

COMPARISON OF ANTIRADICAL ACTIVITY AND TOTAL PHENOLIC CONTENT IN SEEDS OF FIVE SOYBEAN CULTIVARS BY APPLYING DIFFERENT EXTRACTION SOLVENTS

Nikolova M.¹, M. Dimitrova¹, K. Tasheva², R. Todorova³, M. Dimitrova², G. Ravishankar⁴, G. Kosturkova^{2}*

¹*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

²*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

³*Experimental Station of Soya DP Pavlikeni, 5200 Pavlikeni, Bulgaria*

⁴*Dayananda Sagar Institutions, 560078 Bangalore, India*

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Summary: Radical scavenging activity and total phenolic content in seeds of five Bulgarian soybean varieties were analyzed in relation to different types of the extraction solvent. The extraction solvents used were acetonitrile acidified with HCl and 80% methanol. The amount of phenolic compounds in the extracts was estimated by Folin–Ciocalteu reagent. 2,2-diphenyl-1-picryl hydrazyl (DPPH) radicals were used for evaluation of antiradical activity. Statistically significant differences in respect to total phenolic content and antiradical activity were not established among the studied methanol and acetonitrile extracts of one and the same variety. Total phenolic content in soybean cultivars ranged from 7.52 to 12.87 mg GAE/g extract. The cultivars “Rosa”, “Richy” and “Daniela” showed higher levels of phenols. Antiradical activity expressed as the concentration of extracts needed for 50 % inhibition of radicals (IC₅₀) ranged from 2.74 to 3.84 mg/mL. The results from the comparative analysis show that methanol and acetonitrile are interchangeable extraction solvents for determination of antiradical activity and total phenolic content in soybean samples.

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*Corresponding author: georgina_kosturkova@abv.bg

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is an annual plant and one of the most important grain legumes in the world. The species is native to East Asia but widely cultivated in the world because it is globally important source of proteins, oils and secondary metabolites – phenolic compounds and isoflavonoids, such as glycitein, genistein and daidzein possessing antioxidant activity (Fritz et al., 2003; Malenčić et al., 2008; Sammour, 2011).

A widely accepted tool for estimation of antioxidant capacity of plant extracts is the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Alcohol solution of DPPH is purple colored, which after being reduced by an antioxidant, turns into a yellow-colored product. The DPPH assay is considered a valid accurate, easy and economic method which provides first hand information on the overall antioxidant capacity of the test system (Marinova and Batchvarov, 2011; Kedare and Singh, 2011). The antioxidant potential of soybean products and their phenolic content has been extensively evaluated by DPPH assay and Folin–Ciocalteu reagent, respectively (Kim et al., 2006; Malenčić et al., 2007; Dajanta et al., 2011).

In Bulgaria, scientific and applied research in soybean production is concentrated at the Experimental Station of Soya, Pavlikeni. A series of elite soybean varieties with improved yield, nutrition qualities, biotic and abiotic stress tolerance has been released. Seeds of some of these varieties were analyzed previously for their content of phytoestrogen compounds (Sakthivelu et

al., 2008). The present biochemical work is an extension to our former analysis using several new cultivars.

The aim of this study was to compare the efficiency of two extraction solvents for estimation of the total phenolic content and antioxidant activity of soybean seeds. The comparative study was performed with five genotypes cultivated in Bulgaria.

MATERIALS AND METHODS

Material

The study was performed with Bulgarian soybean varieties: “Richy”, “Rosa”, “Srebrina” and “Daniela” and the American variety “Hodson” (world standard) provided by the Experimental Station of Soya DP Pavlikeni, Bulgaria.

Preparation of extracts

Acetonitrile (ACN) extracts. The powdered soybean seeds (2 g) were mixed with 2 mL 0.1 N HCl and 10 mL acetonitrile in a 125-mL flask and stirred for 2 h at room temperature. The solution was filtered and dried under vacuum at a temperature below 30°C (Kim et al., 2005).

Methanol extracts. The powdered soybean samples (2 g) were extracted with 80% methanol by maceration at room temperature for 24 h. After evaporation of the solvent the crude extract was subjected to subsequent analysis.

Determination of total phenolic content

Total phenolic content of the methanol and acetonitrile extracts was determined by methods using Folin–Ciocalteu reagent and gallic acid as a

standard (Nićiforović et al., 2010). Plant extracts were diluted to a concentration of 5 mg mL⁻¹, and aliquots of 0.4 mL were mixed with 2.0 mL of Folin–Ciocalteu reagent (beforehand diluted 10-fold with distilled water) and 1.6 mL of Na₂CO₃ (7.5 %). After 1 h at room temperature, the absorbance of the samples was measured spectrophotometrically at 765 nm versus a blank sample. Total phenols were determined as gallic acid equivalents (mg GA per g of extract) by the following formula:

$$C = c \times V / m$$

where C is total content of phenolic compounds (mg g⁻¹ plant extract) in GAE; c is the concentration of gallic acid established from the calibration curve (mg mL⁻¹); V is the volume of the extract (mL); m is the weight (g) of the pure plant methanolic extract.

