

ANTIOXIDANT ACTIVITY AND MINERAL CONTENT OF ROCKET (*ERUCA SATIVA*) PLANT FROM ITALIAN AND BULGARIAN ORIGINS

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ABSTRACT

Rocket plant (*Eruca sativa*) is a green leafy vegetable with significant levels of bioactive active components. Although, this plant is known in Bulgaria, scanty data concerning chemical composition of representatives with Bulgarian origin is available. The aim of this study was to evaluate and to compare the biological potential of Italian and Bulgarian rockets in terms of their antioxidant activity, total phenolic content and total hydroxycinnamic acid derivatives. The *micro-* and *macro elements* were evaluated as well. The highest content of total phenolic content was detected in Bulgarian rocket – 4.45 mg gallic acid equivalent per g dry weight, while in Italian samples dominated the total hydroxycinnamic acid derivatives – 1.52 mg chlorogenic acid equivalent per g dry weight. The antioxidant activity was not significantly different in both samples. The presence Co and Al was reported for the first time, as in our study their values were higher in a rocket from Italian origin (75.6 and 595 mg/kg). Italian rocket contained arsenic (0.07 mg/g) in comparison with Bulgarian samples where it was not found. The results revealed the sample with Italian origin as a significant better source of antioxidants with potential beneficial effects. Bulgarian rocket was evaluated with smaller amount of some metals as nickel, arsenic, chromium, manganese, aluminum and sodium, but the concentrations of Pb (0.58 mg/kg) and Cd (0.27 mg/kg) were higher than accepted levels by EU.

Keywords: antioxidant activity, micro- and macro elements, rocket salad, total phenolic compounds

INTRODUCTION

With the development of functional foods, the interest of scientists, consumers and industrialists in the raw plant materials rich in biologically active substances has increased. Moreover, consumers require more accurate information about food and drinks quality.

Several biologically active compounds in leafy vegetables were reported to possess beneficial health effects. Vegetables are considered as rich sources of nutrients such as carbohydrates, proteins, vitamins, calcium, iron and concentration of trace elements (Prakash and Pal, 1991; Jimoh and Oladiji, 2005). Most of them contain various pharmaceuticals agents and they are highly beneficial in treatment of various diseases. Some minerals as calcium, phosphorus, iron, magnesium, copper and potassium in vegetables maintain the acid/base balance in body tissues. They help for absorption of vitamins, proteins, fats and carbohydrates (Kalita, 2007; Hussain et al., 2010; Hussain et al., 2011).

Phenolic compounds were reported to possess different bioactivities. They are considered as antioxidants, antibacterial, antimutagenics, anti-inflammatory and antiallergic agents. Such activities contribute significantly for the consumers' well-being. In point of fact, the consumption of foods rich in antioxidants prevents from diabetes, cancer, cardiovascular or neuronal diseases (Zhang et al., 2015).

Hydroxycinnamic acid derivatives (caffeic acid derivatives - CADs) are naturally occurring substances and they are considered as dietary phenolic compounds. The various biological activities like antioxidative, hepatoprotective, anxiolytic, insect repellent, antidiabetic and anticholesterolemic ones were investigated (Sharma, 2011).

Metals have an impact on human health. Some elements, such as Cu, Zn and Mn, are essential micronutrients with requirements that not exceed daily intake more than a few milligrams per day (Ajasa et al., 2004; Atlabachew et al., 2011). On the other hand, the presence of toxic metals in medicinal plants is harmful for the human health (WHO, 1998). Therefore, for consumer's safety, the World Health

Organization approves maximum permission levels in raw plant materials for some elements, such as cadmium (0.3 mg/kg), arsenic (1 mg/kg), and lead (10 mg/kg) (Carvalho et al., 1997).

