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PHENOLIC COMPONENTS AND ANTIOXIDANT ACTIVITIES IN VARIOUS TYPES OF CARROT EXTRACTS

Jelena MLADENOVIĆ^{1*}, Ljiljana BOŠKOVIĆ-RAKOČEVIĆ¹, Milena ĐURIĆ¹, Jasmina ZDRAVKOVIĆ², Nenad PAVLOVIĆ², Marijana DUGALIĆ¹

¹Faculty of Agronomy, University of Kragujevac, Čačak, Serbia

²Institute for Vegetable Crops, Smederevska Palanka, Serbia

*Corresponding author: jelenamala@kg.ac.rs

Abstract

The objective of this study was to evaluate antioxidant activities of vegetable extracts *Daucus carota* L., grown in Serbia. Different experimental models have included the determination content of total phenolics, total flavonoids and antioxidant activities of extracts. From the same material, two extracts were obtained by various methods: maceration and ultrasonic extraction. The highest content of phenolic compounds was detected in *D. carota* L. 50.42 mg GAE/g, ultrasonic extract. The lowest content of phenolic compounds shown *Daucus carota* L. macerat, 17.45 mg GAE/g. The obtained antioxidant activities are in correlation with the content of phenolic components. On the basis of the results obtained, extract of were found to serve as a potential source of natural antioxidants due to their marked activity. The obtained results may be useful in the evaluation of new dietary and food products.

Keywords: *D. carota* L, maceration, ultrasonic extraction, phenolic compounds.

Introduction

Different parts of plants (roots, leaves, flowers, fruit, stem, bark) have been successfully used to treat numerous diseases (Beninger and Hosfield, 2003). Owing to their antioxidant activity, they can influence a number of physiological processes, thus protecting the organism from the damaging effect of free radicals and inhibiting the development of unwanted microorganisms. Several studies have pointed out the possibility to use essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage (Yanishlieva *et al.*, 2006). Our body is exposed to a large number of foreign chemicals everyday. The most of which are man-made and our inability to properly metabolize them negatively affects our health by the generation of free radicals. Free radicals are also generated during normal metabolism of aerobic cells. The oxygen consumption inherent in cells growth leads to the generation of series of oxygen free radicals. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumour (prostate and colon cancers) and coronary heart diseases. Antioxidants can significantly delay or prevent the oxidation of easily oxidizable substances (Macheix and Fleuriet, 1998). Natural antioxidants are classified according to their mechanism of action as chain-breaking antioxidants which scavenge free radicals or inhibit the initiation step or interrupt the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions but do not convert free radicals. However, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), known for their ability to terminate the chain reaction of lipid peroxidation, have been proven to be carcinogenic and to cause liver damage (Prieto *et al.*, 1999). The use of plants in the food industry in place of synthetic preservatives, antioxidants or other food additives has significantly increased over the last few years due to their ability to produce biologically active substances (Merken and Beecher, 2000). There is

growing interest toward natural antioxidants from herbal sources (Beninger and Hosfield, 2003).

Material and Methods

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). The plant material used in the experiment included is grown under plastic-covered greenhouse conditions in Cacak.

Spectrophotometric measurements were performed using an MA9523-SPEKOL 211 UV-VIS spectrophotometer (ISKRA, Horjul, Slovenia).

Plant materials (20 g) were macerated in a mixture of 95% ethanol (250 ml) and 0.5% glacial acetic acid at room temperature for 24 hours. The resulting macerate was filtered and the maceration procedure was repeated once. The extracts obtained were combined and concentrated until dry in a rotary vacuum evaporator to produce the (E₁) extract [5]. The (E₂) extract was obtained by ultrasound-assisted extraction using a Brason B-220 ultrasonic bath (Smith-Kline Company, USA).

The typical procedure involved ultrasound-assisted extraction of crushed plant material with 95% ethanol over a period of 1 hour.

