TOTAL PHENOLIC CONTENT AND HPLC CHARACTERIZATION OF SOME CULINARY HERBS

SIMONA DOBRINAS[1], GABRIELA STANCIU[1], SIMONA LUPSOR[1]

Manuscript received: 13.05.2017; Accepted paper: 01.03.2017;
Published online: 30.06.2017.

Abstract. *The total phenols contents and individual polyphenols in alcoholic extractive solutions of spinach (Spinacia oleracea), common nettle (Urtica dioica), rocket salad /arugula (Eruca sativa), common dandelion (Taraxacum officinale), parsley (Petroselinum crispum), lovage (Levisticum officinale), mint (Mentha piperita), summer savory/satureja (Satureja hortensis) were examined using Folin-Ciocalteu method and an adapted USP30 HPLC method, respectively. The highest concentration in total phenols was registered for lovage (6860.82 mg GAE/100 g f.w.) and rocket salad (620.41 mg GAE/100 g f.w.).HPLC – DAD analysis indicate the presence of nine individual polyphenolic compounds in different concentrations: gallic acid exists in all studied herbs in variable concentrations, from 13.13 mg/100 g f.w.in spinach to 420.02 mg/100g f.w. in rocket salad; ellagic acid in four herbs from 42.71 mg/100 g f.w. in common dandelion to 632.42 mg/100g f.w. in lovage; chlorogenic acid in four herbs from 0.44 mg/100 g f.w. in common nettle to 4.17 mg/100 g f.w. in mint; caffeic, caftaric, coumaric, ferulic, 3-o-methyl-gallic acids were determined in small amounts.*

Keywords: *herbs, antioxidants, HPLC/DAD method, phenolic compounds, gallic acid.*

1. INTRODUCTION

Plants provide abundant natural antioxidants such as ascorbic acid, carotenoids, polyphenols and enzymes with antioxidant activity, which protect the cells from oxidative damage [1-4]. Foods containing culinary herbs are a significant source of nutrients with many benefits for human body [5-7].

Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing, and treatment [5, 8-11]. Gallic acid and esters of gallic acid are antioxidants used in pharmaceutical industry due to their therapeutically effects as cardioprotective, hepatoprotective, anti-diabetic, anti-inflammatory, neuroprotective, anti-ulcerogenic, anti-carcinogenic and anti-nephrotoxicity effects [12-14].

Drying methods influence the composition and biological activity of plant [5]; e.g. treatment at higher temperature decreases content of gallic acid [15-18]. Extracts of herbs, vegetables and other plants rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [17-19].

Due to their widely uses as culinary herbs in the Romanian traditional cuisine, spinach (*Spinacia oleracea*), common nettle (*Urtica dioica*), rocket salad /arugula (*Eruca sativa*), common dandelion (*Taraxacum officinale*), parsley (*Petroselinum crispum*), lovage

[1]Ovidius University of Constanta, Department of Chemistry and Chemical Engineering, 900527 Constanta, Romania. E-mail: gstanciu@univ-ovidius.ro

(*Levisticum officinale*), mint (*Mentha piperita*) and summer savory/satureja (*Satureja hortensis*) have been selected for this study.

To demonstrate their effective antioxidant activities related to the content in polyphenolic compounds, two series of investigations have been done: determination of total phenols concentration using Folin-Ciocâlteau spectrophotometric method and individual polyphenols quantification by HPLC – DAD method.

2. MATERIALS AND METHODS

2.1. MATERIALS

Gallic acid was purchased from Fluka (Buchs, Switzerland), Folin – Ciocalteu reagent (a mixture of phosphowolframate with phosphomolybdate) and ethanol from Merck (Darmstadt, Germany). The 20% Na_2CO_3 solution was prepared by dissolving 20 g of Na_2CO_3 in 80 mL of distilled water. Gallic acid solution (standard phenolic compound) was prepared by dissolving 376 mg of gallic acid in 100 mL of ethanol. Folin – Ciocalteu reagent was diluted with distilled water 1:10 (V/V).

Spectrometric measurements were carried out using a UV-VIS JASCO V550 double beam scanning spectrophotometer.

All reagents used for polyphenols separation and quantification were of HPLC reagent grade. The identification and quantitative determination of phenolic compounds was performed using HPLC - DAD system (Agilent 1200).

2.2. METHODS

Sample preparation

Aerial plants of all eigth studied plants: spinach (*Spinacia oleracea*), common nettle (*Urtica dioica*), rocket salad/arugula (*Eruca sativa*), common dandelion (*Taraxacum officinale*), parsley (*Petroselinum crispum*), lovage (*Levisticum officinale*), mint (*Mentha piperita*) were collected during April 2016 and summer savory/satureja (*Satureja hortensis*) was collected during August 2016 from Constanta City, Dobrogea County.

