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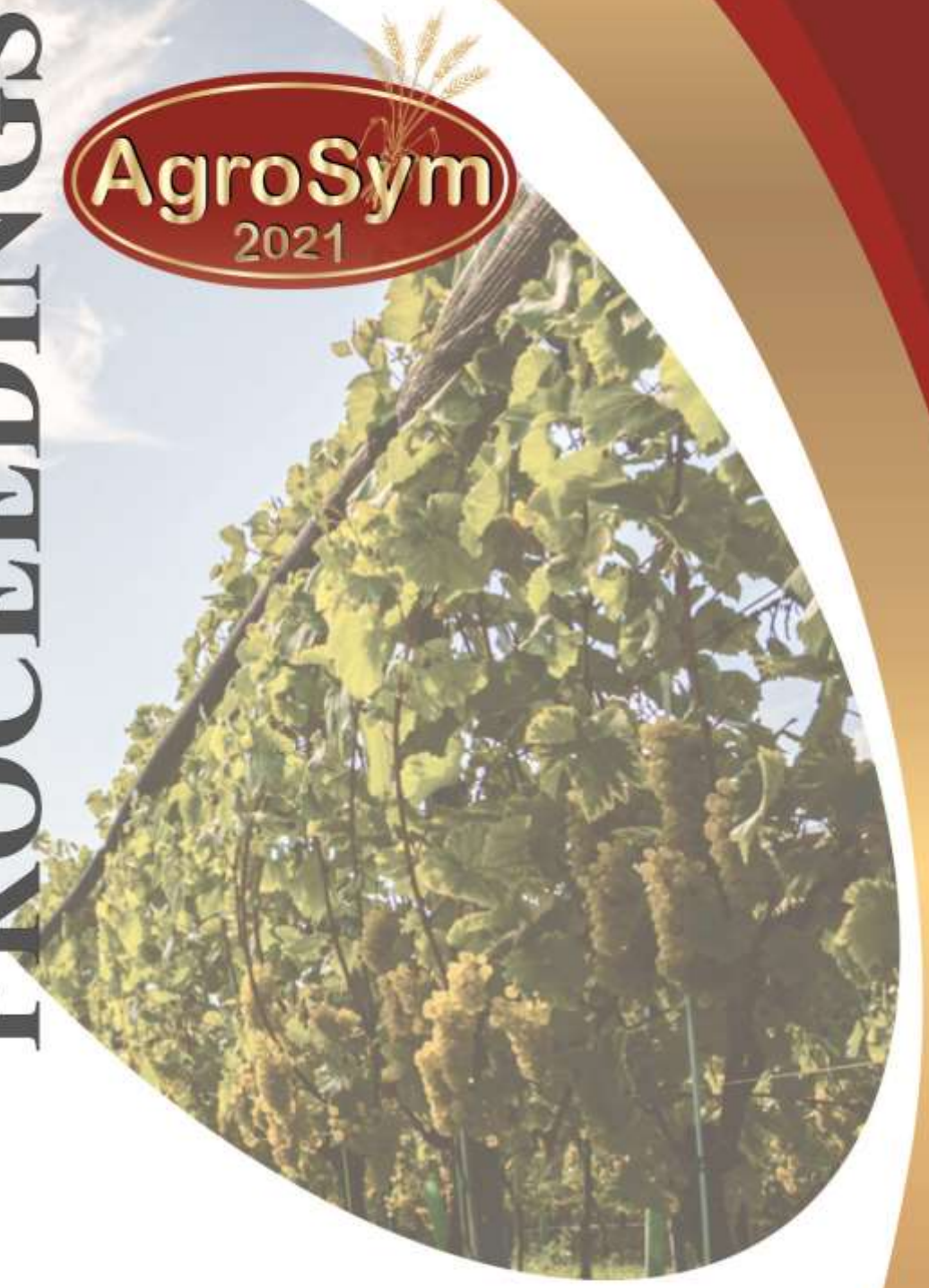
AgroSym 2021



## XII International Scientific Agriculture Symposium "AGROSYM 2021" October 7-10, 2021

agrosym 2021

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# ANTIOXIDANT ACTIVITY ESTIMATION OF INNER AND OUTER SEED FRACTIONS OF THE LEGUMES *VIGNA RADIATA* L. AND *GLYCINE MAX* L.

