Evaluation of Antioxidant Capacities and Phenols Composition of Wild and Cultivated Berries

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The study presents original results concerning analytical characterization of alcoholic extracts of four wild berries (blueberries, blackberries, red currants and raspberries) and two cultivated berries (black cherries and strawberries). Total and some individual phenols concentrations were determined using molecular absorption spectrometry (modified Folin Ciocalteu method) and HPLC-DAD technique. The measurement of lipid-soluble antioxidant capacity (ACL) was done by photochemiluminescence method using trolox as standard.The highest total phenolic content was registered in blueberries (543.5 mg/100g f.w.), while the lowest was found in raspberries (344.5 mg/100g f.w.).HPLC – DAD analysis indicated the presence of six individual polyphenolic compounds in different concentrations: gallic acid (62.664 - 178.821 mg/100 g f.w); chlorogenic acid (30.152 - 243.923 mg/100g f.w.); 3-O-methyl-gallic acid (2.035 - 4.907 mg/100 g f.w.); caffeic acid (0.401 - 5.664 mg/100 g f.w.); p-coumaric acid (4.252 - 10.806 mg/100 g f.w.) and cinnamic acid (0.665-0.958 mg/100 g f.w.). ACL results showed values between 54.00 and 1152 µmols trolox/100 g f.w.

Keywords: berries, total phenols, antioxidant capacity of soluble lipids, HPLC-DAD chromatography

It is well known that the consumption of controlled diets especially high in berries, such as blueberries, blackberries, red currants, raspberries, strawberries and black cherries, increases significantly the human body capacity in the maintenance of health and protection from coronary heart disease, cancer, combating of diabetes, degenerative diseases and protects human organisms against oxidative stress induced by free radical species [1-3].

The antioxidant capacity is mainly given by the content of phenolic compounds but also by the other compounds like minerals, vitamins, phenolic acids, tannins and flavonoids. Different phenolic compounds occur in different berries fruits and their relative concentrations and proportions may also vary. These differences affect the total antioxidant capacity and also influence the therapeutically effects [4,5]. Greater knowledge regarding the cultivars or provenance of berries fruits may influence their market value and also could provide healthier food diet products. This information could be important for pharmaceutical and food industry to decide which berries fruits products to use up and for consumers to decide which products to purchase.

The reduction reactions of free radicals is directly related with the antioxidant compounds contents and reflect the protective capacity for human body cell and tissue protection against oxidative stress and also against other biological effects associated with chronic diseases [6, 7].

Large number of studies suggest that there is a connection between regular polyphenols consumption and reduced risk of developing different types of cancer. Anticancer effect of polyphenols is given by their antioxidant properties which in fact protect DNA (RNA) nucleic acids against oxidative damages inflicted by carcinogenic substances [8, 9].

The main phenols in wild berries are anthocyanins, flavonols, hydroxycinnamic acid, ellagic acid, ellagic tannins (*Rubus* and *Fragaria* genus) and proanthocyanins which mean that berries have bioactive properties with an important capacity against oxidative stress of living cells.

Polyphenolic content of vegetable materials represents a significant interest due to their antioxidant potential and due to anti-inflammatory properties present at cell level [10]. Therefore, it is important to study there compounds activity and also to establish the optimal quantity which can be consumed best. Comparative characterization of the phenolic of wild berries (blueberries, blackberries, red currants, raspberries) versus cultivated berries (black cherries and strawberries) alcoholic extracts could give important information.

The aim of our study was to determine phenolic compounds through various methods for four alcoholic extracts of wild berries (blueberries, blackberries, red currants and raspberries) and two alcoholic extracts of cultivated berries (black cherries and strawberries) and to evaluate their antioxidant capacity of lipids (ACL). The total content of phenolic compounds was measured using the Folin-Ciocalteu method, the phenolic profile of studied extracts was established using high performance liquid chromatography HPLC-DAD and the lipid-soluble antioxidant capacity (ACL) was evaluated by photo-chemiluminiscence method (PLC) using trolox as standard.

