THE CONTENT AND RADICAL SCAVENGING CAPACITY OF PHENOLIC COMPOUNDS FROM BLACK RADISH ROOTS OF VARIOUS SIZES

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ABSTRACT:. In this paper content of phenolic compounds from black radish (*Raphanus sativus* L. var niger) roots of different size were examined. Black radish grown in Serbia, with the same period of development, grouped by size into three groups: (R1) with mass of 350±15 g; (R2): 100±10 g and (R3): 35±5 g, were used. Plant extracts were prepared by using 80% (v/v) ethanol and total phenolic compounds content was determined by spectrometric and the free radical scavenging abilities by DPPH radical method. The phenolic compounds content was in range 42.9 (R1) to 19.7 (R3) μmol chlorogenic acid per g of dried plant material, i.e. 443.7 to 208.6 μmol chlorogenic acid per g of dry extract. The radical scavenging capacity ranged from 88.3 (R1) to 55.6% (R3) and the EC_{50} values were from 1.59 to 2.24 mg/ml. The phenolic compounds content and radical scavenging capacity depended on root size in such a way that bigger root means higher content of phenolic compounds and higher scavenging capacity. By statistical analysis, there was a positive correlation between the phenolic compound content and radical scavenging capacity and Euclidean linkages distances results showed higher similarity between R2 and R3 sample.
Key words: *phenolic compounds, radish, scavenging capacity*

INTRODUCTION

Black radish (*Raphanus sativus* L. var*. niger*) is a plant belonging to *Cruciferae* family. In folk medicine, black radish root has been used since antiquity as a natural drug against the flatulence, formation of gallstones and indigestion (Hänsel, 1985; Pahlow, 1989). Black radish is an effective natural drug for the stimulation of bile function (Böhm, 1959; Ritter, 1984; Weiss, 1985) and significant antiurolithiatic effects of black radish were also reported (Vargas et al.1999). Black radish root contains mixture of various inorganic and organic compounds (minerals, lipids, proteins, carbohydrates, dietary fibres and vitamins).
The biological properties such as anticancer, antimicrobial and antiviral derive from phenolic compounds and sulphur and nitrogen containing organic compounds, such as glucosinolates (Lugasi et. al., 1998). Due to these abilities glucosinolates and phenolic compounds have great attention today. The glucosinolates occur as secondary metabolites of almost all plants of the order Brassicales, derived from glucose and oftenly two amino acids are incorporated. Plants use substances derived from glucosinolates as natural pesticides and as defence against herbivores. These substances are also responsible for bitter or sharp taste of radish (Volder et al., 2009).
Phenolic compounds are product of secondary metabolism of plants which arise biogenetically from the main synthetic pathways: shikimate and the acetate pathway (Bravo, 1998). Phenolic compounds have free radical radical scavenging abilities, which depend on the exposure to stress such as light, temperature, water (Nakamura et al., 2003; Kays et al., 1997), nutritional deficiencies (Dioxin et al., 1995; Robins et al., 1997) type of vegetable tissue (Sonia et al., 2007), mechanical damage such as wounding (Fernando et al., 2007), maturation stages (Ozgen et al., 2009), chemical structure (Lugasi et al., 2003) etc.

With regard to the widespread use of black radish in the diet in this paper the effect of the size of the root on the content of phenolic compounds composition and radical scavenging capacity (SC) were examined. The results could be good indicator for the consumers which size i.e. weight of black radish root for nutritive or healthy benefits is better to consume.

MATERIAL AND METHODS

Plant material

Black radish (*Raphanus sativus* **L**.) var. *niger*) roots, with the same period of development, based on root size i.e. weight, were grouped into R1 (350±15 g), R2 (100±10 g) and R3 (35±5 g) groups. Five samples of roots were collected for each group. The roots were cut in cube shape (1.5x1.5x1.5 cm), dried at 35 ^{o}C during 6 h and left at room temperature for 1 h. Then the five samples were mixed into appropriate group and milled to average particle size of 0.5 mm.Three separate determinations for each group were performed.

Plant material moisture content

The black radish roots moisture content was determined by using the analyzer (Scaltec SMO 01, Scaltec Instruments, Göttingen, Germany). Fresh plant material (3 g) was dried at 110 ^{o}C to a constant weight, and moisture content was read out on the analyzer display.

