

# Antioxidant Activity of *Brassica Oleracea* L., *Allium Cepa* L. and *Beta Vulgaris* L. Extracts

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*Extracts of three vegetables very used in Romanian cuisine, Brassica oleracea L., Allium cepa L. and Beta vulgaris L. were obtained by continuous and ultrasonical extraction methods. Antioxidant activity of extracts was tested using a new flow injection analysis method with chemiluminescence detection (FIA-CL) developed by our group for rapid total antioxidant capacity (TAC) determination. DPPH free radical scavenging method was also used for antioxidant activity. Total phenols (TP), total flavonoids content and anthocyanins were determined spectrophotometrically for obtained extracts. This study illustrates that phenols have a considerable contribution to the antioxidant activity of analyzed vegetables.*

*Keywords: antioxidant activity, DPPH radical scavenging, anthocyanins, flavonoids, polyphenols, chemiluminescence*

Negative effects of pollution on surrounding environment are more and more obvious lately. One of the main consequence is the production of free radicals (FR) that have damaging effects on all living organisms. Humans have complex antioxidant defence systems, but they are not perfect and oxidative damage will occur; the body itself produces free radicals as it processes food.

An important class of bioactive compounds is represented by polyphenols (flavonoids, anthocyanins etc), that, through their action on FR, contribute to prevention of some chronic diseases such as obesity or diabetes [1, 2]; cardiovascular disease and cancer are thought to be particularly the results of oxidative stress, which can lead to damage of the larger biomolecules, such as DNA, lipids, and proteins [3, 4]. Thus, these compounds participate in maintaining or improving health.

Preventing some diseases is a better tactic than their treatment and functional foods like vegetables or fruits a rich source of phytochemicals with antioxidant activity may provide desirable health benefits beyond their basic nutrients content (vitamins, minerals, fibers) [5]. A synergistic effect may be more effective than separately components.

*Brassica oleracea* L. var. *capitata* (*Brassicaceae*, red cabbage), *Allium cepa* L. (*Alliaceae*, red or purple onion) and *Beta vulgaris* L. (*Amaranthaceae*, red beetroot), vegetables very used in Romanian cuisine studied in this work, are on the list of the healthiest foods. All three contain bioactive compounds with significant antioxidant activity: quercetin (red onion) [6, 7], cyanidins (red cabbage) [8, 9], betalains, (beetroot) [10-12]. It was demonstrated that the onion antioxidant effect is more potent than vitamin E [13-15].

In this paper, extracts of red cabbage, red onion and beetroot obtained by Soxhlet and ultrasonically assisted extraction, were tested for their antioxidant activity using a new flow injection analysis with chemiluminescence detection (FIA-CL) and DPPH free radical scavenging methods. Total phenols (TP), total flavonoids and anthocyanins content were also determined spectrophotometrically.

## Experimental part

### Materials and methods

#### Chemicals

Ethanol (S.C. P.A.M. Corporation S.R.L., Romania), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma, Germany), Folin-Ciocalteu reagent (Scharlau, Spain), sodium carbonate (Merck, Germany), gallic acid monohydrate (Riedel-deHaën, China), aluminum chloride hexahydrate (Scharlau, Spain), potassium acetate (Scharlau, Spain), rutin trihydrate (Wako, Japan), cyanin chloride (Sigma, USA), boric acid, cobalt (II) chloride hexahydrate (Reactivul, Romania), hydrogen peroxide 30% (Chimopar, Romania), ethylenediaminetetraacetic acid (EDTA) disodium salt (Loba Chemie, Germany), luminol (Fluka BioChemie, Slovakia), sodium hydroxide (Chemapol, Romania), methanol and acetone (Chemical Company, Romania)

#### Plant materials

Red cabbage, red onion and beetroot (all Romanian products) were purchased from market and used in experiments as fresh vegetables.

#### Plant extracts preparation

Fresh plant material chopped in small pieces was extracted for 2 h in a Soxhlet apparatus with ethanol 96 % (vegetable material /solvent ratio 1:10).

Ultrasonically assisted extraction was carried out using a simple ultrasonic cleaning bath (Langford Sonomatic, 33 kHz, 100 W power), extraction time 2 h, the same extraction solvent and the same ratio vegetable material/solvent were used.

Alcoholic extracts obtained were filtered and alcohol was evaporated under vacuum using a rotary evaporator. Extracts were dried in an oven for 1 hour at 100°C.

Details regarding amounts of dry extracts obtained per 100 g fresh vegetable material are given in results and discussions section.