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## Abstract

Legumes have multiple functions in sustainable agriculture, but also are a favourable ingredient of functional nutrition. Antioxidants in legumes have several beneficial physiological properties and provide protection against chronic diseases. In this study, we compared the antioxidant activities of the seed fractions (outer and inner) for two different legumes: mung bean (*Vigna radiata* L.) and soybean (*Glycine max* L). The antioxidant activity was estimated using a modified DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay in a 96-well microplate. We showed that mung bean hulls possessed significantly higher ($p<0.05$) DPPH free radical scavenging activity (80.80 % ± 0.19) compared to their inner fraction (10.94 % ± 0.23), as well as to both fractions of the soybean. On the other hand, the soybean seeds' inner fractions (21.00 % ± 0.32) exhibited a significantly ($p<0.05$) higher activity than the hulls (8.78 % ± 0.71) and the inner fraction of the mung bean. The obtained results indicated that in each of the two analysed legume species, inner and outer seed fractions exhibited different antioxidant activities regarding to the elimination of the free radicals. The obtained results indicate that antioxidant capacity may be a useful indicator in the estimation of the quality of legume seeds as food and feed.

**Keywords:** *Antioxidant activity, Soybean, Mung bean, DPPH, Food quality.*

## Introduction

Leguminous seeds are considered as significant source of nutrients (proteins, essential amino acids, starch, fibers, minerals, vitamins) but they also, depending on the biological variety of the plants and their origin, contain different bioactive substances including phenolics. Secondary metabolites are involved in protection of the seeds against plant pathogens, wounds caused by insect pests and herbivores, UV radiation and other biotic and abiotic stress conditions (Yusnawan et al. 2019). Antioxidant activity of various phenolic compounds from legume seeds (flavonoids, alkaloids, tannins, phenolic acids) is important both from nutritional and technological points of view (Amarowicz and Pegg 2008). Legumes are excellent functional food ingredients and a great dietary source of antioxidants due to their nutrient composition (Ganesan and Xu, 2018). Health benefits related to antioxidants contained in edible food sources include reduced risk and prevention of developing major chronic diseases such as cancer, diabetes or cardiovascular diseases (Singh et al. 2017). Phenolic antioxidants and polyphenols, that protect the seeds against the harmful effect of oxygen free radicals, are typically present in high content in seed hulls. Mung bean seeds are consumed as a whole or after separation the hulls while soybean seed hulls are usually discarded as a byproduct. This biowaste has a high polyphenol content which could be extracted and used as nutraceuticals, a source of natural antioxidants, or used as animal feed (Singh et al. 2017).

Mung bean (*Vigna radiata* L.), a summer cultivated legume widely distributed and consumed throughout Asia, has a high nutrient value comparable to that of soybean (*Glycine max* L) (Shi et al. 2016; Orak et al. 2018). Mung bean also contains bioactive food components and polyphenols which possess a high antioxidant capacity (Orak et al. 2018). Soybean is an annual self-pollinated diploid leguminous plant, predominantly grown for use in human and animal diet and as a major source of protein and oil (Gaonkar and Rosentrater 2019). Aside from the nutritional value, soybean contains isoflavones and certain phenolic compounds (Wang and Komatsu 2017). The antioxidant potential of soybean has also proven to have health benefits, especially in their seed coat (Peiretti et al. 2019).

In the present study, differences in antioxidant capacity between two seed fractions (inner and outer) of the two different legume species, soybean and mung bean, were examined. Aqueous-ethanol extracts of both seeds and hulls were examined for their respective radical scavenging activity with DPPH (2,2-Diphenyl-1-picrylhydrazyl) reagent. In a reaction with antioxidants, DPPH is reduced to DPPH-H while the color changes from purple to yellow and the decrease in absorbance maximum at 517 nm is spectrophotometrically detected (Khan et al. 2017).

## Material and Methods

### Sample preparation

Soybean and Mung bean were purchased from the local market in Belgrade, Serbia. Seed hulls have been separated from the seed endosperm before homogenization, and considered as outer fractions. The rest of the seed (endosperm and embryo) was the inner fraction. Both fractions of the seed were grinded separately in a mill. The samples were further powdered in a mortar with pestle with liquid nitrogen and stored in the dark at 5 ± 3 °C until use.

### DPPH Assay

Antioxidant activity (AA %) of both legumes (mung bean and soybean) seeds and hulls, was carried out separately using DPPH (Sigma-Aldrich, St. Louis, MO, USA) test (Khan et al. 2017). Compared to the original method, described by Khan et al. 2017, the procedure was modified in terms of absorbance measurement; smaller sample volumes were required since a microplate reader was used instead of UV/VIS Spectrophotometer. This method enables rapid analysis and possibility of screening a large number of samples.

Powdered samples (8 mg) were extracted with 3 ml of 70 % ethanol. To each sample, 1 ml of freshly prepared 0.4 mM DPPH solution in 96 % ethanol was added to a final concentration of 0.1 mM. After 30 minutes of incubation with constant shaking in the dark, 200 µl aliquots were taken and absorbance at 517 nm was measured using a UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). Blank consisted of pure deionized water without any additions. Control contained 0.1 mM DPPH in 96 % ethanol solution. Antioxidant activity was calculated using the following equation (Khan et al. 2017):

$$A\,\% = 100 - \left(\frac{A_{sample} - A_{blank}}{A_{control}}\right) \times 100$$

The results were presented as a percentage of depleted DPPH-reagent. Readings were carried out in four replicates for each analysed seed fraction and, based on obtained data, the standard error was calculated. Mann-Whitney sum rank test has been used for statistical analysis of the obtained results.