Experimental part

Fruit samples

Wild berries (blueberries, blackberries, red currants, and raspberries) collected from local forests and cultivated berries (black cherries and strawberries) brought from local producers from Dobrogea County, Romania have been analysed.

Chemicals

All used reagents were of analytical reagent grade. Gallic acid was purchased from Fluka (Buchs, Switzerland) and Folin - Ciocalteu reagent from Merck (Darmstadt, Germany). Gallic acid (standard phenolic compound) used as 10^{-2} mol×L^{-1} solution was prepared by dissolving 376 mg of gallic acid in 100 mL of ethanol. Folin - Ciocalteu reagent was diluted with distilled water as 1:10 (V:V).

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Sample extracts

Extractions were achieved by maceration of 10 g of fresh berries samples in 100 mL ethylic alcohol 50% (V:V) at room temperature and protected from light during six days. All samples were filtered and analysed.

Determinations and equipments

Total phenolic contents (TPC) were determined according to the Folin-Ciocalteu method [10-14]. Total concentrations of phenolic in extracts were measured by molecular absorption spectrometry according to a colorimetric reduction of a phosphowolframate-phosphomolibdate complex to blue products by soluble phenolic compounds, in sodium carbonate media. Spectrometric measurements were carried out using a UV-Vis Jasco V550 scanning spectrophotometer.

The absorbance of the coloured complex was measured at 675 nm. The absorbance relative to a gallic acid standard curve was measured and results are expressed as gallic acid equivalents (mg GAE/L). All samples were performed in triplicate and the mean value was reported.

The total phenols content of berries was expressed as mg of gallic acid equivalents per 100 gram of fresh weight (mg GAE/100g f.w.). A standard solution of gallic acid prepared as previously mentioned was used for calibration curve.

In a series of 50 mL volumetric flasks volumes of 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 and 3.5 mL of gallic acid standard solution were introduced and were added 1 mL of Folin-Ciocalteu-reagent 1:10 (V:V) and 1 mL of 20% (w/v) aqueous Na_2CO_3; after 10 min the volume was made up to 50 mL with distilled water. After another 30 min. of incubation at 25°C the absorbance was measured at 675 nm. The calibration curve was linear in the range of 37.5 – 262.5 mg GAE/L (R^2 =0.9928) with a standard error of 0.334711 (fig.1).

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

The resulted extractive solution was analysed by an adapted USP30 HPLC method [9] used for separation, identification and quantification of the phenolic compounds as previously described [10, 14]. The identification and quantitative determination of phenolic compounds was performed using HPLC system (Agilent 1200) with 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 flow rate was 1.5 mL/min, the injection volume was 20 µL and the analysis time took 22 minutes. Quantification of phenolic acids was performed using absorbance measurements at 310 nm and 35°C. The retention times and spectra obtained by using diode array detector (DAD) were compared to available authentic standards.

A mixture of standard solutions in 70% methanol was used having the following concentrations:

E – resveratrol = 37 mg/mL, Z – resveratrol = 0.22 mg/L, caffeic acid = 0.36 mg/mL, chlorogenic acid = 0.37 mg/mL, cinnamic acid = 0.58 mg/mL, vanillin = 0.42 mg/mL, gallic acid = 0.39 mg/mL, ferulic acid = 0.48 mg/mL, 3-O-methylgalic acid = 0.34 mg/mL, ellagic acid = 0.43 mg/mL, *p*-coumaric acid = 0.51 mg/mL.

First, the retention times of standard solutions have been determined (table 1).

The lipid-soluble antioxidant capacity (ACL) was measured by photochemiluminescence method (PLC) using the reagents provided in the ALC kit as per the manufacture's protocol. The ACL assay was performed using Photochem® instrument (Analytic Jena, Germany) against the superoxide anion radicals from luminol, as photosensitizer, upon the UV light [15, 16]. In principle, the lipid-soluble antioxidants from the samples partially eliminate the free radicals and the residual radicals and react with luminol to produce luminescence. The measuring signal (volts) produced by luminescence was traced for specified time duration (120 s).