Extractions were achieved by maceration of 10 g of fresh plant material in 100 mL of ethanol at room temperature for 12 h. The mixtures were strongly shaken three times every two hours. After extraction, samples were filtered and used for analyses.

Total polyphenolic content (TPC)

Total phenolic content was determined using the spectrophotometric method based on the reduction of a phosphowolframate – phosphomolibdate complex to blue products by soluble phenolic compounds, in sodium carbonate media and the measurement of the absorption of the formed complex at the wavelength of 675 nm.

The absorbance relative to a gallic acid standard curve was measured and results are expressed as gallic acid equivalents (mg GAE/mL). All samples were performed in triplicate and the mean value was reported.

Calibration curve

To plot the calibration curve in the range of 75 – 262.5 mg GAE/L (Figure 1), 1 mL Folin Ciocalteu reagent 1:10 was added in 50 mL calibrated flasks to different volumes of standard gallic acid solution, then 1 mL sodium carbonate solution 20; the mixture was shaken and allowed to stand for 10 min at room temperature and fill up to the mark with distilled water. Each sample was homogenized and let under room temperature 30 minutes for the color stabilization; after that the absorbance was registered at 675 nm. The obtained correlation coefficient was 0.9928 and standard error = 0.334711.

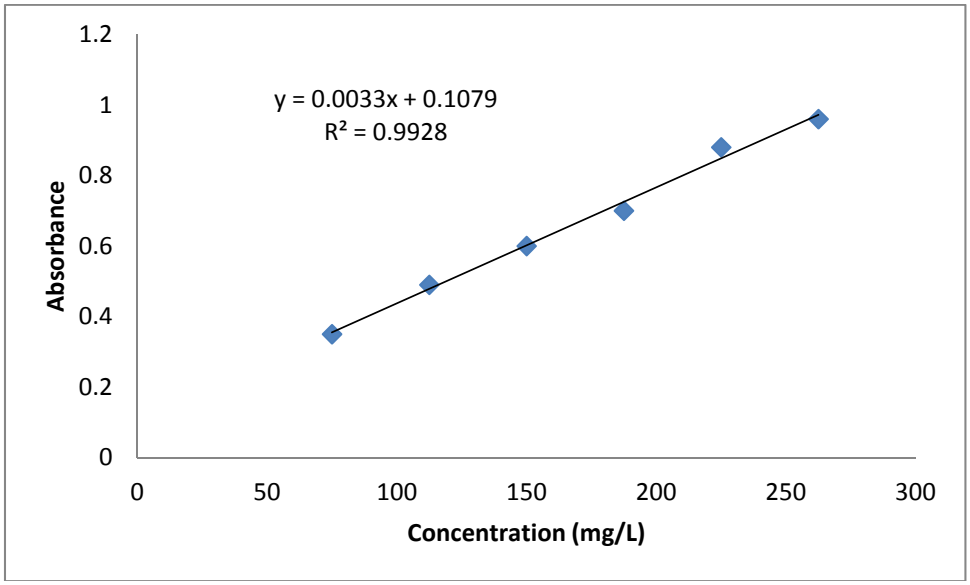

Figure 1. Calibration curve of gallic acid in the range of 75 – 262,5 mg GAE/L at 675 nm.

The calibration curve was linear and corresponds to the equation: y = a + bx, with a = 0.0033 and b = 0.1079.

To measure the total phenols content, 1 mL of each sample were added in 50 mL calibrated flasks, then 1 mL Folin Ciocalteu reagent 1:10, 1 mL sodium carbonate solution 20% and the process was the same like those used for calibration.

HPLC analysis of phenolic compounds

Adapted USP30 HPLC method was used for separation, identification and quantification of the individual phenolic compounds in the resulted extractive solutions [20, 21].

HPLC system (Agilent 1200) had quaternary pump, DAD, auto sampler. Separation was carried out on Zorbax Eclipse XDB-C18 column: 250 mm, 4.6 mm; 5 µm (Agilent Technologies). The gradient elution was phosphoric acid 0.1% in water (solvent A) and acetonitrile (solvent B) as previously described in paper of G. Stanciu et. al. [21].

3. RESULTS AND DISCUSSION

3.1. RESULTS

In Table 1 are presented the mean values of total phenolic compounds (TPC) concentrations, expressed as mg GAE/100 g fresh weight (f.w.) in studied culinary herbs determined by UV-VIS method.

Table 1. TPC concentrations in culinary herbs (mean values).

