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Quantitative determination of some antioxidant compounds from the *Vaccinum myrtills* extracts

Dumitru Condrat[1*], Virgiliu Ciutina[2], Mihaela Meșter[3], Anca Dicu[4], Monica Zdremțan[5], Daniela Diaconescu[6]

[1]*Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, 310130, Elena Drăgoi street, no. 2, Arad, Romania, condrat_dumitru@yahoo.com*
[2]*Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, 310130, Elena Drăgoi street, no. 2, Arad, Romania, virgilciutina@yahoo.com*
[3]*Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, 310130, Elena Drăgoi street, no. 2, Arad, Romania, mihaelamester@yahoo.com*
[4]*Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, 310130, Elena Drăgoi street, no. 2, Arad, Romania, zdremoni@yahoo.com*
[5]*Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, 310130, Elena Drăgoi street, no. 2, Arad, Romania, anca1474@yahoo.com*
[6]*Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, 310130, Elena Drăgoi street, no. 2, Arad, Romania, danieladiac@yahoo.com*

Abstract

The profile of antioxidant compounds of the analyzed extracts was investigated by chromatographic analysis using a Shimadzu Nexera X2 ultra-high performance liquid chromatograph (UHPLC) equipped with a Shimadzu DAD detector and a Nucleosil 100-3-C13 reversed-phase column. Measurements were performed in the wavelength range of 200-600 nm. The plant material subjected to extraction was hawthorn leaves (*Vaccinum myrtills*).

Different solvent extracts were tested and we found that the 70% hydroalcoholic extracts have the highest content in antioxidant compounds, the content of the extracts varying between 8.05 – 3258.73 mg/L.

Keywords: antioxidant, hawthorn, ethanolic extract, high performance liquid chromatography

1.Introduction

Antioxidants work adequately only in close correlation with the structure of the free radical, its properties and level of action and with the concentration of the harmful agent existing in the target tissue [1].

Living organisms have developed a complex system of compounds with an antioxidant character to counteract the reactive species that act to the detriment of life [2-4] of which the most important and most toxic are the reactive/radical species of oxygen.

The great efficiency of compounds with an antioxidant character lies in the synergism of their action, in the summary of their combined action, each operating according to different mechanisms and at various levels of the chain evolution of free radicals in the body.

The presence of antioxidant compounds in plant tissues makes it difficult to measure each antioxidant component separately. That is why several calculation methods for the total antioxidant activity of plant extracts have been developed in recent years.

Recently, several methods have been perfected for the determination of total antioxidant activity, methods based on different reaction mechanisms, such as Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity

* Corresponding author: condrat_dumitru@yahoo.com

(ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and chemiluminescence (LC).

Different extraction environment were also tested to ensure the maximum extraction of antioxidants from the samples (in most cases, alcoholic extractions have been proved more effective than aqueous extractions) [5,6].

The material under study, *Vaccinium myrtillus*, is a shrub from the *Ericaceae* family, a native species of Europe, northern Asia, Greenland, western Canada and western United States; it contains ascorbic acid, tannins, polyphenolic compounds and has various pharmacological actions, such as: astringent, antidiarrheal, diuretic, depurative, antioxidant.

Blueberry leaves and fruits have astringent properties due to tannin [7].

It is recommended in diabetes, gout, enterocolitis, intestinal parasites, urinary infections, uremia, peripheral circulatory disorders, urethritis, somatitis, chronic bleeding ulcers [7].

The literature does not offer a very large range of information regarding the extremely complex chemical composition of plant extracts from the species *Vaccinium myrtillus*, although its therapeutic properties are well known.

Therefore, a more detailed study on the chemical composition of the plant from the *Ericaceae* family, genus Vaccinium rich in antioxidant compounds becomes necessary.

2. Materials and methods

The undertaken studies had as raw material the plant species: *Vaccinium myrtillus* - from Fares S. A. Orăștie.

Reagents: methanol and phosphoric acid for HPLC – Merck, ascorbic acid – Roth, quercetin (≥ 99%), rutin (≥ 99.2%), kaempferol (≥ 99%), caffeic acid (≥ 99%), vanillic acid (≥ 99%), syringic acid (≥ 99%), p-coumaric acid (≥ 99%), catechin (≥ 99%), rosmarinic acid (≥ 99%), ferulic acid - Roth.

The hydroalcoholic extracts were obtained by static extraction [8].

The applied extraction method was uniform, operational parameters, namely the degree of shredding of the plant, the used solvent, the plant/solvent ratio and the extraction temperature were identical.

Were obtained 96% hydroalcoholic and 70% ethanol extracts, with a clear appearance and having a specific color.

Method: High Performance Liquid Chromatography [9].

The profile of polyphenolic compounds of the hydroalcoholic extracts was investigated by chromatographic analysis using a Shimadzu Nexera X2 ultra-high performance liquid chromatograph (UHPLC) equipped with a Shimadzu M30A DAD detector and a Nucleosil 100-3-C18 reversed-phase column (column length 125 mm x 4 mm inner diameter x 3 micrometer particle size, Macherey-Nagel GmbH, Duren, Germany).

