

## COMPARISON OF PHENOLIC AND ANTIOXIDANT SUBSTANCE CONTENT OF SOME KIDNEY BEAN GENOTYPES

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### Abstract

Kidney bean, known for its valuable vegetable proteins, holds a significant place among leguminous plants due to its high protein content, as well as carbohydrates, vitamins, minerals, phenolic compounds, and antioxidant contents. Among leguminous plants identified as the most fundamental food for human consumption worldwide, kidney bean stands out not only for its rich nutritional content but also for significantly improving soil fertility, promoting advantageous dietary and health trends. The study aims to compare 15 different kidney bean genotypes in terms of total phenolics, total flavonoids, tannins, and antioxidant activities. Among the genotypes, genotype A4 has the highest content of total phenolic substance (572.09 mg GAE/g), total flavonoid substance (49.37 mg CE/g), DPPH (92.14%), ABTS (14.26  $\mu$ g TE/g), total hydrolyzable tannin (8.94 mg/kg), and ferric reducing antioxidant activity (10.55 mg AAE/g), while genotype A7 has the lowest value in terms of total phenolic substance (153.01 mg GAE/g), DPPH (28.92%), condensed tannin (102.86 mg/kg), ferric reducing antioxidant activity (3.87 mg AAE/g), and ABTS (4.71  $\mu$ g TE/g). The study concludes that the different colors of the identified genotypes lead to significant differences in phenolic and antioxidant contents.

**Keywords:** antioxidant, kidney bean, phenolic

### 1. INTRODUCTION

Kidney beans, a variety of common beans, rank as the world's second most important legume product after soybeans and are known to be edible legumes rich in protein, carbohydrates, and starch (Vanier et al., 2012). In addition to containing carbohydrates that are vital for human nutrition, it is consumed as an alternative to animal protein in order to prevent health problems related to deficiency of meat consumption. Widely cultivated in Africa, India, Latin America, and Mexico, kidney beans are also a rich source of phytochemicals, including vitamins, anthocyanins, flavonoids, polyphenols, and phenolics (Kan et al., 2018).

Phenolic compounds are phytochemicals found in all plants, consisting of simple phenols such as flavonoids, lignans, tannins and lignins (Khoddami et al., 2013). It is known to be one of the most important factors that make phenolic compounds, which belong to the class of secondary plant metabolites and have radical scavenging ability among its most distinctive features (Djeridane et al., 2007). Antioxidant components include some phenolic compounds as tocopherols, flavonoids, phenolic compounds, carotenoids, and ascorbic acids (Qualities, 2001). Flavonoids are a type of secondary metabolites that are essential for maintaining the relationship between plants and their environment. They have the ability to react with free radicals and neutralize harmful reactive

oxygen species that can be produced during metabolism. This property of flavonoids is crucial in preventing potential damage to the body. (Mathew and Abraham, 2006; Atınç and Kalkan, 2018). Natural antioxidants, which are responsible for avoiding the negative effects of oxidative stress, are considered to be dependent on the presence of substances such as alkaloids, glycosides, flavonoids, and tannins as determined by phytochemical assays (Mathew and Abraham, 2006). Studies have shown that condensed tannins can hinder the function of several digestive enzymes, including pectinase, amylase, lipase, cellulose, and microbial enzymes that ferment grains (Ergezer and Çam, 2008). Studies have shown that condensed tannins can hinder the function of several digestive enzymes, including pectinase, amylase, lipase, cellulose, and microbial enzymes that ferment grains (Ergezer and Çam, 2008). The production of tannins and phenolic compounds by plants, which offer numerous health benefits to humans, is influenced by their genetic makeup, physiological requirements, and defense mechanisms. They have been known to vary in biological activity due to differences in the nutritional and phytochemical composition of certain varieties (Germ et al., 2010).

## 2. MATERIALS AND METHODS

### Extraction preparation and analysis methods

Xu and Cahng, (2007), in their study, different solvents and protocols mentioned were tested on kidney bean samples, and the extraction solvent (80:20 /acetone: water) that gave the best results was determined and the resulting extracts were used for further analyses. The total phenolic content was analyzed using the method described by Singleton and Rossi (1965), with results expressed as mg GAE g<sup>-1</sup> of dry sample. The total flavonoid content was evaluated following the method of Zhisten et al. (1999), and results were reported as mg CE 100 g<sup>-1</sup> of dry sample. The ferrous ion chelating activity of the extracts was assessed using the methodologies outlined by Rival et al. (2001) and Duh et al. (2001). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to the procedure specified by He et al. (2011), and the activity was expressed as % inhibition. Ferric reducing antioxidant power (FRAP) was measured as described by Kaplan et al. (2021). The ABTS<sup>+</sup> radical scavenging activity was determined based on the method developed by Sun et al. (1998), with results expressed as % inhibition. Condensed tannin content was quantified using the approach recommended by Kaplan et al. (2023), with results provided in mg/kg dry sample. Hydrolyzable tannins were analyzed using the procedure specified by Kaplan et al. (2023).