Sample	TPC, mg GAE/100 g f.w.
Spinach (*Spinacia oleracea*)	56.98
Common nettle (*Urtica dioica*)	120.92
Rocket Salad (*Eruca sativa*)	620.41
Common dandelion (*Taraxacum officinale*)	98.12
Parsley (*Petroselinum crispum*)	68.65
Lovage (*Levisticum officinale*)	860.82
Mint (*Mentha piperita*)	84.36
Summer savory/Satureja (*Satureja hortensis*)	140.58

In Figs. 2-9 there are presented the HPLC chromatograms of each investigated culinary herbs ethanolic extracts.

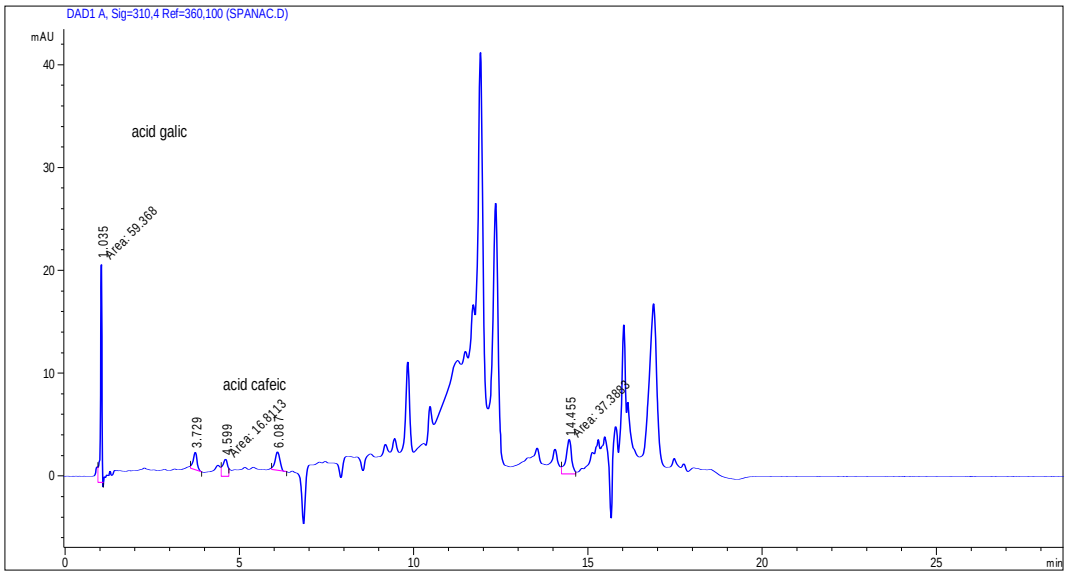

Figure 2. HPLC chromatogram of Spinach (*Spinacia oleracea*).

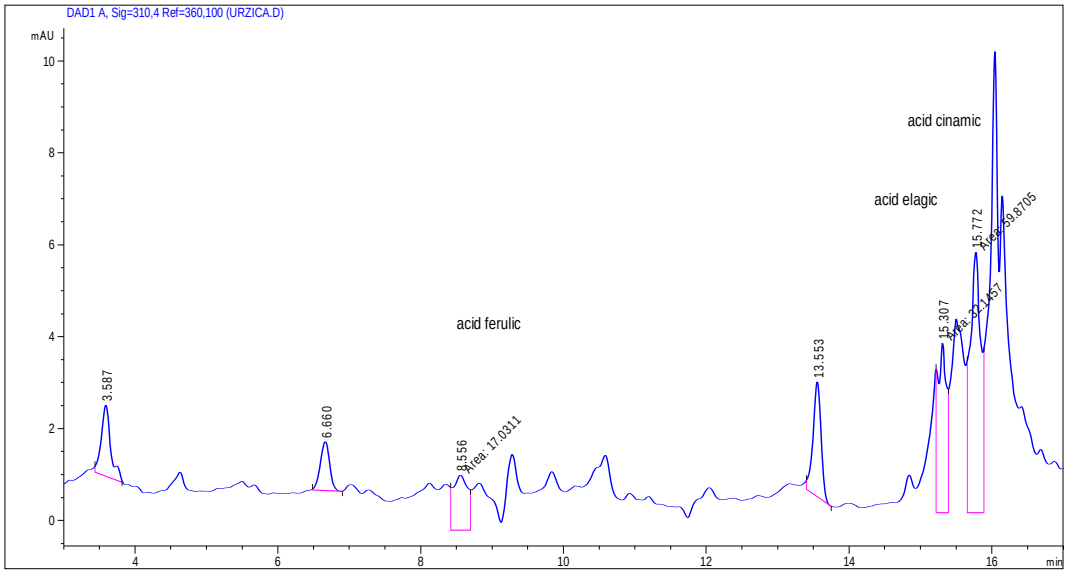

Figure 3. HPLC chromatogram of Common nettle (*Urtica dioica*).