Extracts preparation

Plant extracts preparation for measurements of the phenolic compounds content were done according to Dokhani et al. (2005) procedure. Dried and milled black radish roots sample (3 g) was measured and 80 mL of 80% (v/v) ethanol was added. The mixture was stirred by MR1 magnetic stirrer (IKA-Werke, Staufen, Germany) for 10 minutes at 200 min^{-1} and vacuum filtered through No. 54 Wathman filter paper (GE Healthcare, Brondby, Denmark). The solids were re-extracted with 60 mL of 80% (v/v) ethanol, the filtrates combined and made to a final volume of 250 mL. For the measurement of phenolic compounds content, 10 mL of each extract was filtered through a 0.45 μm membrane filter (Agilent Technologies, Wilimington, Delaware, USA). For radical scavenging capacity (SC) measurements and HPLC analysis, 200 mL of each extract was evaporated in vacuum at 45 ^{o}C until dry and dissolved in 10 mL of 96% (v/v) ethanol.

Phenolic compounds content

For phenolic compounds content (PCC) measurement, a standard curve for five concentrations of chlorogenic acid (Sigma Chemical, St. Louis, Missouri, USA) concentrations, covering the range from 50 to 1500 μmol, was made. In a test tube, 0.25 mL of 0.1% (w/v) HCl in 95% (v/v) ethanol, 4.50 mL of 2% (w/v) HCl and 0.25 mL of chlorogenic acid standard solutions were added, mixed by vortex and allowed to stand for approximately 15 min. Then the absorbance (*A*) was read at 280 nm using UV 21 000 Sprectrophotometer (Cole Parmer Instruments Company, Vernon Hills, Illinois, USA) as described by Glories (1998). Based on standard curve, the equation for PCC determination was obtained as:

$$PCC = \frac{A - 0.1083}{4.890 \times 10^{-4}} \quad (1)$$

For measuring phenolic compounds content in black radish extracts, into test tube 0.25 mL of 0.1% HCl in 95% (v/v) ethanol, 4.50 mL of 2% (w/v) HCl and 0.25 mL of filtered extracts were added and further treated as standard solutions of chlorogenic acid. The PCC was obtained based on equation (1) and presented as μmol of chlorogenic acid per g of dry plant material and μmol of chlorogenic acid per g of dry extract.

Radical scavenging capacity

The radical scavenging capacity (SC) of extract diluted by ethanol to concentrations ranging from 0.2 to 6 mg/mL, was determined by the DPPH test (Mensor et al., 2001). Ethanol solution of DPPH radicals, 1 mL of a 0.3 mM, was added to 2.5 mL ethanol solution of given

concentration of investigated extract and allowed to react at room temperature during 30 min. Then the *A* value was measured at 518 nm on UV 21 000 Sprectrophotometer (Cole Parmer Instruments Company), and converted into the percentage of radical SC by using the equation (Mensor et al., 2001):

$$SC = 100 - \frac{(A_{sample} - A_{blank})}{A_{control}} \times 100 \quad (2)$$

where A_{sample} is the absorbance at 518 nm of the ethanol solution of the extract treated with the DPPH radical solution; A_{blank} is absorbance at 518 nm of the ethanol solution of the extract (1 mL of ethanol added to 2.5 mL of extract), and $A_{control}$ is absorbance at 518 nm of ethanol solution of DPPH radical (1 mL of a 0.3 mM/L added to 2.5 mL of ethanol). The final results are presented as EC_{50} value, i.e. concentration of investigated extracts sufficient to decrease the initial DPPH concentration by 50%.

Statistical analysis

Statistica version 5.0 software (StatSoft, Tulsa, Oklahoma, USA) was used to perform the statistical analysis: the mean, standard deviations and the correlation coefficients. The mean and standard deviations were obtained by Descriptive Statistics, marking the Median & Quartiles and Confirm Limits for Means and the correlation coefficients were obtained by correlations matrix analysis with displaying p and N value. The Euclidean distances were obtained by the cluster analysis and the Euclidean method with the complete linkage.

RESULTS AND DISCUSSION

Extract yield, phenolic compounds content and scavenging capacity

The results of moisture content, extract yield, the PCC and radical SC of phenolic extracts from black radish roots are shown in table 1. The results are mean values of three determinations. The dependence of total scavenging capacity on concentration of phenolic compounds content in extract, are presented in figure 1.

The results showed that the PCC varied in range from 42.9 (R1) to 19.7 (R3) μmol chlorogenic acid per g of dried plant material, i.e. 443.7 to 208.6 μmol chlorogenic acid per g of dry extract. Based on results presented on figure 1, the radical scavenging capacity ranged from 88.3 (R1) to 55.6% (R3) and the appropriate EC_{50} values were 1.59 and 2.24 mg/ml, respectively.