Eruca sativa (*Brassica eruca* L.) is an edible annual plant, commonly known as rocket salad or arugula (rucola, rucoli, rugula, rugula, colewort, and roquette). It is a green leafy vegetable, member of Brassicaceae family that originates from Mediterranean countries. Rocket is much appreciated vegetable in Europe, North America, Argentina and South Africa. It has been cultivated in Central and Western Asia for seed oil production (Yaniv et al., 1998). This plant is consumed in several ways as a flavoring in salads, spice and in boiled or baked dishes (Kim et al., 2006). Several recipes provide the preparation of pureed, sauces and pesto from its leaves (Tripathi et al., 2017).

The researchers reported the nutrient composition and concentration of phytochemicals in this plant (Bouis, 1996). In this respects, the representatives from Brassicaceae family play significant role in human diet and they are rich sources of bioactive compounds such as glucosinolates, flavonoids, phenolic acids, fibers, vitamins and carotenoids (Martinez-Sanchez et al., 2006; Jin et al., 2009; Durazzo et al., 2013). The consumption of rocket plant increases constantly due to its low prices, easy cultivation, short harvest time, high mineral content and many health promoting substances (Kawashima and Valente-Soares, 2003; Esiyok et al., 2010). *E. sativa* was treated as a plant with powerful active components that might be effective in increasing human health and as a novel preventing cancer agent (Michael, 2011).

However, the quality and nutritional potential of rocket plant available on Bulgarian market are faintly investigated and controlled. The imported plants can be a potential source of disease or present a treat for consumers. Because of that, the screening of their bioactive compounds and mineral content in important for human health. The microelements content in rocket leaves were not detailed investigated, especially Bulgarian plants.

Therefore, the current study aims was to evaluate the bioactive compounds and mineral content in the rocket plants leaves (*E. sativa*) available on the Bulgarian

market and to compare the samples from Italian and Bulgarian origins in terms of antioxidant activity and mineral content.

MATERIAL AND METHODS

Plant material

Eruca sativa leaves from Italian and Bulgarian origin were purchased fresh from the local market in Plovdiv (Bulgaria). The plant material was then air-dried, ground and stored at close-tight containers until further uses.

Extraction procedure

Each dried and ground plant material (1 g) was extracted with 50 mL hexane: petroleum ether mixture (1:1, v/v) for 1 h and then was filtrated. The residue was dried and subsequently extracted with 50 mL 50% ethanol in order to dissolve the total hydroxycinnamic acid (caffeic acid) derivatives for 30 min at 30 °C in ultrasound water bath (ultrasonic bath SIEL UST 5.7 150 (Gabrovo, Bulgaria) operating at 45 kHz).

Determination of total phenolic content (TPC)

The TPC was analyzed using the method of **Kujala et al. (2000)** with some modifications. Each extract was mixed with Folin-Ciocalteu's reagent and 7.5 % Na₂CO₃. The mixture was vortexed and left for 5 min at 50°C. After incubation at room temperature, the absorbance was measured at 765 nm against control. The TPC was expressed as mg gallic acid equivalents (GAE) per g dry weight (dw) plant material.

Determination of total hydroxycinnamic acid derivatives

Total hydroxycinnamic acid content (including caffeic acid derivatives) was expressed as chlorogenic acid according to the **European Pharmacopoeia 8th edition (2014)**. The extract (1 mL) was added to 2 mL 0.5 M HCl, 2 mL Arnov's reagent (10 % sodium nitrite and 10 % sodium molybdate in distilled water), 2 mL 2.125 M NaOH and 3 cm³ of distilled water. Each sample was measured against control sample prepared by above mentioned procedure without Arnov's reagent and with additional volume of 2 mL distilled water. Absorbance was read at 525 nm. The content of each plant was calculated and expressed as mg chlorogenic acid equivalents (CAE) per g dw plant material.

Determination of antioxidant activity

ABTS⁺ radical scavenging assay

The radical scavenging activity of the extracts against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) was estimated according to **Re et al. (1999)**. The results were expressed as TEAC value (μM TE/g dw).

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of **Benzie and Strain (1999)** with a slight modification. The FRAP reagent was prepared freshly and it was warmed at 37°C before use. The absorbance was measured at 593 nm and the results were expressed as μM TE/g dw.