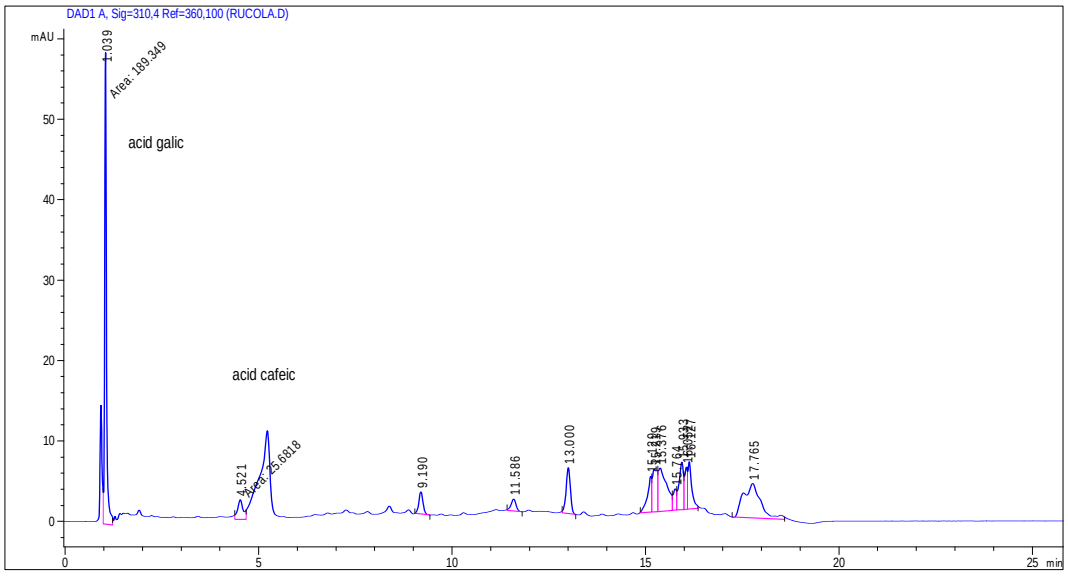

Figure 4. HPLC chromatogram of Rocket Salad /Arugula (*Eruca sativa*).

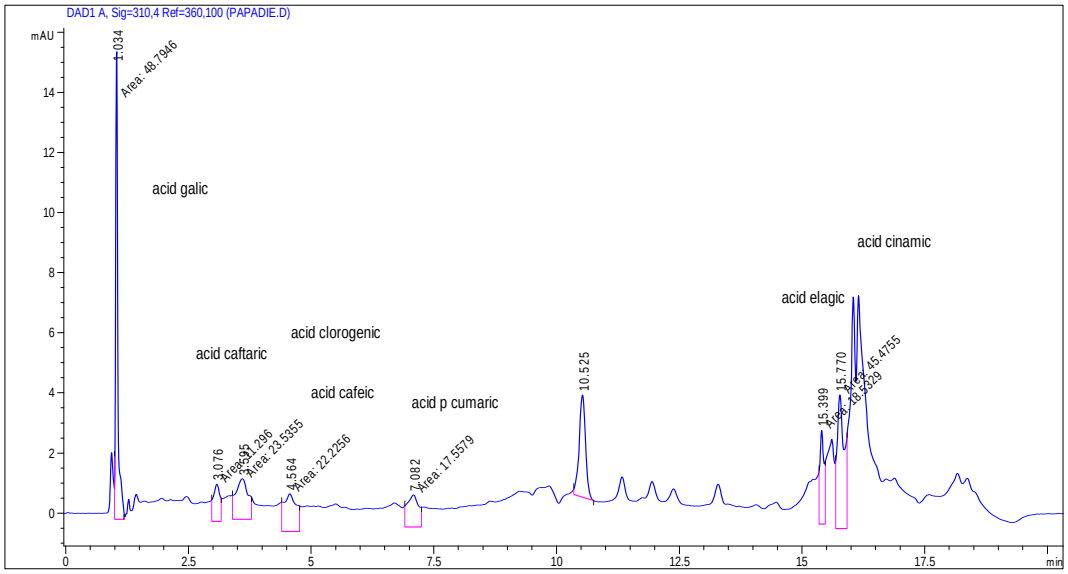

Figure 5. HPLC chromatogram of Common Dandelion (*Taraxacum officinale*).