Table 1. Moisture content, extract yield, phenolic compounds content and radical scavenging capacity in black radish roots of various weights

Group of parsnip roots	R1 (350±15 g)	R2 (100±10 g)	R3 (35±5 g)
Fresh plant material moisture content (%)	68.2±1.9	71.9±1.3	70.8 ±1.6
Extract yield (g/kg)	48.2±3.5	47.1±3.6	46.4±3.4
Phenolic compounds content (μmol/g of dry plant material)	42.9±2.6	23.1±1.8	19.7±1.6
Phenolic compounds content (μmol/g of dry extract)	443.7±15	241.3±12	208.6±16
EC_{50} (mg/mL)	1.59±0.4	2.98±0.5	3.54±0.5

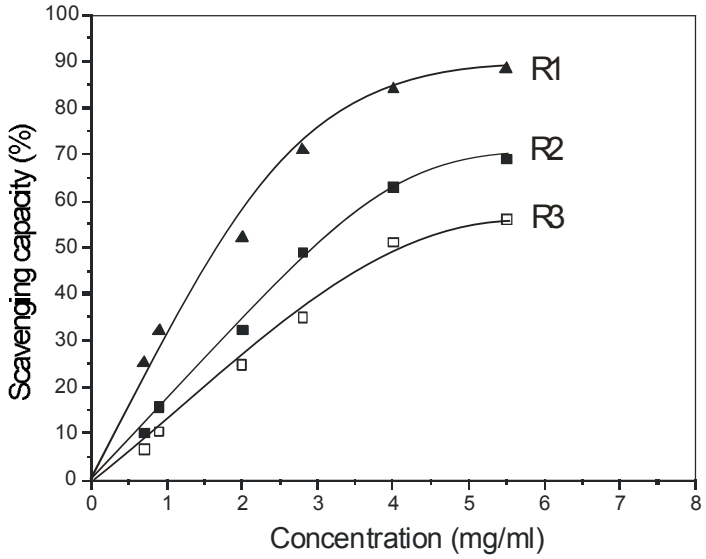

Figure 1. Total scavenging capacity of 80% (v/v) ethanol extract from black radish root of various sizes

Statistical analysis

The correlation coefficients were obtained based on five parameters (1- extract yield, EY; 2- phenolic compounds content as μmol of chlorogenic acid per g of dry plant material, PPC1; 3. phenolic compounds content as μmol of chlorogenic acid per g of dry extract, PPC2; 4- radical scavenging capacity, SC and 5- concentration of investigated extract sufficient to decrease the initial DPPH concentration by 50%, EC_{50}).

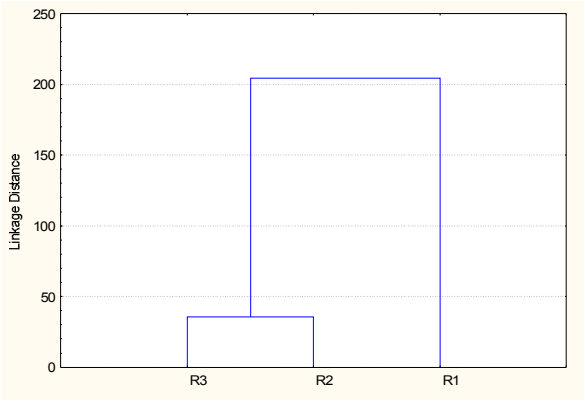

Figure 2. Total scavenging capacity of 80% (v/v) ethanol extract from black radish root of various sizes

The sample size was six (*N* = 6, three groups and their minimum and maximum value of determinations). The results show the high PCC was associated with high (the correlation coefficient was 0.96) and low EC_{50} values (the correlation coefficient was -0.99). As the lower EC_{50} value indicates higher antioxidant capacity, the obtained correlations show that higher phenolic compounds content in black radish root means higher scavenging capacity.

The results of Euclidean linkages distances based on the same parameters as the obtained correlation coefficients, showed higher similarity between R2 and R3 samples than between others (Figure 2), and this is evident from data in Table 1 and figure 1.

CONCLUSIONS

The phenolic compounds content and radical scavenging capacity depended on black radish root size in such a way roots with higher weight had higher content of phenolic compounds and better antioxidant capacity. There was a positive correlation between the phenolic compounds content and total radical scavenging capacity. The black radish root with weight higher than 100 g are recommended for human nutrition for healthy benefits.