Total phenols were estimated using the Folin-Ciocalteu method (Duh *et al.*, 1999). Plant extracts were diluted to a concentration of 1 mg/mL, and aliquots of 0.5 mL were mixed with 2.5 mL of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water) and 2 mL of NaHCO₃ (7.5%), (Yan *et al.*, 2006). After heating for 15 min at 45°C, the absorbance was measured at 765 nm in a spectrophotometer against blank sample. Total phenols were determined as gallic acid equivalents (mg GA/g extract), and the values are presented as means of triplicate analyses (Hsu *et al.*, 2008). The method used by (Brighente *et al.*, 2007), was adopted with suitable modifications from (Daker *et al.*, 2008). DPPH (8 mg) was dissolved in C₂H₅OH (100 mL) to obtain a concentration of 80 mg/mL. Serial dilutions were carried out with the stock solutions (1 mg/mL) of the extracts. Solutions (2 mL each) were then mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur, and the absorbance was measured at 510 nm. Ascorbic acid (AA), gallic acid (GA) and butylated hydroxytoluene (BHT) were used as reference standards and dissolved in methanol to make a stock solution at the same concentration (1 mg/mL), (Yanishlieva *et al.*, 2006). Control sample was prepared containing the same volume without test compounds or reference antioxidants (Tandon *et al.*, 1995). Ninety-five percent ethanol was used as blank. The DPPH free radical scavenging activity (%) was calculated using the following equation:

$$\% \text{ inhibition} = [(A_c - A_s) / A_c] 100.$$

Results and Discussion

The total phenolic content of the (E₁) ultrasonic extraction 50.42 mg GAE/100g of sample, and that of the (E₂) maceration extract was 17.45 mg GAE/100g of sample. The results obtained were calculated as average values of five parallel measurements. High values of antioxidant activity were identified, being 92.67 % and 89.67 % for the E₁ and E₂ extracts, respectively. Figures 1. and 2. show graphic presentation of the antioxidant activity of the E₁ and E₂ extracts.