For the calibration curve, the standard reagent kit, Analytik Jena Standard was used: R1 (dilution solvent - Merck ethanol), R2 (buffer reagent), R3 (photosensitizer), R4 (standard solution). The calibration curve was plotted using standard solutions containing 1.0, 2.0, 3.0 and 4.0 nmoles Trolox (R4, Hoffman, La Roche, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed as µmoles equivalent Trolox/g fresh weight. Measurements were done using 5 µL volumes of each undiluted sample and 5µ L volumes of diluted sample (1:25). ACL value was calculated from the area under the curve at different concentrations. Three individual measurements were performed and the mean value was reported.

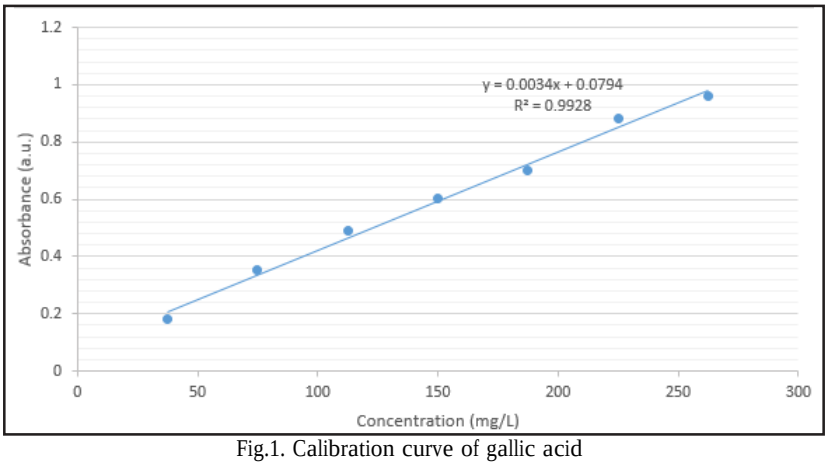


Fig.1. Calibration curve of gallic acid

Table 1

THE RETENTION TIME OF STANDARDS

Standard	Retention time ± SD*, min.
gallic acid	0.990 ± 0.025
3-O-methylgallic acid	2.606± 0.008
chlorogenic acid	3.501 ± 0.015
caffeic acid	4.598 ± 0.036
vanillin	6.919 ± 0.051
p-coumaric acid	7.187± 0.019
feluric acid	8.565± 0.058
E - resveratrol	14.467 ± 0.017
ellagic acid	15.303± 0.027
Z - resveratrol	15.751 ± 0.058
cinnamic acid	15.867 ± 0.007

(*standard deviation (SD) for six injections)

Results and discussions

Total phenolic content (TPC)

The results of total phenolic content determined for four alcoholic extracts of wild berries versus two alcoholic extract of cultivated berries are presented in table 2.

The highest result of total phenolic content of was registered for blueberries (543.5 mg/100g f.w.), followed by black cherries, red currants, blackberries and strawberries. The lowest, but still rich, total phenolic content was obtained for raspberries (344.5 mg/100g f.w.). Regarding the total phenolic content of wild berries versus cultivated berries we noticed small difference between the TPC concentration found in black cherries extracts and in blueberries. This fact indicates that wild berries as well as cultivated berries are valuable sources of phenolic compounds important for their therapeutically benefits.

Table 2

TOTAL PHENOLIC CONTENT FROM WILD AND CULTIVATED BERRIES SAMPLES

No.	Sample	Concentration (mg GAE/100g f.w.)
1	blueberries	543.5
2	raspberries	344.5
3	blackberries	425.0
4	red currants	432.3
5	black cherries	518.5
6	strawberries	378.2

Phenolic compounds separation, identification and quantification

HPLC – DAD analysis in the ethanolic extracts of studied berries consisted in the identification and quantification of six individual phenolic compounds in different concentrations.