Cupric ion reducing antioxidant capacity (CUPRAC) assay

The CUPRAC assay was carried out according to the procedure of **Ak and Gülcin (2008)**. Absorbance against a blank reagent was measured at 450 nm after 30 min. The results were expressed as μM TE/g dw.

Analysis of micro- and macro-elements

Mineralization of rocket leaves (0.5 g) was performed in a heat-controlled microwave system with 9 mL of 65 % HNO₃ in a closed vessel system. The microwave-assisted acid digestion was carried out on Milestone ETHOS PLUS with MPR-300/12S medium pressure rotor and heating in two stages (5 and 10 min at 180°C up to 1000 W power, respectively). The microelements including some heavy metals accumulated in leaves (Ni, Cu, Zn, As, Cd, Pb, Cr, Mn, Co, Al, Na and K) of the investigated *E. sativa* samples were analyzed using ISP-MS Agilent 7500 (G3272B) (Agilent Technologies, Inc., Japan). The results were expressed in mg/kg using calibration standards according to BSS EN ISO 17294-2 (2016). The reference material used was SRM 1547 - Peach Leaves of National Institute of Standards and Technology (Gaithersburg, USA).

RESULTS AND DISCUSSION

The frequent consumption of *E. sativa* and the control of its quality contribute to the current research. On Bulgarian market was available rocket plants with Italian and Bulgarian origin. Because of the scanty data for chemical composition of the rocket leaves, the biological activity and nutrient composition of the both plants were assessed and compared.

The total phenolic content was studied in order to evaluate the biological potential of the samples (Table 1). The results revealed that the plant from Bulgarian origin contained higher value for total phenolic compounds – 4.45 ± 0.14 mg GAE/g dw compared with the Italian plant material (3.62 ± 0.16 mg GAE/g dw). Other authors reported rocket leaves and flowers as sources of significant amount of phenolic compounds (23.07 ± 0.11 and 19.9 ± 0.3 mg GAE/g dw, respectively) (**Sadiq et al., 2014**) and (0.9 and 4.7 mg/g fresh weight) (**Heimler et al., 2007**). The significant differences in the values were due to the use of young, fresh plants for consumption and analyses. However, our results indicated that rocket leaves was a source of phenolic compounds. This finding supported its use in most of the regions where people consume this vegetable as a whole plant (leaf, flowers, stem and seed) or various combinations in the form of fresh salad.

In order to compare both plants from Italian and Bulgarian origin, the hydroxycinnamic acid (caffeic acid) derivatives content as a part from phenolic compounds were also evaluated. *E. sativa* of Italian origin consisted of higher total dihydroxycinnamic derivatives 1.52 ± 0.30 mg CAE/g dw, almost twice higher than *E. sativa* of Bulgarian origin (0.91 ± 0.20 mg CAE/g dw) (Table 1). Total hydroxycinnamic derivatives presented about 41.44% of total phenolic compounds for the Italian origin plants and 20.22% for the Bulgarian ones. It is known that caffeic acid and its esters such as caffeic acid phenyl ester and octyl caffeate are potential antioxidants that possess some important anti-inflammatory action (**da Cunha et al., 2004**).

To evaluate the antioxidant activity of the rocket plant materials several assays based on different principles were applied (Table 1). Among them, the FRAP method showed the highest values followed by the ABTS and the CUPRAC methods. However, despite of the higher total phenolic content in the Bulgarian rocket, the antioxidant activity tested by the three assays was lower when the values of analyses were compared. The antioxidant potential in respect of FRAP assay was 35.85 ± 0.65 and 29.82 ± 0.75 μM TE/g dw, respectively. The high antioxidant activity was also demonstrated for rocket plants by DPPH and FRAP methods (**Heimler et al., 2007; Durazzo et al., 2013**). This could be due to the phenolic compounds having different antioxidant activity with each single method. The extraction procedures strongly influenced on the composition of the extracts and also influence on the antioxidant activity results (**Halliwell, 1997; Schwarz et al., 2001; Trojakova et al., 2001**). In addition, the effect of the antioxidant compounds in a food matrix may be significantly different than the activity of a purified extract (**Brewer, 2011**).