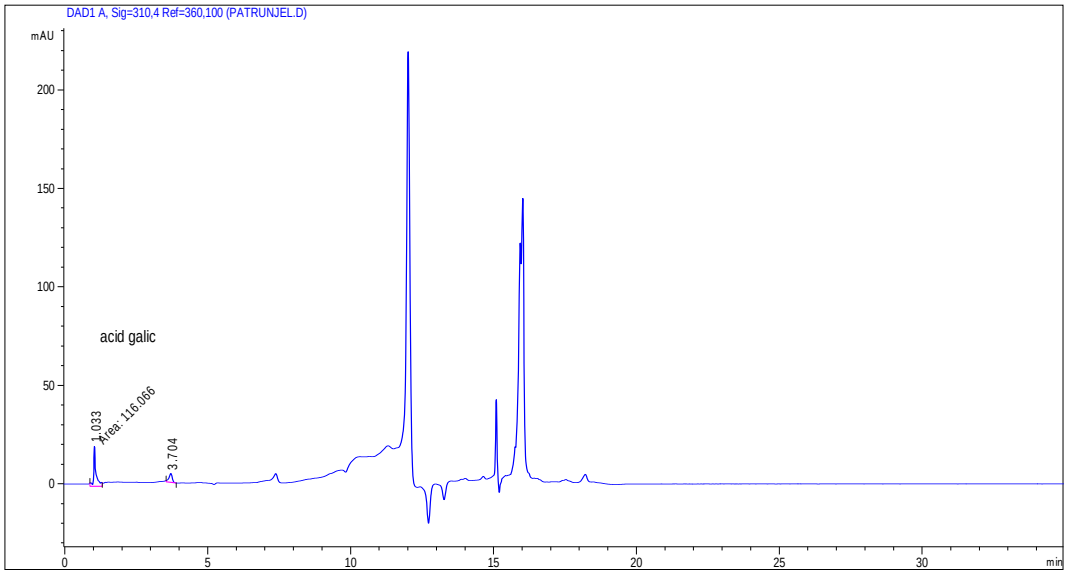

Figure 6. HPLC chromatogram of Parsley (*Petroselinum crispum*).

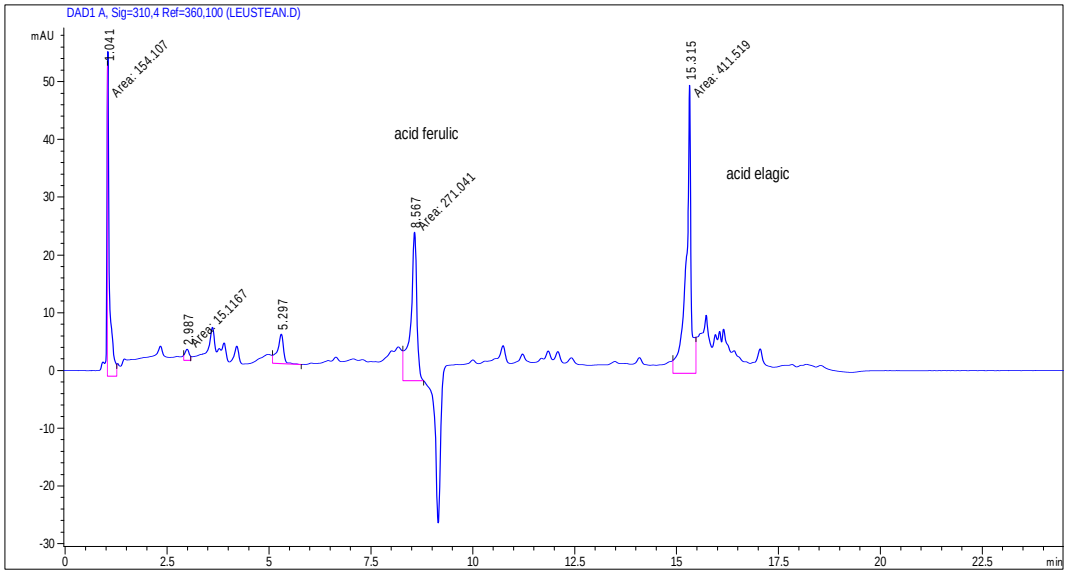

Figure 7. HPLC chromatogram of Lovage (*Levisticum officinale*).