ACKNOWLEDGEMENTS

This paper is a result of the research within the project Grant No.172047 „Prirodni proizvodi biljaka i lišajeva: izolovanje, identifikacija, biološka aktivnost i primena" (Natural products from plants and lichens: isolation, identification, biological activity- the possibility for final application) by the Ministry of Education and Sciences Republic of Serbia.

REFERENCES

1. Böhm, K. (1959). Untersuchungen über choleretische Wirkungen einiger Arzneipflanzen. *Arzneimittelforschung*, *9*, 376–378.
2. Bravo, L. (1998). Polyphenols: chemistry, dietary source, metabolism and nutritional significance. *Nutrition Reviews, 56,* 317-333.
3. Choi, C.W., Kim, S.C., Hwang, S.S., Choi, B.K., Ahn, H.J., Lee, M.Y., Park, S.H., Kim, S.K. (2002). Antioxidant capacity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science, 163,* 1161-1168.
4. Dioxin, R.A., Paiava, N.L., (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell, 7,* 1085-1097.
5. Dokhani S., Cottrel, T., Khajeddin, J. and Mazza, G. (2005). Analysis of aroma and phenolics components of selected Achillea species. *Plant Foods for Human Nutrition, 60*, 55-62.
6. Fernando, R.L., Emilio, V.J., Cisneros-Zevallos, L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable. *Food Chemistry, 101,* 1254-1262.
7. Glories Y. (1978). Reserches sur la matiere colorante des vins rouges. *Bullten Chimistry, 9,* 2649-2655.
8. Hänsel, R. (1985). Pflanzliche cholagoga. *Deutsche Apotheker Zeitung, 125,* 1373–1378.
9. Kays, S. (1997). Stress in harvested products. Postharvest physiology of perishable plant products*, Exon press, Athens, GA.*
10. Lugasi, A., Dworschák, E., Blázovics, A., Kéry, Á. (1998). Antioxidant and free radical scavenging properties of squeezed juice from black radish (*Raphanus sativus* L. var *niger*) root, *Phytotherapy Research, 12* 502-506.
11. Lugasi, A., Hóvári, J. Sági, K.V. Bíró, L. (2003). The role of antioxidant phytonutritients in the prevention of diseases. *Acta Biologica Szegediensis,* 47, 119-125.
12. Mensor, L.M., Menezes, F.S., Leitão, G.G., Reis, A.S., dos Santos, T.C., Coube, C.S., Leitão, S.G. (2001). Sreening of Brazilian plant extracts for antioxidant capacity by the use of DPPH free radical method. *Phytotherpy Researrch, 15,* 127-130.
13. Nakamura, Y., Watanabe, S., Miyake, N., Kohno, H., Osawa, T. (2003). Dihydrochalcones: evaluation as novel radical scavenging antioxidants. *Journal of Agricultural and Food Chemistry, 51,* 3309-3312.
14. Özgen, M., Torun, A., Ercisli, S., Serce, S. (2009). Changes in chemical composition, antioxidant activities and total phenolic content of arbuts andrachine fruit at different maturation stages. *Journal of Food Science, 21,* 65-73.
15. Pahlow, M. (1989). Heilpflanze Heute, *Grafe und Unzer GmbH, Munchen, Germany*.
16. Ritter, U. (1984). Therapie mit Choleretika and Cholekinetika. *MedPharm, 7*, 99–104.
17. Robins, R., Tomas-Barden, F. (1997). Phytochemistry of fruits and vegetables, Oxford University Press, London, GB.
18. Sonia, Z.V., Alicia, R.C. (2007). Respiratory activity and phenolic compounds in pre-cut black radish. *Food Chemistry, 100,* 654-1660.
19. Vargas, R., Perez, R.M., Perez, S. (1999). Antiurolithiatic activity of Raphanus sativus aqueous extract on rats. *Journal of Ethnopharmacology, **68,*** 335–338.
20. Volden, J., Borge, G.A., hansen M., Wicklund T., Bengtsson G.B. (2009) Processing (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (Brassica oleracea L. ssp. botrytis), *LWT - Food Science and Technology, 42,* 63–73.
21. Weiss, R.F. (1985). Lehrbuch der Phytotherapie. 4. Auflage. *Hippokrates-Verlag, Stuttgart, Germany.*