### Statistical Analysis

The experimental design was completely randomized design with three replications. Data were analyzed using the SAS packet program.

## 3. RESULTS AND DISCUSSIONS

The outcomes are presented in Table 1. Based on the findings, it was discovered that the A4 genotype exhibited the highest levels of phenolic and antioxidant substances compared to the other kidney bean genotypes investigated.

A4 genotype has a higher level in terms of total phenolic substance (572.09 mg GAE/g), total flavonoid substance (49.37 mg CE/g), DPPH (92.14%), ABTS (14.26 µg TE/g), total hydrolyzable tannin (8.94 mg/kg) and ferric reducing antioxidant activity (10.55 mg AAE/g) content as depicts in Table 1. The findings obtained by Aydar et al. (2022) were similar regarding antioxidant activity.

**Table 1: Total phenolic, flavonoid, tannin and antiradical and antioxidant activity values of some kidney bean genotypes**

Genotypes	TPC	TFC	DPPH	ICA	CT	THT	FRAA	ABTS
A1	431.37 <sup>b</sup>	37.59 <sup>b</sup>	81.49 <sup>b</sup>	91.70 <sup>a</sup>	851.90 <sup>c</sup>	0.73 <sup>g</sup>	8.15 <sup>b</sup>	8.09 <sup>cde</sup>
A2	340.67 <sup>e</sup>	30.94 <sup>d</sup>	64.19 <sup>d</sup>	87.24 <sup>ef</sup>	661.07 <sup>d</sup>	2.32 <sup>d</sup>	7.82 <sup>bc</sup>	10.44 <sup>bc</sup>
A3	316.09 <sup>f</sup>	15.32 <sup>gh</sup>	58.58 <sup>ef</sup>	83.04 <sup>g</sup>	2426.90 <sup>a</sup>	1.99 <sup>e</sup>	7.72 <sup>bc</sup>	9.37 <sup>cd</sup>
A4	572.09 <sup>a</sup>	49.37 <sup>a</sup>	92.14 <sup>a</sup>	89.22 <sup>bcde</sup>	1240.72 <sup>b</sup>	8.94 <sup>a</sup>	10.55 <sup>a</sup>	14.26 <sup>a</sup>
A5	254.13 <sup>h</sup>	18.41 <sup>fg</sup>	45.25 <sup>h</sup>	88.50 <sup>cdef</sup>	286.55 <sup>j</sup>	1.59 <sup>f</sup>	5.18 <sup>ef</sup>	6.63 <sup>efg</sup>
A6	324.70 <sup>f</sup>	26.84 <sup>e</sup>	61.29 <sup>edf</sup>	89.69 <sup>abcd</sup>	524.76 <sup>ef</sup>	0.77 <sup>g</sup>	6.36 <sup>d</sup>	7.39 <sup>def</sup>
A7	153.01 <sup>k</sup>	12.22 <sup>h</sup>	28.92 <sup>j</sup>	87.67 <sup>def</sup>	102.86 <sup>l</sup>	0.20 <sup>h</sup>	3.87 <sup>h</sup>	4.71 <sup>g</sup>
A8	251.39 <sup>h</sup>	20.39 <sup>f</sup>	44.87 <sup>h</sup>	91.26 <sup>ab</sup>	313.69 <sup>i</sup>	0.63 <sup>g</sup>	5.09 <sup>ef</sup>	7.43 <sup>def</sup>
A9	252.72 <sup>h</sup>	20.31 <sup>f</sup>	51.10 <sup>g</sup>	89.62 <sup>abcd</sup>	333.09 <sup>i</sup>	0.020 <sup>h</sup>	5.43 <sup>e</sup>	6.43 <sup>efg</sup>
A10	209.12 <sup>i</sup>	16.29 <sup>g</sup>	41.04 <sup>hi</sup>	91.46 <sup>a</sup>	384.40 <sup>h</sup>	0.043 <sup>h</sup>	4.33 <sup>gh</sup>	6.51 <sup>efg</sup>
A11	189.51 <sup>j</sup>	7.92 <sup>i</sup>	38.2 <sup>i</sup>	86.65 <sup>f</sup>	246.67 <sup>k</sup>	0.077 <sup>h</sup>	4.76 <sup>gf</sup>	5.48 <sup>fg</sup>
A12	364.32 <sup>d</sup>	35.23 <sup>bc</sup>	62.45 <sup>de</sup>	90.54 <sup>abc</sup>	543.57 <sup>e</sup>	0.13 <sup>h</sup>	6.53 <sup>d</sup>	12.49 <sup>ab</sup>
A13	280.9 <sup>g</sup>	17.55 <sup>fg</sup>	55.89 <sup>gf</sup>	82.15 <sup>g</sup>	510.36 <sup>f</sup>	2.60 <sup>c</sup>	7.88 <sup>bc</sup>	7.75 <sup>efg</sup>
A14	381.12 <sup>c</sup>	30.14 <sup>de</sup>	74.70 <sup>c</sup>	89.65 <sup>abcd</sup>	856.07 <sup>c</sup>	4.10 <sup>b</sup>	7.52 <sup>c</sup>	10.36 <sup>bc</sup>
A15	324.04 <sup>f</sup>	33.42 <sup>cd</sup>	61.58 <sup>de</sup>	88.55 <sup>cdef</sup>	455.95 <sup>g</sup>	1.71 <sup>f</sup>	6.44 <sup>d</sup>	10.35 <sup>bc</sup>
Sg Df.	**	**	**	**	**	**	**	**
<b>LSD</b>	<b>9.9816</b>	<b>3.42</b>	<b>5.5169</b>	<b>2.0839</b>	<b>20.59</b>	<b>0.2716</b>	<b>0.585</b>	<b>2.444</b>