Fig. 1. Antioxidant activity of the E₁ extract

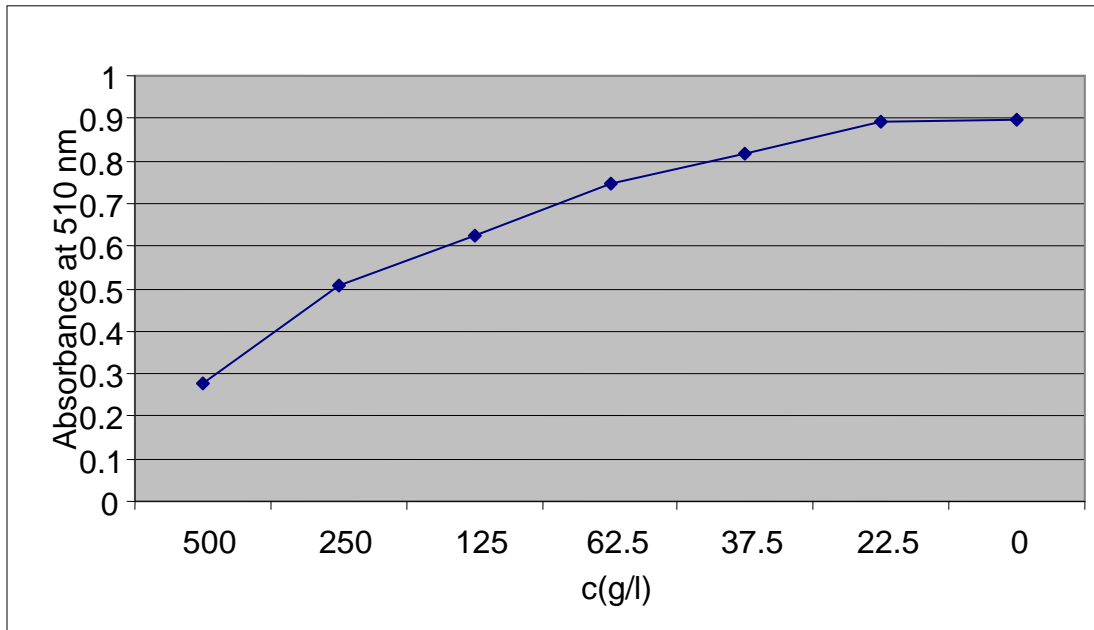
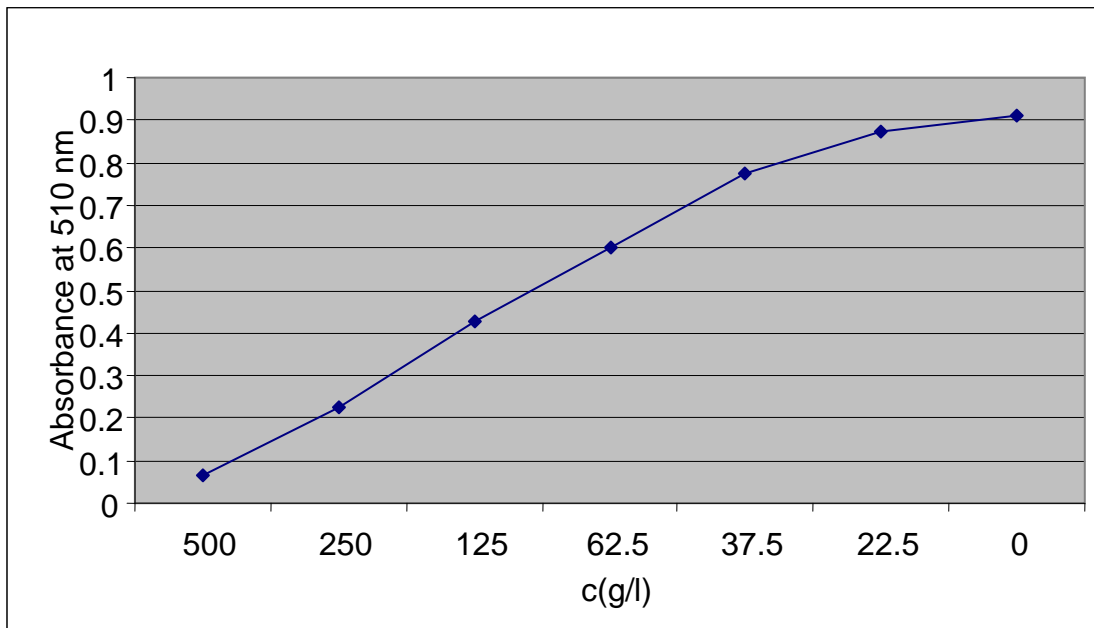


Fig. 2. Antioxidant activity of the E₂ extract



The DPPH capacity values were calculated relative to rutin, trolox and quercetin (Table 1). The values were calculated based on the graph representing dependence between standard concentration (mg/g DPPH) in the X-axis and capacity of DPPH radicals (%) in the Y-axis.

$$\text{Rutin} \quad c = (\% \text{ DPPH} - 1.29481) / 0.37472$$

$$\text{Trolox} \quad c = (\% \text{ DPPH} - 0.24856) / 0.38094$$

$$\text{Quercetin} \quad c = (\% \text{ DPPH} - 12.09185) / 5.2032$$

Table 1. DPPH mg/g relative to rutin, trolox and quercetin

extract	mg/g DPPH	standard
Ethanol E ₁	0.24385	Rutin
Ethanol E ₂	0.24118	Rutin
Ethanol E ₁	0.24261	Trolox
Ethanol E ₂	0.23999	Trolox
Ethanol E ₁	0.01549	Quercetin
Ethanol E ₂	0.01529	Quercetin

Conclusions

The importance of this study lies in a preliminary examination of whether carrot extracts can be used as a source of natural preservatives in the food industry. Results show significant antioxidant activity in the vegetable extracts tested. Moreover, the E₁ and E₂ extracts were found to have significant antioxidant activity which correlated with the total phenolic content. Total phenols, hence and antioxidant activity, are dependent upon the method and time of extraction, which is most likely due to their instability. Therefore, this fact should be considered when selecting and obtaining natural antioxidants.

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