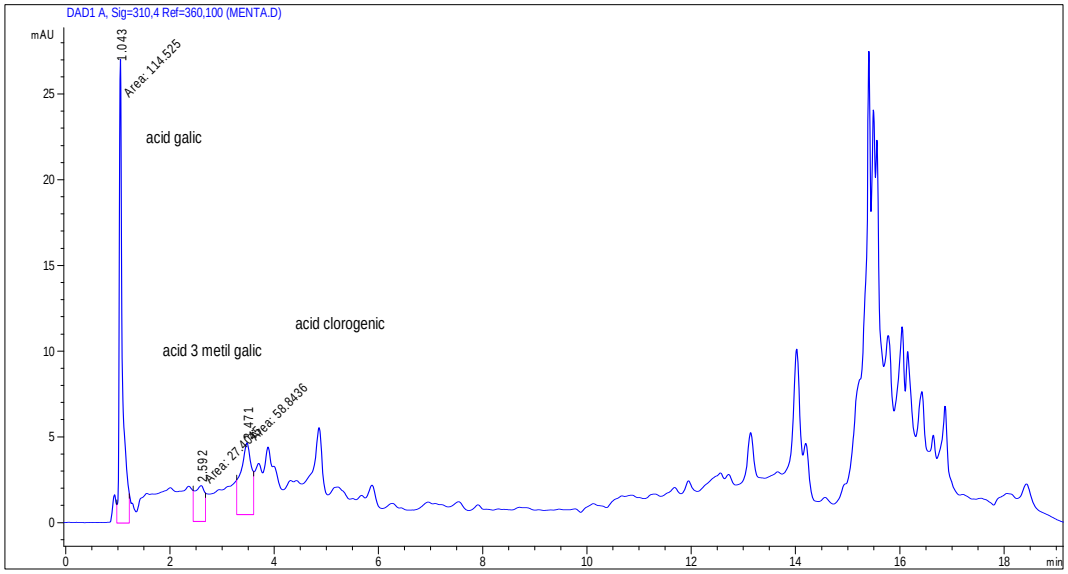

Figure 8. HPLC chromatogram of Mint (*Mentha piperita*).

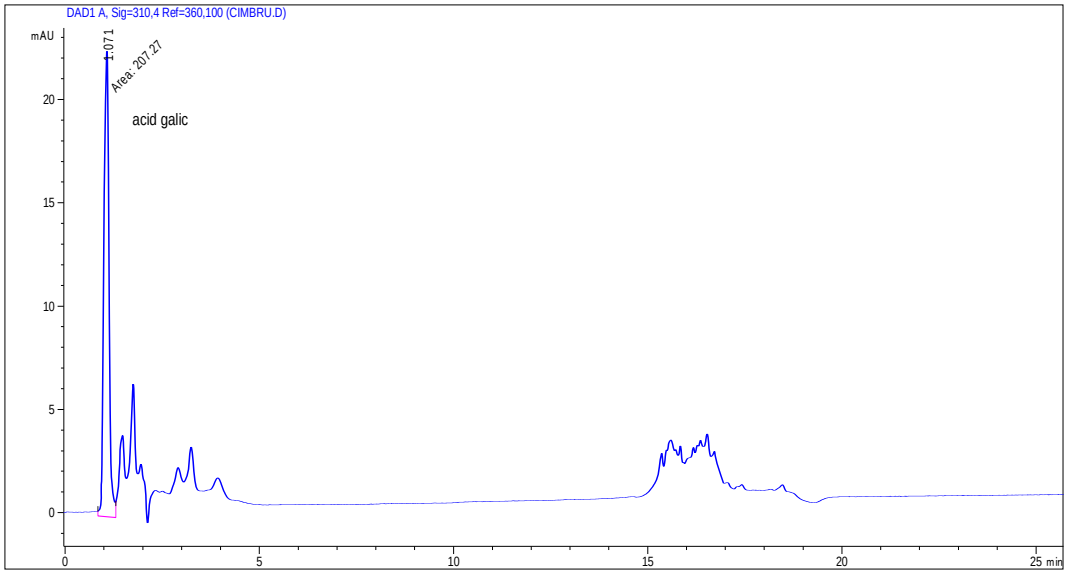

Figure 9. HPLC chromatogram of Summer savory/Satureja (*Satureja hortensis*).

In Table 2 the individual polyphenols concentrations determined by HPLC – DAD in each ethanolic extract of studied culinary herbs in mg/100 g f.w. and percentages (%) are presented.

3.2. DISSCUSION

The highest total polyphenolic content was registered for lovage (860.82 mg GAE/100 g f.w.) and rocket salad (620.41 mg GAE/100 g f.w.). The lowest concentration of total phenols was obtained for spinach (56.98 mg GAE/100g f.w.). All others culinary herbs registered TPC concentrations in the range of 68.65 – 140.58 mg GAE/100g f.w.).

TPC concentration in ethanolic extracts of studied culinary herbs expressed in mg GAE/100g f.w. decreases in order: lovage > rocket salad > summer savory > common nettle > common dandelion > mint > parsley> spinach.