**TFC:** Total flavonoid content (mg CE/g), **TPC:** Total phenolic content (mg GAE/g), **DPPH:** DPPH scavenging activity (%), **CT:** Condensed tannin (mg/kg), **THT:** Total hydrolyzable tannin (mg/kg), **ICA:** Iron chelating activity (%), **FRAA:** Ferric reducing antioxidant activity (mg AAE/g), **ABTS:** ABTS scavenging activity (μg TE/g)

Among the genotypes, the A7 was determined to be the genotype with the lowest value in terms of total phenolic substance (153.01 mg GAE/g), DPPH (28.92%), condensed tannin (102.86 mg/kg), ferric reducing antioxidant activity (3.87 mg AAE/g) and ABTS (4.71 μg TE/g). It was found that the A11 genotype had the lowest total flavonoid substance content, measuring at 7.92 mg CE/g. Additionally, it was discovered that the phenolic substance content and total flavonoid substance content of kidney bean seeds were higher compared to the values obtained in the studies conducted by Kan et al. (2017) and Carbas et al. (2020). This difference in content can be attributed to the different genotypes used in the study.

The hydrolyzable tannin rate ranged from 0.02 mg/kg to 8.94 mg/kg, with the lowest rate found in the A9 genotype. The A1 (91.70%) and A10 (91.46%) genotypes had the highest iron chelating activity rates, while A13 (82.15%) and A3 (83.04%) had the lowest values.



**Figure 1: 15 Kidney bean genotypes used in the study**

The condensed tannin ratio varied between 2426.90- 102.86 mg/kg. The highest value was detected in the A3 (2426.90 mg/kg) genotype. A study conducted by Caldas and Blair, (2009) showed that the condensed tannin content in kidney bean seeds is related to the seed coat, which showed similar results that are given in Figure 1.

#### 4. CONCLUSIONS

This study aimed to conduct a comparative analysis of the total phenolic, total flavonoid, tannin, and antioxidant activities of 15 different kidney bean genotypes. The results revealed that genotype A4 had the highest content of phenolic and antioxidant compounds, while the lowest values were obtained from genotype A7. Condensed tannin and hydrolysable tannin contents were highest in genotypes A3 and A4, respectively. Considering the seed coat color of the genotypes that yielded the highest results among the parameters examined, it was observed that genotypes with dark-colored seed coats provided higher values. Based on the current findings, it was determined that the seed coat color of kidney beans affects the phenolic content, antioxidant content, and tannin content.

