DETERMINATION OF POLYPHENOLIC COMPOUNDS OF *LYSIMACHIA NUMMULARIA* L.

Suciu Felicia[1]
Roșca Adrian Cosmin[2]
Lupu Carmen[3]
Popescu Antoanela[4]
Badea Victoria[5]
[1, 2, 3, 4] Faculty of Pharmacy, Ovidius University of Constanța, Romania
[5] Faculty of Dentistry, Ovidius University of Constanța, Romania

ABSTRACT

The history of medicinal plants is associated with the evolution of civilization.

In all regions of the world, the history of nations shows that these plants have always occupied an important place in medicine, in cosmetic products, and culinary preparations.

The paper aims to determine the total polyphenols in different parts of the species *Lysimachia nummularia* L.

In our study, we focused on the extraction of polyphenolic compounds in different solvents.

The solvents used in the extraction were: 40% ethanol, concentrated methanol, in water.

The total polyphenol content was determined by spectrophotometric methods, a method from the European Pharmacopoeia 10.0, with minor modifications.

The total polyphenol content of different extracts varied depending on the extraction process.

Different parts of the plant and different solvents were used in the determinations carried out to establish the optimal extraction method for the organs of *Lysimachia nummularia* L.

Keywords: *Lysimachia, polyphenolic compounds, active principle, spectrophotometry, extracts*

INTRODUCTION

Polyphenols are a large family of naturally occurring organic compounds characterized by multiples of phenol units, they include flavonoids, tannins, anthocyanins, proanthocyanins, stilbenoids, some of which have been used historically as dyes and for tanning garments [1], [2]. Polyphenolic compounds are abundant in many plants and structurally diverse, they are the most important active principles with antioxidant action [3].

From the specialized literature it is known the presence of phenolic compounds in the species *Lysimachia nummularia* L. [4].

Thus, in various studies, appreciable amounts of active principles was determined in all plant organs as follows: polyphenolic compounds, 1.77-3.00 (g / 100g expressed in pyrogallol), flavonosides 0.36-1.77 (g / 100g, expressed in hyperosides), tannins 0.97 -1.95 (g / 100g), hydroxycinnamic acids 0.63-1.25 (g / 100g, expressed as rosmarinic acid) [5].

The highest amount of polyphenols was found for *Lysimachia vulgaris* L. (76.122±0.35 mg/g) and also the content of flavonoids (26.42±1.3mg/g) [6].

Different hungarian samples of *Lysimachia nummularia* L. and *Lysimachia vulgaris* harvested in early and late flowering stage showed the highest total polyphenolic content of these two species (34.2±0.15mg/g and 30.0±0.12mg/g respectively) [7].

Lysimachia nummularia L. is a species of the *Primulaceae* family, known for its antioxidant properties correlated with the content in polyphenolic compounds [8].

MATERIALS AND METHODS

Lysimachia nummularia L., were collected in July 2020, from the edge of Lake Tău-Brazi in the Roșia Montană area.

The determination of total polyphenols was made on plant products obtained from the species *Lysimachia nummularia* L.: *Lysimachiae radix, Lysimachiae herba* and *Lysimachiae flores*.

The vegetable products were obtained from the species *Lysimachia nummularia* L. after drying and sorting and then crushing them.

Solutions to be analyzed: The vegetable products (three samples) were refluxed for 30 minutes with 150 mL of solvent, the quantities listed in Table I. For the vegetal product *Lysimachiae flores*, smaller amounts of vegetal product were used due to the fact that the flowers were collected in smaller quantities.

Table 1. *Working protocol*

Sample	Vegetal products	Solvent	The amount of vegetal product (g)	The amount of vegetal product (g)	The amount of vegetal product (g)
1.	*Lysimachiae herba*	Ethanol 40% (v/v)	1,0233	1,0021	1,0452
2.	*Lysimachiae herba*	Ethanol 96º	1,0494	1,0693	0,9965
3.	*Lysimachiae herba*	Water	1,0534	1,0237	1,0005
4.	*Lysimachiae radix*	Ethanol 40% (v/v)	1,1097	1,0025	1,0597
5.	*Lysimachiae radix*	Ethanol 96º	1,0390	1,0889	1,0875
6.	*Lysimachiae radix*	Water	1,2066	1,0058	1,1052
7.	*Lysimachiae flores*	Ethanol 40% (v/v)	0,2052	0,2144	0,2311
8.	*Lysimachiae flores*	Ethanol 96º	0,200	0,210	0,2245
9.	*Lysimachiae flores*	Water	0,2349 dried vegetal product	0,2233 dried vegetal product	0,2147 dried vegetal product

The spectrophotometric method was used in this study for the development of the analytical procedure for assessing TPC (total polyphenolic content) by Folin-Ciocâlteu method. The used method is based on the general procedure recommended by the European Pharmacopoeia (European Pharmacopoeia 2020) [9] for the determination of total tannins with slight modifications.