The column temperature was maintained at 30 degrees Celsius and the flow rate at 1 ml/min.

The solvents used for the chromatographic elution consisted of the 0.1% aqueous solution of trifluoroacetic acid, pH=3 (A) and acetonitrile (B).

The chromatographic elution schedule used in this analysis was as follows: 95% A and 5% B, then the linear gradient increased to 35% B and held for 5 min, followed by a linear gradient of 42% B in 30 minutes. After that, the eluent was changed to the initial composition, consisting of a linear gradient of 95% A and 5% B for 5 minutes.

Measurements were performed in the wavelength range of 200-600 nm.

The equilibrium curve for the identified compounds was expressed in mg/L.

3.Results and discussions

Once the retention time was established, calibration lines were drawn for each analyzed standard using the series of solutions of known concentrations.

The chromatograms of the analyzed samples are shown in figure 1.

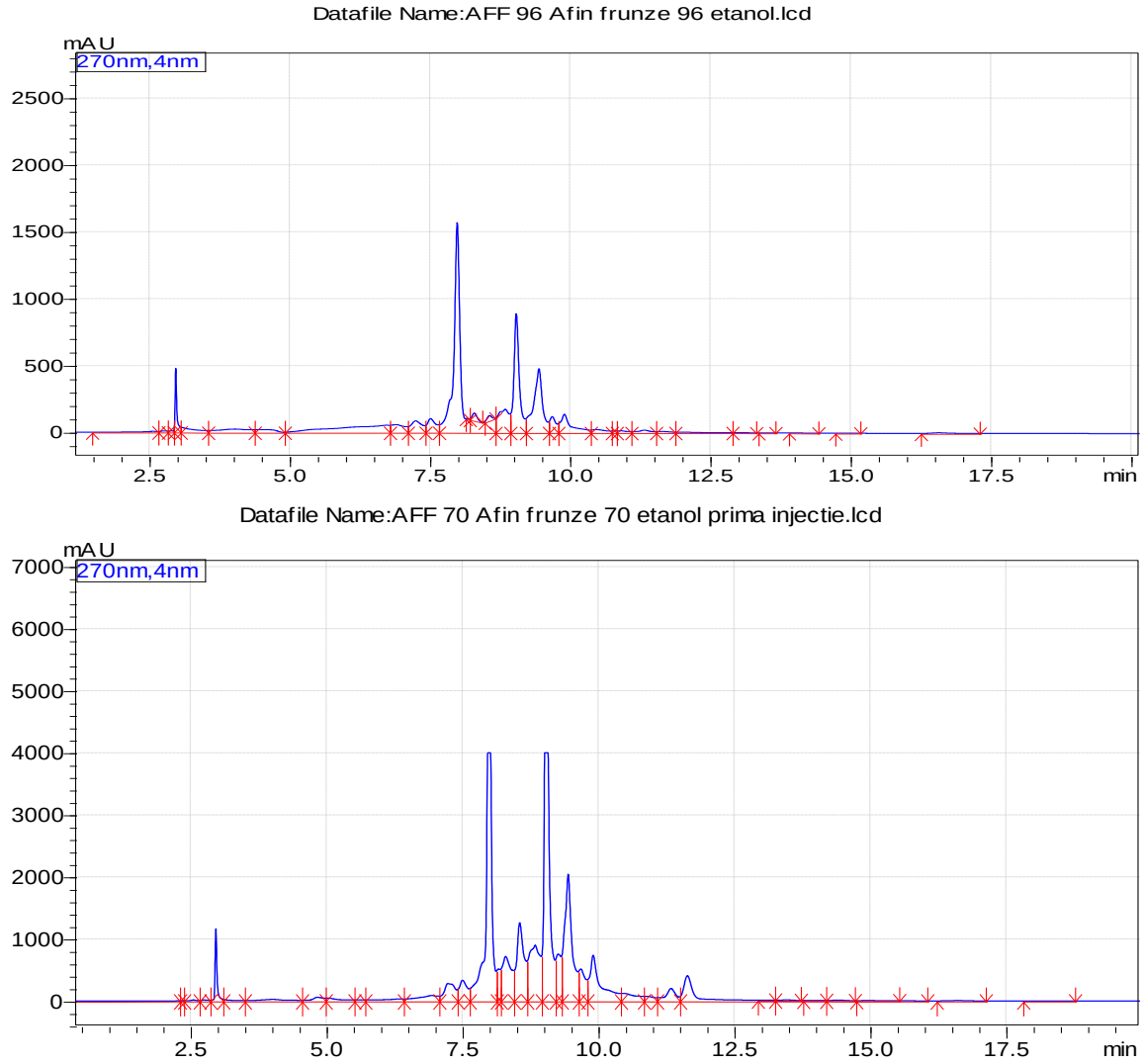

Figure 1. Sample chromatograms

Table 1. The content of compounds with an antioxidant character in the investigated plant extracts