#### 5. REFERENCES

- Atınç, M., & Kalkan, İ., Flavonoids and their effects on health, *Aydın Gastronomy*, 2(1), 31–38, (2018).
- Aydar, E. F., Mertdinç, Z., Demircan, E., Koca Çetinkaya, S., & Özçelik, B. (2023). *Innovative Food Science and Emerging Technologies*, 83,(2022).
- Caldas, G. V., & Blair, M. W., Kidney bean (*Phaseolus vulgaris* L.) milk substitute as a novel plant-based drink: Fatty acid profile, antioxidant activity, in-vitro phenolic bio-accessibility and sensory characteristics., *Theo. and Applied Genetics*, 119(1), 131–142,(2009).
- Carbas, B., Machado, N., Oppolzer, D., Ferreira, L., Queiroz, M., Brites, C., Rosa, E. A. S., & Barros, A. I. R. N. A., Nutrients, antinutrients, phenolic composition, and antioxidant activity of common bean cultivars and their potential for food applications. *Antioxidants*, 9(2), (2020).
- Duh, P.D., Yen, G.C., Yen, W.J., Chang, L.W., J., Antioxidant effects of water extracts from barley (*Hordeum vulgare* L.) prepared under different roasting temperatures., *Agric. Food Chem.* 49, 1455–1463, (2001).
- Djeridane, A., Yousfi, M., Nadjemi, B., Vidal, N., Lesgards, J. F., & Stocker, P., Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. *European Food Research and Technology*, 224(6), 801–809. (2007).
- Ergezer, H., & Çam, M., Tannins: Classification, structure and health effects., *Türkiye*, 10, 229-232, (2008).
- Germ, M., Stibilj, V., Kreft, S., Gaberščik, A., & Kreft, I., Flavonoid, tannin and hypericin concentrations in the leaves of St. John's wort (*Hypericum perforatum* L.) are affected by UV-B radiation levels. *Food Chemistry*, 122(3), 471–474. (2010).

- He, L., Xu, H., Liu, X., He, W., Yuan, F., Hou, Z., Identification of phenolic compounds from pomegranate (*Punica granatum* L.) seed residues and investigation into their antioxidant capacities by HPLC–ABTS+ assay. *Food Res. Int.* 44, 1161–1167, (2011).
- Kan, L., Nie, S., Hu, J., Wang, S., Bai, Z., Wang, J., Zhou, Y., Jiang, J., Zeng, Q., & Song, K., *Food Chemistry*, 260(December 2017), 317–326. (2018).
- Kan, L., Nie, S., Hu, J., Wang, S., Cui, S. W., Li, Y., Xu, S., Wu, Y., Wang, J., Bai, Z., & Xie, M., *Food and Chemical Toxicology*, 108, 467–477, (2017).
- Kaplan, M., Yüksel, F., Karaman, K., *In vitro* glycemic index, antioxidant capacity and some physicochemical characteristics of deep-fried sorghum based gluten free chips. *J. Food Sci. Technol.* 58 (10), 3725–3733, (2021).
- Kaplan, M., Say, R., Köprü, S., Karaman, K., Sunulu, S., & Yılmaz, M. M., Morphological, physicochemical, and bioactive characteristics of *Hypericum perforatum* genotypes, and their classification by GT-Biplot approach. *Biochemical Systematics and Ecology*, 111, 104721, (2023).
- Khoddami, A., Wilkes, M. A., & Roberts, T. H., Techniques for analysis of plant phenolic compounds. *Molecules*, 18(2), 2328–2375. (2013).
- Mathew, S., Abraham, T.E., Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food Chem.*, (2006), 94, 520–528.
- Rival, S.G., Boerriu, C.G., Wichers, H.J., Caseins and casein hydrolysates. 2. Antioxidative properties and relevance to lipoxygenase inhibition. *J. Agric. Food. Chem.* 49, 295–302, (2001).
- Singleton, V. L., & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144–158, (1965).
- Sun, B., Ricardo-da-Silva, J.M., Spranger, I., Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. and Food Chem.*, 46, 4267–4274, (1998).
- Vanier, N. L., Da Rosa Zavareze, E., Pinto, V. Z., Klein, B., Botelho, F. T., Dias, A. R. G., & Elias, M. C. Physicochemical, crystallinity, pasting and morphological properties of bean starch oxidised by different concentrations of sodium hypochlorite. *Food Chemistry*, 131(4), 1255–1262 (2012).
- Zhishen, J., Mengcheng, T., & Jianming, W., In *Food Chemistry* (Vol. 64, Issue 4, pp. 555–559), (1999).
- Qualities, N. S., Sensory and nutritive qualities of food antioxidant potential of pea beans (*Phaseolus vulgaris* L.) *Science*. (2001).
- Xu, B. J., & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science*, 72(2).