#### Total Antioxidant Capacity (TAC) determination

TAC determination of extracts has been made according to the FIA-CL method described in our previous paper [16].

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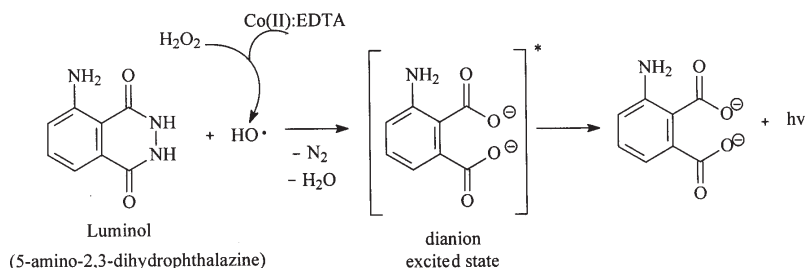


Fig. 1. CL reaction of luminol with hydrogen peroxide in the presence of Co(II)

This method combines the advantages of flow injection method with the sensitive detection by chemiluminescence [16, 17].

The proposed method is based on the reaction of hydroxyl radicals (generated by a Fenton-type reaction of  $H_2O_2$  with Co(II) ions released in a very low concentration from Co(II)/EDTA complex) with luminol. The reaction with luminol leads to diazaquinone luminol form which reacts with  $O_2$  generating the hydroperoxide carbanion, that rearranges to a transient endoperoxide of luminol. This compound releases nitrogen and leads to 3-aminophthalate dianion in an electronically excited state. The excited dianion emits a photon, which is observed as visible light, producing a chemiluminescence blue radiation (fig. 1).

In the absence of an antioxidant, a relatively constant CL signal of certain intensity is registered (as a plateau), while in the presence of an antioxidant in reaction medium the CL signal decreases [16, 18-20].

For TAC determination, 0.1 g dry extract dissolved in 5 mL ethanol 80% v/v in EDTA  $2 \times 10^{-4}$  M solution was moved to Falcon tubes, added solvent to 10 mL (ethanol 80% (v/v) in EDTA  $2 \times 10^{-4}$  M) and sonicated 1 min before analysis. Calibration curve for gallic acid (for concentrations between 2.5–300  $\mu$ M) was drawn. The TAC values obtained are given as mg equivalents gallic acid /100 g fresh plant weigh.

#### Free radical scavenging activity

For free radical scavenging activity determination of extracts, DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used [21]. This method is based on the reduction of purple DPPH, where the radical centre is situated on nitrogen atom, by an antioxidant (A-H) [22-24]. Yellow coloured DPPH-H (1,1-diphenyl-2-picrylhydrazine) molecule resulted, shows maximum absorption at 520 nm (fig. 2).

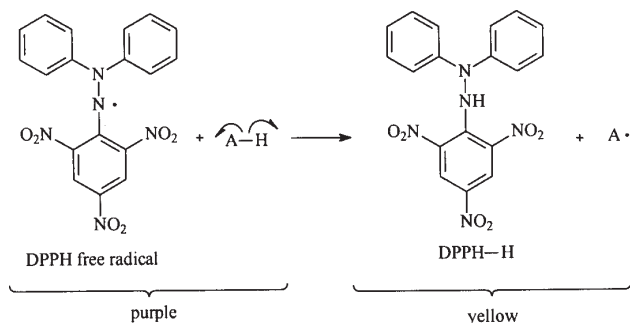


Fig. 2. Principle of DPPH method

To 1 mL extract (obtained from 0.01 g dry extract dissolved in 10 mL methanol 50% v/v) was added 1 mL DPPH (0.0135 mM in methanol 50% v/v) solution. After 30 min. in dark at room temperature, absorbance was measured at 520 nm against methanol used as blank. Standard for comparison was rutin (0.1 mg/mL in methanol 50%). Because red cabbage and red onion contain anthocyanins, compounds known as having strong antioxidant activity, cyanin was also tested for DPPH radical scavenge activity as another standard for comparison. The

absorbance of the samples was measured on a Single Beam Cole Palmer 1100 RS spectrophotometer.

The capacity of the scavenging free radicals was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = 1 - [A_{\text{sample}}/A_{\text{control}}] \times 100 \quad (1)$$

where:

$A_{\text{sample}}$  is the absorbance of sample/standard,  
 $A_{\text{control}}$  - the absorbance of the control (methanolic solution of DPPH).