Antioxidant compounds	**Blueberry hydroalcoholic extracts**	
	96% ethanol [mg/l]	**70% ethanol [mg/l]**
Kaempferol	-	-
Quercetin	-	-
Rutin	-	-
Ascorbic acid	39,98	110,80
Caffeic acid	487,13	3258,73
Vanillic acid	79,78	373,76
Syringic acid	113,38	599,03
p – cumaric acid	77,57	237,20
Catechin	148,79	1293,04
Rosmarinic acid	14,48	32,93
Ferulic acid	7,25	10,84

The calibration lines of the standards:

a) kaempferol, the correlation coefficient $r^2 = 0.9942$;
b) quercetin, the correlation coefficient $r^2 = 0.9995$;
c) rutin, the correlation coefficient $r^2 = 0.9766$;
d) ascorbic acid, the correlation coefficient $r^2 = 0,9948$;
e) caffeic acid, the correlation coefficient $r^2 = 0,9964$;
f) vanillic acid, the correlation coefficient $r^2 = 0,9946$;
g) syringic acid, the correlation coefficient $r^2 = 0,9932$;
h) p-coumaric acid, the correlation coefficient $r^2 = 0,9965$;
i) catechin, the correlation coefficient $r^2 = 0,9925$;
j) rosmarinic acid, the correlation coefficient $r^2 = 0,9918$;
k) ferulic acid, the correlation coefficient $r^2 = 0,9935$.

The equations that describe the calibration lines are given by the relations:

a) y = 86551x +11018.
b) y = 87093x + 49.623 .
c) y = 43364x +2670,8.
d) y = 240458x +33383.
e) y = 106645x-2526,3
f) y = 90926x - 31497.
g) y = 53835x - 14960.
h) y = 189328x – 74775
i) y = 8765,5x + 644662.
j) y = 97771x – 1E+06
k) y = 2,20E + 0,6x – 1,56E + 07

From the data contained in table 1, the existence of a difference in the standards content of the studied extracts can be found. This difference is given only by the solvent from which the extracts were obtained.

The results presented in table 1 show that the extracts obtained by static extraction using 70% ethanol as solvent have the highest content in the analyzed compounds.

It was found that the hydroalcoholic 70% ethanol extract has the highest content of caffeic acid, and the lowest content is ferulic acid in the 96% hydroalcoholic extract.

The three flavonoids (kampherol, quercetin, rutin) were not present in the two types of hydroalcoholic extracts analyzed, instead the other analyzed compounds with an antioxidant character are found in significant quantities.

4. Conclusions

Knowing the chemical composition of the blueberry is necessary, because the determined substances are responsible for the antioxidant and antibacterial properties of the shrub.

In the hydroalcoholic extracts analyzed, high-performance liquid chromatography compounds with an antioxidant character were quantitatively determined - ascorbic, caffeic, vanillic, syringic, p-coumaric, rosmarinic, ferulic acids, and coumarin.

As a result of the analysis carried out, it can be stated that the 70% ethanol hydroalcoholic extracts indicate significantly higher amounts of antioxidant compounds compared to the 90% ethanol hydroalcoholic extracts obtained in the same way.

Regardless of the used extract, kaempferol, quercetin and rutin were not found in the analyzed samples.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. Thomas, C., *Approaches and Rationale for the Design of Synthetic Antioxidants as therapeutic agent,* in Handbook of Synthetic Antioxidants, Ed. L. Parcker, E. Cadenas, Marcel Dekker Inc., New York, 1997, pp. 3-5
2. Prior, R. L., Cao, G., In vivo total antioxidant capacity: comparison of different analytical methods, *Free Radical Biololgy & Medicine* **1999**, *27*(11-12), 1173-1181, doi:10.1016/S0891-5849(99)00203-8
3. Gey, K. F., Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease, *British Medical Bulletin* **1993**, *49*(3), 679-699, doi:10.1093/oxfordjournals.bmb.a072640
4. Hennekens, C. H., Antioxidant vitamins and cancer, *The American Journal of Medicine* **1994** *97*(3A), 2S-4S, doi:10.1016/0002-9343(94)90291-7
5. Kaur, C., Harish, C. K., Antioxidant activity and total phenolic content of some Asian vegetables, *International Journal of Food Science and Technology* **2002**, *37*(2), 153–161, doi:10.1046/j.1365-2621.2002.00552.x
6. Pineda, A.D., Salucci, M., Lazaro, R., Maiani, G., Ferro – Luzzi, A., Capacidad antioxidante y potencial de sinergismo entre los principales constituyentes antioxidantes de algunos alimentos, *Revista cubana alimentacion y nutricion* **1999**, *13*(2), 104-11
7. Duță, V., *Tratamente naturiste*, Editura Ștefan, București, 2007
8. ****Farmacopeea Română Ediția a -X – a*, Editura Medicală, Bucureşti, 1994, 309-718
9. Condrat, D., *Contribuţii la obţinerea unor extracte din plante (phanerogame) cu efect antioxidant*, Teză de doctorat, Seria 4, Nr.36, Editura Politehnica, Timișoara, 2010