The samples chromatograms indicate the presence of some individual polyphenolic compounds in different concentrations. Gallic acid exists in all studied herbs in variable concentrations. The highest concentration of gallic acid has been determined in rocket salad (420.02 mg/100g f.w.) and the lowest concentration in spinach (13.13 mg/100 g f.w.).

Lovage presents the highest concentration of ellagic acid (632.42 mg/100g f.w.). Considerable concentrations of ellagic acid were detected as well as in case of rocket salad (129.13 mg/100 g f.w.), common nettle (49.33 mg/100 g f.w.) and common dandelion (42.71 mg/100 g f.w.).

Chlorogenic acid, known for his cardioprotective and anti-diabetic effects was identified in mint (4.17 mg/100 g f.w.), common dandelion (1.67 mg/100 g f.w.) and common nettle (0.44 mg/100 g f.w.).

Coumaric acid was identified only in small quantity in common dandelion (0.44 mg/100 g f.w.).

Small quantities of caffeic acid were determined in spinach (0.27 mg/100 g f.w.), common nettle (0.4 mg/100g f.w.) and common dandelion (0.33 mg/100 g f.w.).

Table 2. Contents (mg/100g f.w.) and percentages (%) of phenolic compounds of selected herbs determined by HPLC.

Phenolic compound	Spinach		Common nettle		Rocket Salad		Common Dandelion		Parsley		Lovage		Mint		Summer savory	
	mg/ 100g f.w.	%	mg/ 100g f.w.	%	mg/ 100g f.w.	%	mg/ 100g f.w.	%	mg/ 100g f.w.	%	mg/ 100g f.w.	%	mg/ 100g f.w.	%	mg/ 100g f.w.	%
Gallic acid	13.13	97.97	29.86	37.328	420.02	76.77	16.23	25.79	35.62	100	34.13	5.11	38.10	80.75	98.42	100
3-*o*-Methyl -gallic acid	-	-	-	-	-	-	-	-	-		-	-	4.91	10.41	-	-
Ferulic acid	-	-	0.004	0.005	-	-	-	-	-		0.059	0.01	-	-	-	-
Ellagic acid	-	-	49.33	61.667	129.13	23.60	42.71	67.86	-		632.42	94.69	-	-	-	-
p-Coumaric acid	-	-	-	-	-	-	0.44	0.70	-		-	-	-	-	-	-
Chlorogenic acid	0.002	0.015	0.44	0.50	-	-	1.67	2.65	-		-	-	4.17	8.84	-	-
Caffeic acid	0.27	2.015	0.40	0.50	-	-	0.33	0.52	-		-	-	-	-	-	-
Caftaric acid	-	-	-	-	-	-	1.56	2.48	-		1.28	0.19	-	-	-	-
Total Phenols	13.40	100	79.99	100	549.15	100	62.94	100	35.62	100	667.87	100	47.18	100	98.42	100

Caftaric acid was identified in small quantity in common dandelion (1.56 mg/100 g f.w.) and lovage (1.28 mg/100 g f.w.).

E-Resveratrol was not identified in the studied samples.

The results obtained by the two methods applied in this study, showed that there is a strong positive correlation between them (the Pearson correlation coefficient - r = 0.996). Also, the results are significant correlated (p = 0.00001 <0.05).

To determine if the differences between values of total phenolic compounds concentrations obtained by both methods were significant, a pair t-test was performed. All statistical analyses were carried out at the 95% confidence level and two-tailed hypothesis was applied. According to this test, were not found statistically significant differences values of total phenolic compounds determined by both methods ($t_{calculated}$ = 1,75 < t_{table} = 2,36). In the same time, p = 0.12 > 0,05 which means that differences are not significant.

4. CONCLUSIONS

Seven culinary herbs widely used in the Romanian traditional cuisine: spinach (*Spinacia oleracea*), common nettle (*Urtica dioica*), rocket salad /arugula (*Eruca sativa*), common dandelion (*Taraxacum officinale*), parsley (*Petroselinum crispum*), lovage (*Levisticum officinale*), mint (*Mentha piperita*) and summer savory/satureja (*Satureja hortensis*) have been studied to demonstrate their effective antioxidant activities related to the content in polyphenolic compounds.

TPC analysis show that lovage (860.82 mg GAE/100 g f.w.) and rocket salad (620.41 mg GAE/100 g f.w.) have the higest levels of total polyphenolic compounds concentrations.

TPC concentration in ethanolic extracts of studied culinary herbs expressed in mg GAE/100g f.w. decreases in order: lovage > rocket salad > summer savory > common nettle > common dandelion > mint > parsley> spinach.