#### Total Phenols (TP) content determination

TP content of plant extracts was determined colorimetrically according to the Folin-Ciocalteu method (FCM) [25]. Samples were prepared by mixing 0.5 mL of extract (10 mg dry extract dissolved in 10 mL methanol 50% v/v; red cabbage was diluted again 1:10 with methanol 50% v/v) with 5 mL aqueous solution of Folin-Ciocalteu (10% v/v) and 4 mL sodium bicarbonate solution (1 mol/L). Mixture reaction was allowed to stand at room temperature for 15 min. and absorbance was measured at 746 nm against blank (methanol 50% v/v). Calibration curve absorbance vs. concentration of gallic acid (50-150 mg/mL in methanol 50% v/v) was drawn. Equation curve was  $y = 0.0065x + 0.0225$  (where  $y$  = absorbance,  $x$  = concentration of extract) and  $r^2/n = 0.9990/5$  ( $r^2/n$  = correlation coefficient/number of determinations). The results were expressed as mg equivalents gallic acid /100g of fresh plant material.

#### Total flavonoids determination

The total flavonoids content was determined using the aluminum chloride colorimetric method [26]. 1 mL extract (5 mg/mL in methanol 50% v/v) was mixed with 0.2 mL of 10% w/v aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. After the 30 min at room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 430 nm. Concentrations between 20-80 mg/L of rutin standard solutions were used for calibration curve; equation curve was  $y = 0.0034x - 0.0015$  (where  $y$  = absorbance,  $x$  = concentration of extract) and  $r^2/n = 0.9984/6$  ( $r^2/n$  = correlation coefficient/number of determinations). The total flavonoids content was expressed as mg equivalents rutin /100g of fresh plant weight.

#### Total anthocyanins determination

Vanillin colorimetric assay was used for anthocyanins content determination [27]. 1 mL of extract, 2.5 mL of 1% (w/v) vanillin in methanol, and 2.5 mL of 9.0 N HCl in methanol, were mixed. After incubation at room temperature for 30 min, absorbance was measured at 500 nm. Cyanin chloride (0.1 mg/mL in methanol 50% v/v) was used as standard. The results are given in mg equivalents cyanin/100 g fresh plant material. Concentrations of cyanin extracts were measured using the relation (2).

**Table 1**  
THE YIELDS OF THE EXTRACTS OBTAINED BY THE TWO METHODS

Plant extract	Yield of dry extract (g/100 g of fresh plant material)	
	Continuous extraction	Ultrasounds extraction
red cabbage	5.2	4.1
red onion	7.4	3.1
beetroot	1.7	2.3

**Table 2**  
TOTAL ANTIOXIDANT CAPACITY (TAC) OF THE EXTRACTS

Plant extract	TAC* (mg equivalents gallic acid /100g fresh plant)	
	Continuous extraction	Ultrasounds extraction
red cabbage	379	586
red onion	109	46.7
beetroot	37.5	35.5

\*Values are expressed as mean of measurements ( $n = 2$ ).

**Table 3**  
DECREASE OF DPPH ABSORBANCE (100%) IN THE PRESENCE OF PLANT EXTRACTS

Vegetable Extract	Sample concentration mg/mL		DPPH absorbance reduction* (%)	
	Continuous extraction	Ultrasounds extraction	Continuous extraction	Ultrasounds extraction
red cabbage	0.1	0.1	55.4	77.4
red onion	1.0	1.0	76.8	76.5
beetroot	1.0	1.0	58.2	24.4
rutin		0.1		88.7
cyanin		0.1		63.3

\*Values are expressed as mean of measurements ( $n = 2$ ).

$$c_{\text{sample}} = (A_{\text{sample}} \times c_{\text{standard}}) / A_{\text{standard}} \quad (2)$$

where:

- $c_{\text{sample}}$  - concentration of sample determined
- $A_{\text{sample}}$  - absorbance measured of sample against blank (methanol 50% v/v)
- $c_{\text{standard}}$  - concentration of standard (cyanin chloride)
- $A_{\text{standard}}$  - absorbance of standard (cyanin chloride) measured against blank (methanol 50% v/v)

## Results and discussions

The amounts of the dry extracts obtained per 100 g of fresh plant material are given in table 1.

The experimental data show that the highest quantity of extract (7.4 g dry extract/100 g of fresh plant material) was obtained for red onion extract, obtained by continuous extraction. For the other samples there are no significant differences between the amounts of extracts obtained by the two extraction methods.

### TAC determination

The TAC values of the analyzed extracts are shown in table 2.

It can be observed that the highest TAC values are for red cabbage, for both extraction methods. TAC values for analyzed extracts varied in the following order: red cabbage > red onion > beetroot. Literature data [28] also report red cabbage extract as having the highest antioxidant activity.