The solutions were filtered through a filter material in 250 mL volumetric flasks. The solutions were made up to the mark by washing the vegetable products with the solvent used (solution A).

In 25 mL volumetric flasks were added 5 mL of solution A (all solutions), filtered through filter paper, and made up to the mark with water (solution B).

Exceptions were samples 7, 8 and 9 which were not diluted, further used in this form.

We put 2 mL of solution B (for samples 1-6), and 2 mL of solution A (for samples 7-9) were added to 25 mL flasks, over which 1 mL of Folin-Ciocâlteu reagent, 10 mL, was added water and Na_2CO_3 290g / L at 25 mL. After the stage, a blue coloration has formed. The samples were left to stand for 30 minutes and the

absorbances at the wavelength λ = 760 nm were read, using water as the compensation liquid.

The spectrophotometric determination was performed on a Jasco V650 spectrophotometer.

Pyrogallol standard. In a 100 mL volumetric flask 50 mg of pyrogallol were brought and dissolved in 50 mL of water. It was then filled to the brim with water (stock solution). In a 100 mL volumetric flask, dilute 5 mL of the pyrogalol stock solution with water and makeup to the mark with the same solvent (dilute pyrogallol solution). In a 25 mL volumetric flask, 2 mL of the dilute pyrogallol solution was added to which was added 1 mL of Folin-Ciocâlteu reagent, 10 mL of water and 292 g / L Na_2CO_3 at 25 mL. A blue coloration has formed. It was left to stand for 30 minutes and the absorbance (A2) was read at the wavelength λ = 760 nm using water as the compensating liquid.

Pyrogallol was purchased from Sigma-Aldrich and Folin-Ciocalteau reagent from Merck (Darmstadt, Germany).

***Table. 2**. Determination of total polyphenols. Working protocol*

Sample	**Solution**	**Folin Ciocâlteu reagent**	**Water**	**Sodium carbonate 290g/L**	**Absorbance** λ = 760 nm
1.1	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4350
1.2	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4145
1.3	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4449
2.1	2 mL sol. B	1 mL	10 mL	la 25 mL	0,2001
2.2	2 mL sol. B	1 mL	10 mL	la 25 mL	0,2563
2.3	2 mL sol. B	1 mL	10 mL	la 25 mL	0,2214
3.1	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4221
3.2	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4220
3.3	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4140
4.1	2 mL sol. B	1 mL	10 mL	la 25 mL	0,2158
4.2	2 mL sol. B	1 mL	10 mL	la 25 mL	0,2225
4.3	2 mL sol. B	1 mL	10 mL	la 25 mL	0,2405
5.1	2 mL sol. B	1 mL	10 mL	la 25 mL	0,1951
5.2	2 mL sol. B	1 mL	10 mL	la 25 mL	0,2007
5.3	2 mL sol. B	1 mL	10 mL	la 25 mL	0,1998
6.1	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4562
6.2	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4443

6.3	2 mL sol. B	1 mL	10 mL	la 25 mL	0,4621
7.1	2 mL sol. A	1 mL	10 mL	la 25 mL	0,4143
7.2	2 mL sol. A	1 mL	10 mL	la 25 mL	0,4122
7.3	2 mL sol. A	1 mL	10 mL	la 25 mL	0,4206
8.1	2 mL sol. A	1 mL	10 mL	la 25 mL	0,2021
8.2	2 mL sol. A	1 mL	10 mL	la 25 mL	0,2022
8.3	2 mL sol. A	1 mL	10 mL	la 25 mL	0,2051
9.1	2 mL sol. A	1 mL	10 mL	la 25 mL	0,4091
9.2	2 mL sol. A	1 mL	10 mL	la 25 mL	0,4086
9.3	2 mL sol. A	1 mL	10 mL	la 25 mL	0,4177
Standard - Pyrogallol	2 mL sol. diluted pyrogalol	1 mL	10 mL	la 25 mL	0,3905

The percentage content of total polyphenols for samples 1-6 was calculated according to the following formula:

$$\% \text{ total polyphenols} = \frac{62,5 \times A1 \times m2}{A2 \times m1}$$

A modified formula is used to determine the polyphenols in samples 7-9, as one of the dilutions has not been made:

$$\% \text{ total polyphenols} = \frac{12,5 \times A1 \times m2}{A2 \times m1}$$

m_1 = mass of the sample taken in work, in grams

m_2 = pyrogallol mass, in grams (0.05 g)

A_1= absorbance of the sample

A_1= absorbance of the standard

Statistical evaluation was performed with using GraphPad Prism 9 software (GraphPad, USA).