## Results and Discussion

Images of mung bean and soybean seeds are shown in Figure 1. The obtained results indicate that mung bean seed coats possessed significantly higher ($p<0.05$) DPPH radical scavenging activity, according to Mann-Whitney test ($p=0.021$), 80.80 % ± 0.19, compared to their inner fraction of the seed being only 10.94 % ± 0.23 (Figure 2). Our results are consistent with previously reported studies showing that mung bean hull contained higher antioxidant capacity compared to the inner fraction of the seed (Singh et al. 2017). Pigmented grain such as mung bean contains a higher concentration of secondary metabolites especially in the seed coat. These phytochemicals contributing to antioxidant activity, such as phenolic acids and flavonoids (flavones, flavonoids, isoflavone, and isoflavonoids), are distributed unevenly through the seed fractions (Yusnawan et al. 2019). Some of the major polyphenols (caffeic acid, syringic acid, chlorogenic acid, ferulic acid and *p*-coumaric acid) have been detected in mung bean (Singh et al. 2017). Furthermore, water-soluble polysaccharides (containing mannose, rhamnose, and galactose) from mung bean hulls exhibit high DPPH radical-scaveninging activities (Ganesan and Xu, 2018).

Figure 1. Image of mung bean (left) and soybean (right) seeds.

Figure 2 shows antioxidant activity (AA %) of both inner and outer fractions of soybean. Soybean seeds' inner fraction showed a significantly higher antioxidant activity compared to the soybean seed coats, 21.00 % ± 0.32 and 8.78 % ± 0.71, respectively. These results are in accordance with Lim et al. (2021), who showed higher antioxidant activity in cotyledon compared to the seed coat.

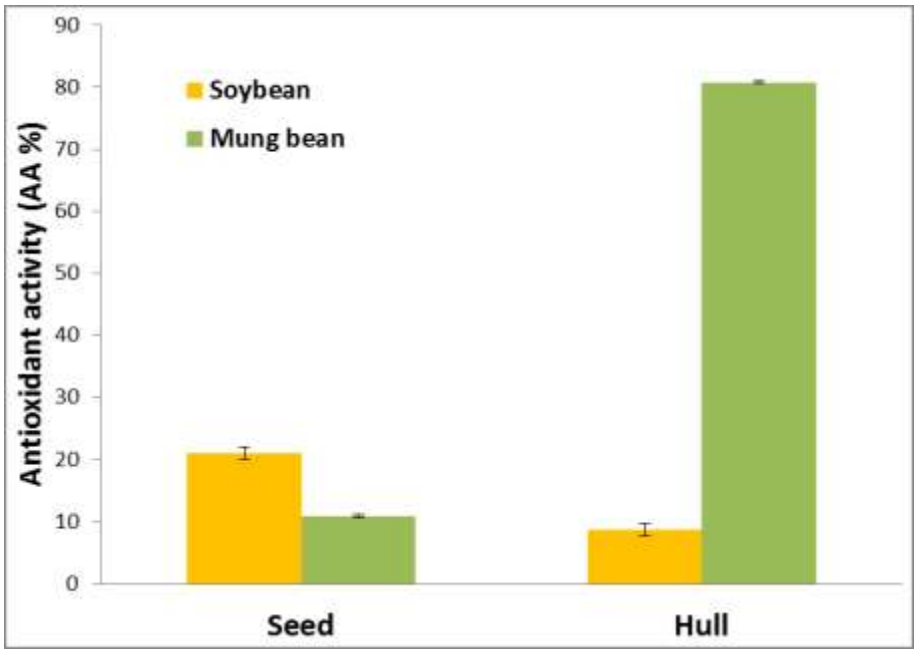


Figure 2. Antioxidant activity (AA %) of both inner and outer fractions of soybean and mung bean. Each value is expressed as the means out of 4 replicates with standard error.

## Conclusions

The obtained results indicate that comparing the two analysed legumes, the highest antioxidant capacity was exhibited in the mung bean hulls, almost 10 times higher than the activity of the same seed fraction of soybean. Each of the analysed legume seed fractions had a different antioxidant capacity. Antioxidant activity may be a reliable indicator of seed quality. Efficient radical scavenging activity of the outer fraction of the mung bean suggests that this legume may be evaluated as a functional food ingredient. The seeds possessing higher antioxidant potential could be suggested as a better food source due to their health benefits. The growing interest in the functional properties of mung bean and soybean has focused the attention of research studies towards bioactive compounds and their health benefits.

## Acknowledgement

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