Determination of DPPH radical-scavenging activity

The effect of methanolic extract and acetonitrile on DPPH radicals was estimated according to Stanojević et al. (2009) with minor modifications. Different concentrations of plant extract in methanol (1, 2, 5, 10 and 20 mg/mL) were added at an equal volume (2.5 mL) to methanol solution of DPPH (0.3 mM, 1 mL). After 30 min at room temperature, the Ab values were measured at 517 nm on a spectrophotometer (Jenway 6320D) and converted into percentage antioxidant activity using the following equation:

$$\text{DPPH antiradical scavenging capacity (\%)} = [1 - (\text{Ab}_{\text{sample}} - \text{Ab}_{\text{blank}}) / \text{Ab}_{\text{control}}] \times 100$$

Methanol (1.0 mL) plus plant extract solution (2.5 mL) was used as a blank, while DPPH solution plus methanol was

used as a control. The IC₅₀ values were calculated using Software Prizm 3.00.

Statistical analysis

All assays were performed in three independent experiments and are presented as mean value ± standard deviation (SD). Statistical analysis was carried out using Excel. Significance levels were defined at p<0.05 as analyzed by *t*-test.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

Two types of extracts - methanol (ME) and acetonitrile (AE) from five soybean varieties were evaluated for their antiradical potential using the DPPH method. The results are presented as IC₅₀ values (mg/mL) - extract concentration providing 50% inhibition of the DPPH solution. The antiradical activity of the studied extracts ranged from 2.74 mg/mL to 3.84 mg/mL. Differences between the methanol and acetonitrile extracts were detected but they were not statistically significant (p<0.05) even for cultivars “Hodson” and “Daniela” where the differences between the two extractions were greatest. The highest antiradical activity was observed in the acetonitrile extract (AE) of cv. “Srebrina” followed by very similar values of the extracts of varieties “Richy” (AE and ME), “Hodson” (ME) and “Daniela” (ME). The lowest antiradical activity exhibited the acetonitrile extract of cv. “Daniela” (Fig. 1). The obtained values were close to those reported in the literature for soybean with black and yellow kernel coat and cooked soybean (Haiwei, 2010; Dajanta et al., 2011; Žilić et al., 2011; Sefatie et al., 2013). The obtained data

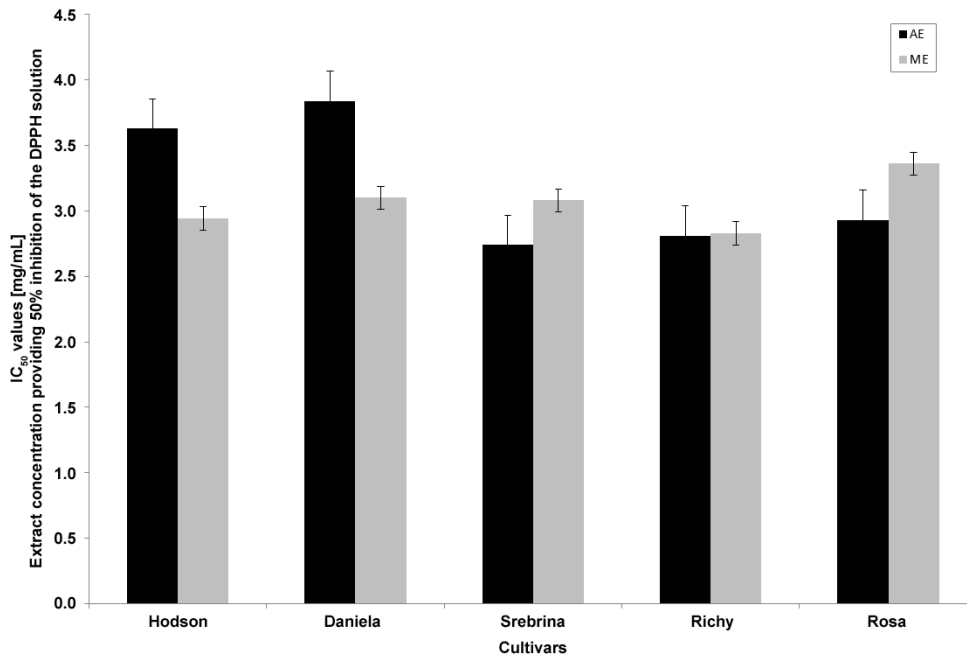


Figure 1. Antiradical activity of extracts from different soybean cultivars.

for antiradical properties of the studied cultivars are better compared with those reported for Croatian soybean seed cultivars and a Thai soybean (Mujić et al., 2011; Samruan et al., 2012).