RESULTS AND DISCUSSIONS

The amount of polyphenols obtained is shown in the table below:

Table 3. *Determination of total polyphenols*

Sample	Total polyphenols g% dried vegetable grodus	Mean	Standard deviation
1.1	3,7195	3,6877	0,0594
1.2	3,6192		
1.3	3,7245		
2.1	1,6684	1,9032	0,2173
2.2	2,0973		
2.3	1,9440		
3.1	3,5061	3,5779	0,0626
3.2	3,6070		
3.3	3,6206		
4.1	1,7027	1,8778	0,1531
4.2	1,9433		
4.3	1,9872		
5.1	1,6442	1,6222	0,0192
5.2	1,6138		
5.3	1,6087		
6.1	3,3105	3,6131	0,2817
6.2	3,8678		
6.3	3,6610		
7.1	3,4932	3,3228	0,1722
7.2	3,3264		
7.3	3,1489		
8.1	1,7483	1,6650	0,0838
8.2	1,6659		
8.3	1,5807		
9.1	2,7874	2,9433	0,1637
9.2	2,9287		
9.3	3,1138		

All samples were performed in triplicate and the mean value was reported.

The amount of polyphenols varied significantly depending on the extraction solvent and the plant product.

The highest amount of polyphenols was determined in *Lysimachiae herba*, in the order of 40% alcohol> water> ethanol. In *Lysimachiae radix* the smallest amount of polyphenolic compounds was obtained as follows: water%> alcohol 40%> ethanol. In *Lysimachiae flores* an average amount of polyphenolic compounds was obtained in the following order: alcohol 40%> water> ethanol.

From the results we can deduce that alcohol 40% is the best extractive solvent for all vegetal products of the species except the root, but the amount of polyphenols in alcohol % is large enough to choose the solvent, alcohol 40, for the extraction of total polyphenols.

The amount of total polyphenols falls in the range, 1.5-3%, mentioned in the literature [5], [6], [7].

Results represent the average of three replicates, from three independent determinations. Results are presented as mean ± standard deviation (SD) and were statistically analysed using GraphPad Prism 9 software (GraphPad, USA), by means of two-way ANOVA followed by Tukey's multiple comparisons test. Differences between the groups were considered statistically significant at $p < 0.05$.

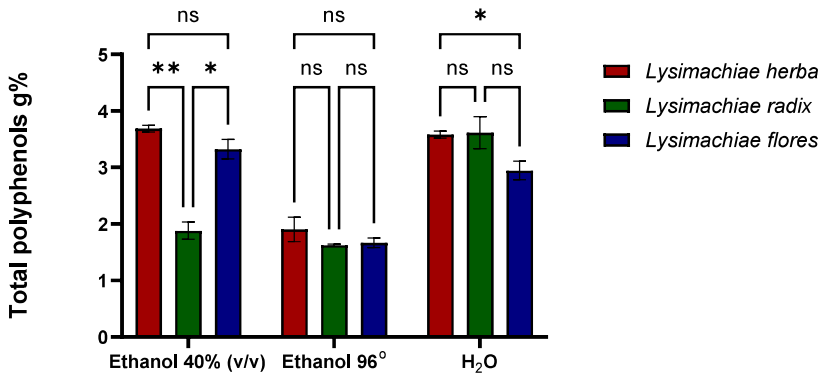

Fig. 1. *Multiple comparison of total polyphenols content of Lysimachia nummularia L.*

Values were expressed as Mean ± SD ($n = 3$). Significance is indicated by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns: $p > 0.05$ (Fig.1).

CONCLUSION

Polyphenolic compounds were determined in all organs of the plant *Lyzimachia nummularia* L. (*Primulaceae*).

The largest amount of polyphenolic compounds was determined in the aerial part of the species *Lyzimachia nummularia* L.

The amount of polyphenolic compounds determined in alcohol 40 % led us to say that this solvent is the best extraction solvent of the three solvents tested.

Thus, in order to obtain an extract from all the organs of the species Lyzimachia nummularia L. to be standardized in polyphenolic compounds, we can further use ethanol 40 % as extraction solvent.

The large amount of polyphenolic compounds determined in ethanol 40 %, leads us to believe that the extract we will obtain will have very good antioxidant

properties, and which could be used in the therapy of many diseases, which require a high consumption of antioxidants.

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