proliferative effects on tumor cells [44,45]. Instead, the caffeic acid phenethyl ester can accelerate wound re-epithelization and increase keratinocytes' proliferation [46], while gallic acid, which belongs to the hydroxybenzoic acids, can increase the migration and proliferation of fibroblasts and keratinocytes in a dose-dependent manner [47].

An anti-obesity effect was demonstrated in *Lysimachia* species [48], and the *trans*-resveratrol identified and quantitatively determined in this study by HPLC-RP analysis could be responsible for this effect, because the role of this compound in reducing obesity in humans has been demonstrated [49].

5. Conclusions

According to the experimental results, the plant products *Lysimachiae herba*, *Lysimachiae radix* and *Lysimachiae flores* show marked antioxidant properties, correlated with the content of total polyphenols and phenolic compounds contained, but especially with the presence of resveratrol, an active compound with strong antioxidant properties. Since polyphenols are soluble in ethanol, it was deduced that the optimal methodology for obtaining the dry extract consists of Soxhlet extraction with 70% ethanol, while this does not affect the stability of the polyphenols.

The presence of these compounds with antioxidant potential in all parts of the *Lyzimachia nummularia* L. species leads us to the conclusion that the species are of interest and can be researched for therapeutic use. With these considerations, we believe that the antioxidant properties of the polyphenols contained in the extracts obtained from *Lysimachia nummularia* L., as identified in the current study, represent the possibility of obtaining some antioxidant medicinal products for the treatment of various diseases caused by oxidative stress and free radicals, including the oro-dental rehabilitation of patients.

Author Contributions: Conceptualization, F.S., A.P. and M.M.; methodology, I.S., E.C.L. and A.M.M.; software, A.C.R.; validation, F.C.B., A.C. and V.B.; formal analysis, D.-E.D. and A.M.M.; investigation, I.S. and D.-E.D.; resources, E.C.L.; data curation, D.-E.D.; writing—original draft preparation, F.S., A.P. and M.M.; writing—review and editing, A.M.M.; visualization, A.C.R.; supervision, V.B. and A.C.; project administration, F.S., A.P. and M.M.; funding acquisition, F.C.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work is supported by the project PROINVENT within the framework of the Human Resources Development Operational Programme 2014-2020, financed by the European Social Fund under the contract number 62487/03.06.2022 POCU 993/6/13/– Cod SMIS: 153299 in Romania.

Conflicts of Interest: The authors declare no conflict of interest.

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