Results are also confirmed by DPPH radical scavenging analysis as can be seen below.

### DPPH radical scavenging activity

Evaluation of antioxidant activity of red cabbage, red onion and beetroot extracts compared to rutin as standard through DPPH free radical scavenging (DPPH absorbance reduction %) can be seen in table 3. At different concentrations, extracts exhibited DPPH absorbance reduction between 24.4% and 77.4%.

The highest DPPH radical scavenging activity was found for red cabbage extract (77.4% for concentration of 0.1 mg/mL) obtained using ultrasounds, compared to rutin as standard (88.7% for 0.1 mg/mL).

When compared to cyanin scavenging activity, which at concentration of 0.1 mg/mL scavenge 63% of DPPH free radical, we can conclude that not only anthocyanins are responsible of total antioxidant activity of red cabbage; the other polyphenols also takes part to this activity.

Radical scavenging activity of the analyzed extracts decreases in the same order with TAC.

### TP content determination

TP values reported in figure 3 show that all extracts contain a considerable amount of phenols varying from 61.3 to 521 mg equivalent gallic acid/100 g fresh plant.

The highest values were obtained for red cabbage extract. TP content of analyzed extracts are in concordance with the TAC values, which demonstrate the contribution of polyphenols at total antioxidant activity of plants and also validate FIA-CL as an efficient method for antioxidant activity determination.

### Total flavonoids determination

As can be seen from figure 3, flavonoids content of analyzed extracts varied from 4.32 to 79.9 mg equivalents rutin/100 g fresh plant, when ultrasounds were used as extraction method and from 13.4 to 108 mg equivalents rutin/100 g fresh plant when continuous extraction was utilized. The flavonoids content is higher when continuous method is used for extraction. From all extracts, red cabbage extracts are rich in flavonoids, followed by red onion and beetroot extracts.

### Total anthocyanins determination

The values of anthocyanins content are between 7.29 and 164 mg equivalents cyanin/100 g fresh plant (fig. 3). Red onion and beetroot contain lower amount of anthocyanins compared to red cabbage.

A comparison between total phenols, flavonoids and anthocyanins contents is shown in figure 3.

As can be seen, red cabbage is richer in polyphenols compounds than red onion and beetroot.

Our results are in agreement with literature [24, 28-32] as follows: for red cabbage data reported per 100 g of fresh weight are TP = 197 mg equivalents gallic acid [29]

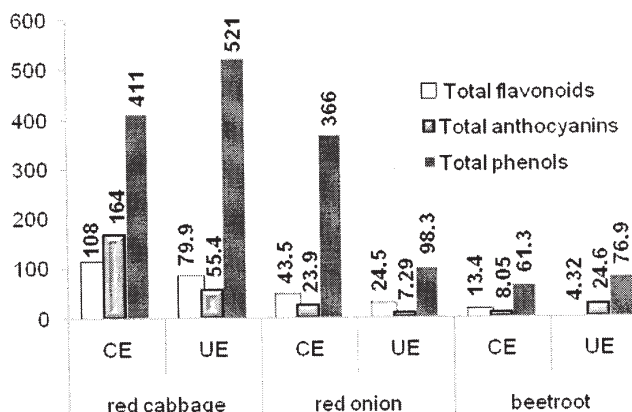


Fig. 3. Total phenols, flavonoids and anthocyanins content of plant extracts. TP: total phenols, CE: continuous extraction, UE: ultrasounds extraction (Values are expressed as mean of measurements (n = 2).)

anthocyanins = 72.9 mg cyanidin (HPLC) [F30]; for red onion others found TP = 422 mg gallic acid equivalents [31], total anthocyanins 7-21 mg [32], 6.2 mg cyanidin (HPLC) [30] per 100 g fresh weight; for beetroot, it was determined TP = 169 mg equivalents gallic acid/ 100 g fresh weight material [28].

The determined amounts of polyphenols, flavonoids and anthocyanins, with strong antioxidant activity, demonstrate that these vegetables can be used as natural antioxidant sources.

### Conclusions

Extracts obtained by continuous and ultrasonic extraction from red cabbage, red onion and beetroot were studied for their total antioxidant activity using a new FIA-CL method and DPPH free radical scavenging. Total phenols, flavonoids and anthocyanins were also determined.

Comparison between obtained data regarding antioxidant activity of the extracts confirms FIA-CL method as appropriate for this type of analysis, sensitive, rapid and efficient.

Our study also illustrate that phenols have a considerable contribution to the antioxidant activity of analyzed extracts and demonstrated that these vegetables can be used as rich natural antioxidant sources.

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