Table 1 Total phenolic content (TPC, mg GAE/g dw), total hydroxycinnamic acid derivatives (THAD, mg CAE/g dw) and antioxidant activities (TEAC, μM TE/g dw) in the *E. sativa* extracts

Samples	TPC	THAD	TEAC		
			FRAP	ABTS	CUPRAC
Italian <i>E. sativa</i>	3.62±0.16	1.52±0.30	35.85±0.65	32.07±0.64	25.03±0.22
Bulgarian <i>E. sativa</i>	4.45±0.14	0.91±0.20	29.82±0.75	28.76±0.26	19.74±0.25

As it was known mineral deficiencies can lead to many health disorders. The mineral composition can influence on the antioxidant activity as some metal ions act as pro-oxidants. Minerals are presented usually in all parts of body and are essential for life (**WHO and FAO, 2004; Soetan et al., 2010**). The detailed information in respect of micro- and macro-elements composition of the investigated *E. sativa* samples was summarized (Table 2). The results revealed that the most frequently found metals were K and Na as macro-elements in accordance with **Villatoro-Pulido et al. (2012), Barlas et al. (2011), Tripodi et al. (2017)**. Rocket salad nutritional values for 100 g of fresh leaves (based on USDA Nutrient Database) include K, Ca, Mg and Na as major elements in rocket leaves, followed by P, Fe and Zn (**Tripodi et al. 2017**). In addition, among the micro-elements Mn, Al and Zn were in the highest content for the both samples.

Manganese content in Italian rocket was approximately 2.5 higher than in Bulgarian rocket.

Arsenic was detected only in Italian rocket and absent in Bulgarian plant. Moreover, in the current study the presence of Co (cobalt) (0.1 mg/kg) and Al (aluminum) in rocket was established for the first time. The amount of Al in the Italian sample (595.0 mg/kg) was almost 8 times higher than the Bulgarian sample (75.60 mg/kg). However, the differences among the two samples were observed. Zinc was evaluated in significantly higher amount (6 times) in the Bulgarian plant (91.05 mg/kg) than the Italian sample (15.07 mg/kg).

The established quantities of manganese (Mn) (144.5 – 365.5 mg/kg) were 10 times higher than those reported in other studies (**Bukhsh et al., 2007; Barlas et al., 2011; Villatoro-Pulido et al., 2012**). In addition, the concentrations of copper (Cu) and chromium (Cr) were approximately 2 to 4 times lower (3.0 – 8.0

mg/kg and 0.6 – 1.4 mg/kg, respectively) in comparison with the results of Bukhsh et al. (2007) and Villatoro-Pulido et al. (2012).

However, the geochemical properties of the soil, aerial, and/or aquatic environment as well as the capacity of plants to accumulate elements selectively from their surroundings are known factors influencing the elements content in plants. It had to be noted that the established Pb and Cd content did not exceed the values in the standards for maximum levels of heavy metals (mg/kg of fresh product) for leafy vegetables and fresh herbs with respect to *E. sativa* with Italian origin. Regarding the Bulgarian origin plant the values were exceeding the maximum allowed concentrations (MACs) approved by the European Union

(EU) food standards (EC, 2006) as follow: Pb – 0.3 and Cd 0.2 mg/kg, respectively. Cd may accumulate in the human body and induces kidney disfunction, skeletal damage and reproductive deficiencies (Commission of the European Communities, 2001). Although Cd is a highly toxic metal occurred naturally in soil, it was distributed in the environment because of human activities (Alam et al., 2003). Concentrations of metals analyzed in the samples could be related to their concentration in the corresponding soils. Therefore, to protect humans from harmful effects of heavy metals, the daily intake should not exceed the levels approved by the authorities.