Table 3 presents the individual phenols concentrations determined by HPLC - DAD expressed in mg/100 g f.w. and percentages (%).

In blueberries extract there were identified four phenolic acids. Chlorogenic acid and gallic acid have registered high concentrations (175.894 mg/100g f.w., respectively 88.307 mg/100 g f.w.). On contrary, small quantities of 3-O-methyl-gallic acid and caffeic acid were measured in blueberries. A similar situation was observed in raspberries extract, where were determined four individual phenolic acids, as gallic acid (178.821 mg/100 g f.w.) and chlorogenic acid (34.916 mg/100g f.w.) in high concentrations while *p*-coumaric acid and 3-O-methyl-gallic acid were in small concentrations.

The strawberries extract had five individual polyphenolic compounds: chlorogenic acid (243.923 mg/100g f.w.) and gallic acid (62.664 mg/100g f.w.) in high amount, whereas the caffeic acid, 3-O-methyl-gallic acid and cinnamic acid were in small amounts. Five individual polyphenolic compounds there were recorded also in blackberries extract: gallic acid (168.782 mg/100g f.w.), chlorogenic acid (30.152 mg/100g f.w.) in concentrations higher than caffeic acid *p*-coumaric acid and 3-O-methyl-gallic acid. In the case of red currant extract there were identified and quantified four individual phenolic compounds: gallic acid (169.131 mg/100g f.w.) and chlorogenic acid (30.320 mg/100g f.w.) in higher amounts than 3-O-methyl-gallic acid and cinnamic acid. The results of HPLC-DAD analyses performed to black cherries extract indicated the presence of only one individual polyphenolic compound: gallic acid; that means that black cherries may contain different phenolic compounds other than the used standards. The highest gallic acid concentration was found in black cherries extract, followed by red currant and blackberries, while the lowest gallic acid concentration was registered for strawberries (62.664 mg/100g f.w.).

Table 3

CONTENTS (mg/100g F.W.) AND WEIGHT PERCENTAGES (WT%) OF INDIVIDUAL PHENOLIC COMPOUNDS OF TESTED BERRIES DETERMINED BY HPLC-DAD

Phenolic compound	Blueberries		Raspberries		Blackberries		Red currants		Black Cherries		Strawberries	
	mg/ 100g F.W.	wt%	mg/ 100g F.W.	wt%	mg/ 100g F.W.	wt%	mg/ 100g F.W.	wt%	mg/ 100g F.W.	wt%	mg/ 100g F.W.	wt%
Chlorogenic acid	175.894	65.321	34.916	15.227	30.152	14.332	30.320	14.824	-	-	243.923	77.380
Caffeic acid	0.401	0.149	-	-	2.286	1.087	-	-	-	-	5.665	1.780
Gallic acid	88.307	32.794	178.821	77.985	168.782	80.228	169.131	82.690	447.514	100	62.664	19.880
3-O-Methyl -gallic acid	4.674	1.736	4.798	2.092	4.907	2.332	4.418	2.161	-	-	2.035	0.646
Cinnamic acid	-	-	-	-	-	-	0.661	0.323	-	-	0.958	0.305
p-coumaric acid	-	-	10.806	4.712	4.252	2.021	-	-	-	-	-	-
Total Phenols	269.276	100	229.341	100	210.378	100	204.536	100	447.514	100	315.245	100

No.	Sample	Total phenols identified and quantified wt %	The major phenolic compound identified and quantified	
			phenolic compound	wt%
1	blueberries	49.54	chlorogenic acid	32.36
2	raspberries	66.57	gallic acid	51.90
3	blackberries	49.50	gallic acid	39.71
4	red currants	47.31	gallic acid	39.12
5	black cherries	86.31	gallic acid	86.31
6	strawberries	83.35	chlorogenic acid	64.50