HPLC – DAD analysis indicate the presence of nine individual polyphenolic compounds in different concentrations: gallic acid exists in all studied herbs in variable concentrations, from 13.13 mg/100 g f.w.in spinach to 420.02 mg/100g f.w. in rocket salad; ellagic acid in four herbs from 42.71 mg/100 g f.w. in common dandelion to 632.42 mg/100g f.w. in lovage; chlorogenic acid in four herbs from 0.44 mg/100 g f.w. in common nettle to 4.17 mg/100 g f.w. in mint; caffeic, caftaric, coumaric, ferulic, 3-*o*-methyl-gallic acids were determined in small amounts.

The presence of these phenolic compounds in the studied herbs indicates them as a proper antioxidant source wich can be used for special diets.

Determination of TPC concentration using Folin-Ciocâlteau spectrophotometric method and individual polyphenols quantification by HPLC – DAD method in the ethanolic extracts of culinary herbs indicate that they have important concentrations in phenolic compounds which recommend them to be included in various foods. Consumption of these herbs is recommended not only for health benefits but also for low-calorie diets.

REFERENCES

[1] Vabkova, J., Neugebauerova, J., *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, **LX**(1), 167, 2012.
[2] Chrpova, D., Kourimska, L., Gordon, M.H., Veronika Hermanova, V., Roubickova, I., Panek, J., *Czech J. Food Sci*., **28**(4), 317, 2010.
[3] Tabaraki, R., Sadeghinejad, N., *Iranian Journal of Pharmaceutical Sciences*, **9**(1), 47, 2013.
[4] Tomsone, L., Kruma, Z., *9th Baltic Conference on Food Science and Technology*, *FOODBALT*, 191, 2014.
[5] Butnariu, M., Butu, A., *Handbook of Food Chemistry*, P.C.K. Cheung, B.M. Mehta (eds.), Springer-Verlag Berlin Heidelberg, 627, 2015.
[6] Kazimierczak, R., Hallmann, E., Kazimierczyk, M., Sokołowska, O., Rembiałkowska, E., *Vegetable Crops Research Bulletin*, **75**, 133, 2011.
[7] Miłek, M., Jaroslav Legáth, J., *Res J. Chem. Environ. Sci.*, **3**(6), 59, 2015
[8] García-Carrasco, B., Fernandez-Dacosta, R., Dávalos, A., Ordovás, J.M., Rodriguez-Casado, A., *Med. Sci.*, **3**, 38, 2015.
[9] Pădureţ, S., Amariei, S., Gutt, G., Piscuc, B., *Romanian Biotechnological Letters*, **21**(3), 2016.
[10] Rodino, S., Butu, A., Butu, M., P. C. Cornea, P.C., *Digest Journal of Nanomaterials and Biostructures*, **10**(3), 947, 2015.
[11] Lee, S., Han, S., Kim, H.M., Lee, J.M., Mok, S.-Y., Lee, S., *J. Korean Soc. Appl. Biol. Chem*., **54**(1), 73, 2011.
[12] Popovici, C., Capcanari, T., Deseatnicova, O., Sturza, R., *Sibiu Alma Mater University Conference*, *Fifth Edition*, **1**, 313, 2011.
[13] Pasini, F., Verardo, V., Caboni, M.F., D'Antuono, L.F., *Food Chemistry*, 133, 1025, 2012.
[14] Elena Degl'Innoocenti, E., Pardossi, A., Tattini, M., Guidi, L., *Journal of Food Biochemistry*, **32**, 642, 2008.
[15] Ismail, A., Marjan, Z.M., Foong, C.W., *Food Chemistry*, **87**, 581, 2004.
[16] Bunea, A., Andjelkovic, M., Socaciu, C., Otilia Bobis, O., Neacsu, M., Verhe, R., Van Camp, J., *Food Chemistry*, **108**, 649, 2008.
[17] Kamath, S.D., Arunkumar, D., Avinash, N.G., Samshuddin, S., *Adv. Appl. Sci. Res.*, **6**(6), 99, 2015.
[18] Nencu, I., Istudor, V., Ilieş D.-C., Rădulescu, V., *Farmacia,* **61**(2), 2013.
[19] Kukrića, Z. Z., Topalić-Trivunović, L.N., Kukavica, B.M., Matoš, S.B., Pavičić, S.S., Boroja, M.M., Savić, A.V., *Acta periodica Tehnologica*, *APTEFF*, **43**, 1-342, 2012.
[20] ***** NF 25, *United States Pharmacopeia and the National Formulary (USP 30/NF 25)*, Rockville (MD), The United States Pharmacopeial Convention Inc., 914, 2007.
[21] Stanciu, G., Lupsor, S., Popescu, A., Oancea, A.I., *Journal of Science and Arts*, **1**(38), 107, 2017.