Table 2 Analysis of the micro-elements (Ni, Cu, Zn, As, Cd, Pb, Cr, Mn, Al and Co) and macro elements (Na and K) in the investigated *E. sativa* extracts, mg/kg

Elements	Bulgarian <i>E. sativa</i>	Italian <i>E. sativa</i>	References
Microelements, mg/kg			
Nickel (Ni)	0.38±0.06	0.78±0.10	0.17±0.10 ^a 21.00±5.60 ^a
Copper (Cu)	8.37±0.90	3.58±0.40	10.10±0.40 ^b 5.37 ^c 1.12±0.09 ^a
Zinc (Zn)	91.05±0.65	15.07±0.60	42.10±0.40 ^b 64.86 ^c
Arsenic (As)	ND	0.07±0.01	-
Cadmium (Cd)	0.27±0.03	0.12±0.01	0.0053 ^a
Lead (Pb)	0.58±0.03	0.28±0.01	-
Chromium (Cr)	0.56±0.06	1.43±0.10	4.72±0.03 ^a 10.60±2.80 ^a
Manganese (Mn)	144.50±7.00	365.50±18.00	5.34±0.12 ^b 40.58 ^c
Cobalt (Co)	0.10±0.01	0.13±0.01	-
Aluminum (Al)	75.60 ±7.30	595.00 ±60.00	-
Microelements, g/kg			
Sodium (Na)	2.51±0.25	4.12±0.41	1.90±0.04 ^b ; 0.79 ^c
Potassium (K)	47.26± 4.72	43.56± 4.35	49.60±1.00 ^b ; 51.40 ^c

Legend: ND – not detected; ^a – Bukhsh et al. (2007); ^b – Villatoro-Pulido et al. (2012); ^c – Barlas et al. (2011)

However, the intake of heavy metal-contaminated vegetables may pose a risk to the human health. Food contamination with heavy metal is one of the most important aspects of food quality assurance. The accumulation of heavy metals in vegetables depended on some factors as climate conditions, soils, water irrigation, maturity of the plants, harvest and post harvest conditions (Atanasova et al., 2015).

Many reports were connected with growing rocket plants in contaminated with toxic metals soils (Atanasova et al., 2015). Saleh (2001) claimed that rocket had the potential to accumulate minerals from soils in significant amounts. The increase of Cd and Pb levels in soils was observed their accumulation in plant without further toxic effect, but improvement in biomass, chlorophylls content and enzyme activity of the plant. Shobha and Kumar (2016) reported for *E. sativa* form Mysore district Cd content of 0.1 mg/kg and Pb content of 0.81 mg/kg.

According to Demirezen and Aksoy (2006) heavy metal levels in vegetables in Turkey were within safe limits for Cu, Zn, Ni and exceeded for Cd and Pb. Hussain et al. (2013) established for *E. sativa* leaves from Pakistan the micro-elements (ppm; Fe, Cu, Mn, Cr, Cd, and Pb) and macro-elements (Mg and Na) content as follow Fe - 7.96 ± 0.12; Cu- 3.97 ± 0.17; Mn - 0.75 ± 0.01; Cr - 0.94 ± 0.03; Cd - 0.09 ± 0.00; Mg- 25.65 ± 0.21; Na- 40.56 ± 0.24. The values for Pb and Cd were below the health-based guidance values (EU standards), as the Na and Mg had the highest concentrations.

CONCLUSION

The current research evaluated rocket as rich sources of bioactive compounds. The evaluation of antioxidant activity and presented dihydroxycinnamic derivatives revealed the potential application of this plant as source of natural antioxidants. The levels of micro- and macroelements in rocket plant with Italian and Bulgarian origin were determined. The results suggested that significant differences existed in the element concentrations between both samples. The presence of Co and Al was established for the first time. The obtained results for the *E. sativa* of Bulgarian origin showed that concentrations of some metals (Pb and Cd) exceeded the recommended maximum acceptable levels proposed by EU. The predominant antioxidant activity of the rocket from Italy revealed the sample as a significant source of biologically active substances.

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