Table 4
THE WEIGHT PERCENTAGES (%) OF TOTAL PHENOLS AND OF MAJOR PHENOLIC COMPOUND IDENTIFIED IN TESTED ALCOHOLIC BERRIES EXTRACTS DETERMINED BY HPLC-DAD REPORTED TO TPC VALUES

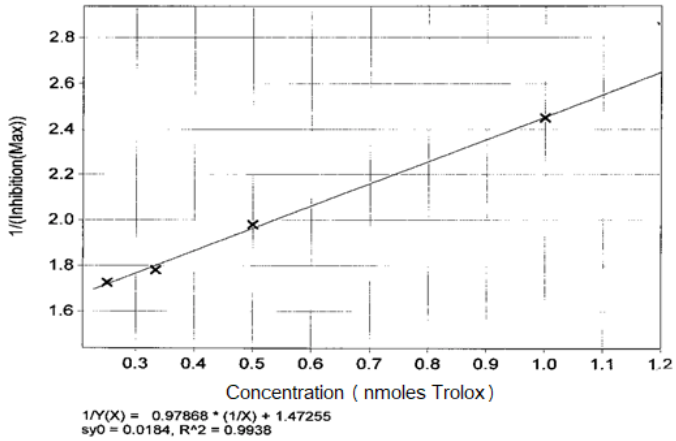


Fig. 2. Calibration curve for Trolox in the calculation of Trolox equivalents for ACL measurements (generated by PCLsoft®)

In most cases, the available authentic standards used have allowed the determination of the phenolic compounds present in the analyzed samples.

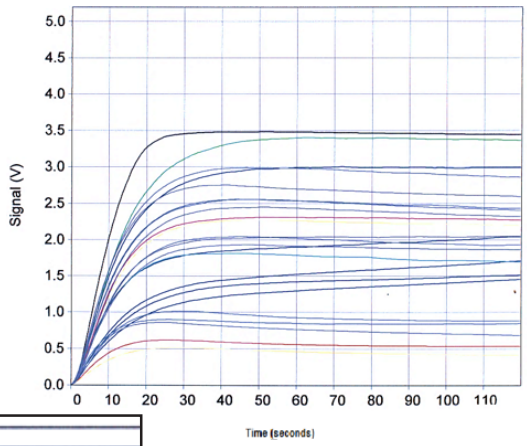
Table 4 shows the concentrations found by HPLC-DAD of total phenolic compounds and major compound in case of each sample being reported to TPC values.

It is clearly that the gallic acid is the main phenolic compound in the majority of the analyzed samples. It was found that the higher percentages are those of gallic acid in black cherries (86.31%) and of chlorogenic acid in strawberries (64.50%). The difference between values of total phenolic compounds (TPC) and the identified and quantified individual phenolic compounds by HPLC-DAD is due to the presence of other phenolic acids than the available authentic standards used.

The antioxidant capacity (ACL) of the lipophilic antioxidants (tocopherols, tocotrienols and carotenoids) from the alcoholic extracts of berries was evaluated by photochemiluminiscence method.

The calibration curve was constructed by measuring a series of standard solutions containing 1.0, 2.0, 3.0 and 4.0 nmoles Trolox (suitable for 5–30µL R4) (fig. 2).

The shapes of lipophilic antioxidants content (nmols trolox/mg f.w.) of studied alcoholic berries samples were measured using Photochem instrument and are presented in figure 3.