Total phenolic content

The results on total phenolic content determination in the methanol and acetonitrile extracts of the studied cultivars evaluated by the Folin - Ciocalteu method, are presented in Fig. 2. The phenolic content of the analyzed extracts ranged between 7.52 and 12.87 mg GAE/g extract. Differences between the methanol and acetonitrile extracts of one cultivar were observed but they were not statistically significant ($p < 0.05$). In most cases, the methanol extract had slightly higher content of phenols in comparison to acetonitrile of one and the same cultivar, with the exception of the extracts of cv. “Srebrina”. The

highest content of phenols was found in the methanol extract of cv. “Rosa” and the lowest in the methanol extract of cv. “Srebrina”. The obtained results on total phenolic content of the analyzed cultivars were in the range of the values reported for other soybean cultivars where the reported values varied from 6.4 to 81.7 mg GAE/g extract (Pracas et al., 2007).

Correlation between total phenolic content and antiradical activity

A positive correlation between total phenolic content and antiradical activity was found in the extracts (ME and AE) of cultivars “Hodson” and “Daniela”. Although there were significant differences in the phenolic content between methanol and acetonitrile extracts of cv. “Richy” differences in antiradical activity were not established. The methanol extract of cv. “Rosa” had high phenolic content, but showed low antiradical activity. The

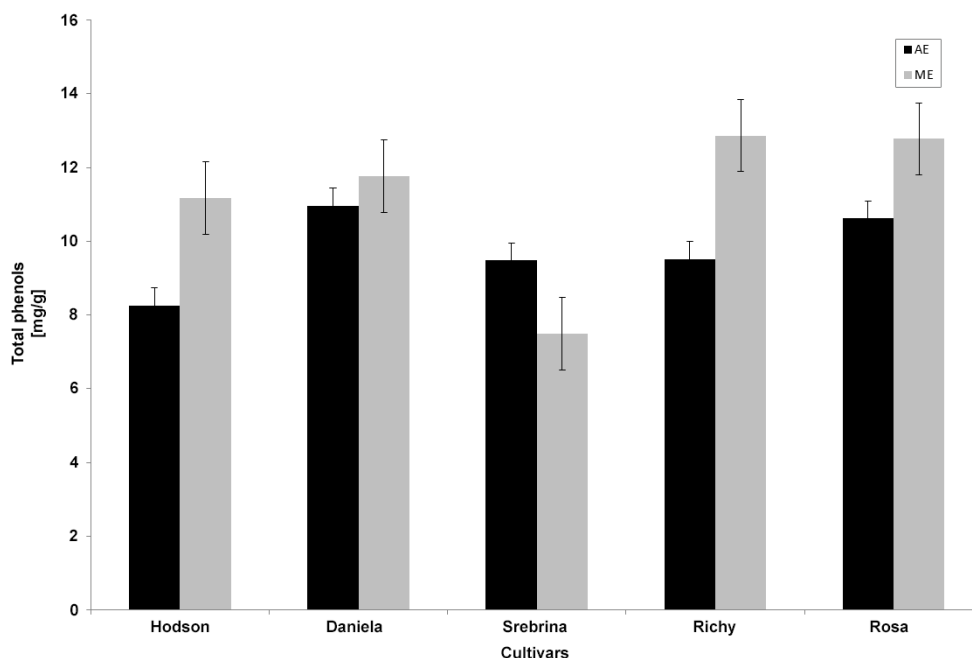


Figure 2. Total phenolic content of extracts from different soybean cultivars.

lack of positive correlation between total phenolic content and antiradical activity was detected in the extracts (ME and AE) of cv. “Srebrina”.

Although most of the authors have found a positive correlation between the content of phenolic compounds and antioxidant activity in soybean samples (Kim et al., 2006; Malenčić et al., 2007) the cases where there is not such a dependence are not an exception (Ried et al., 2007; Dajanta et al., 2011; Carson et al., 2012). The absence of such a correlation has been explained with the presence of some other antioxidant compounds such as oligoproteins, free amino acids, peptides and melanoidins (Carson et al., 2012; Dajanta et al., 2011). On the other hand, data are available showing that isoflavonoid aglycones (i.e., daidzein and genistein) can be inactive substrates in the radical scavenging assay (Dajanta et al., 2011).

CONCLUSION

Statistically significant differences ($p < 0.05$) with respect to total phenolic content and antiradical activity were not established among the studied methanol and acetonitrile extracts of one and the same soybean variety. This result shows that methanol and acetonitrile are equally effective in the analysis of total phenolic content and antiradical activity of soybean samples. Both solvents are interchangeable in the analysis of antiradical activity and total phenolic content. However, low cost methanol could be preferable for large scale analyses.

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