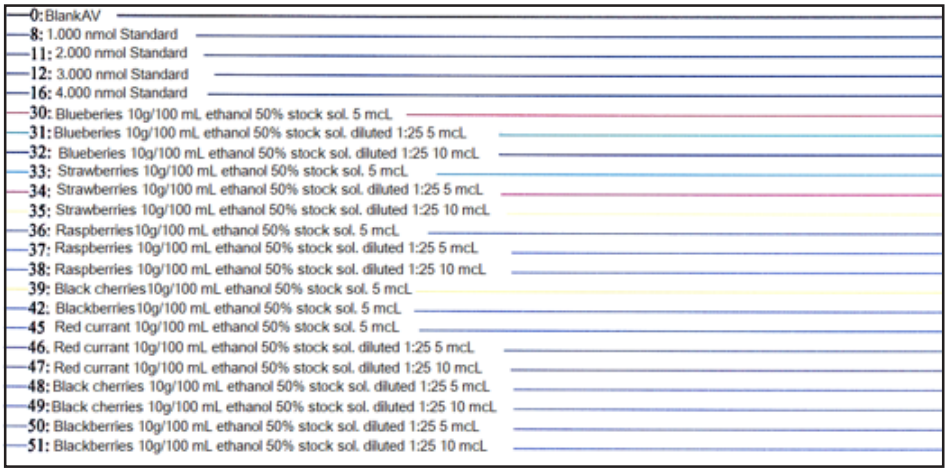


Fig.3. Antioxidant capacity (nmoles trolox /mg fresh weight) for undiluted sample and diluted samples 1:25 measured using Photochem instrument

Because of the high concentration of phenolic compounds present in undiluted berries extract a dilution of 1:25 was done for all samples. Measurements were performed for 5 µL volumes of each undiluted sample and 5 mL volumes of diluted sample (1:25) (fig. 3).

The highest antioxidant capacity of lipid-soluble phenolic compounds was found in black cherries extract (1152 µmoles trolox/100 g f.w.), followed by raspberries extract, strawberries extract and red currants extract (table 5).

Table 5
ACL VALUES REGISTERED FOR WILD AND CULTIVATED BERRIES

No.	Sample	ACL (nmoles Trolox/mL)	ACL (µmoles Trolox /g f.w.)
1.	blueberries	6000	54.00
2.	raspberries	104250	938.25
3.	blackberries	8082	72.74
4.	red currants	7150	643.5
5.	black cherries	128000	1152
6.	strawberries	74608	671.47

The lowest antioxidant capacity of lipid-soluble polyphenolic compounds was found in blueberries (54.00 µmoles trolox/100 g f.w.).

Regarding the ACL values for wild versus cultivated berries we notice that black cherries extract present the highest ACL compared to all tested wild berries extracts.

The obtained results are similar with previous published researches concerning the lipid-soluble phenolic compounds ACL in berries [16-20]. The measured analytes concentrations (total phenols, individual polyphenolic acids) and antioxidant capacity of the studied berries alcoholic extracts indicate important ACL values that recommend them as a proper antioxidant source which can be used for special diets and also for its health benefits.

Conclusions

Characterization of four wild berries (blueberries, blackberries, red currants and raspberries) and two cultivated berries (black cherries and strawberries) alcoholic extracts was done.

The highest total phenolic content determined using molecular absorption spectrometry (modified Folin Ciocalteu method) was registered in blueberries and black cherries, while the lowest was found in raspberries.

HPLC - DAD analysis indicated the presence of six individual polyphenolic compounds in different concentrations: gallic acid exists in all studied berries in variable concentrations (62.664 - 178.821 mg/100 g f.w); chlorogenic acid (30.152 - 243.923 mg/100g f.w.); 3-O-methyl-gallic acid (2.035 - 4.907 mg/100 g f.w.); caffeic acid (0.401 - 5.664 mg/100 g f.w.); *p*-coumaric acid (4.252 - 10.806 mg/100 g f.w.) and cinnamic acid (0.661-0.958 mg/100 g f.w.).

Lipid-soluble antioxidant capacity (ACL), determined by the photochemiluminescence method showed values between 54.00 and 1152 µmols trolox/100 g f.w.

Considering the phenolic compounds concentrations found in wild berries (blueberries, blackberries, red currants and raspberries) versus cultivated berries (black cherries and strawberries) we noticed that the highest concentration of total phenolic compounds and ACL values were obtained for black cherries, that recommends them as the highest source of antioxidants from all the tested berries.

The obtained results indicate that all studied berries extracts are rich in antioxidant compounds and can be used in diets or to get health benefits.

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