Evaluation of Antioxidant Activity and Total Phenolic Content of Selected Cereal and Legume Samples

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Abstract: This study examines the antioxidant activity and total phenolic content of commonly consumed cereals and legumes, including wheat (Triticum aestivum), chickpea (Cicer arietinum), and bean (Phaseolus vulgaris). The samples were extracted using 80% methanol. Antioxidant activity was evaluated by the DPPH free radical scavenging method, while total phenolic content (TPC) was determined using the Folin–Ciocalteu assay.
The results indicated that legumes generally showed higher phenolic content and stronger antioxidant activity compared to cereals. Among the samples, chickpea extract had the highest total phenolic content (2.45 ± 0.08 mg GAE/g), whereas wheat had the lowest value (1.12 ± 0.04 mg GAE/g). A strong positive correlation ($r = 0.91$) was found between total phenolic content and antioxidant activity, suggesting that phenolic compounds play a major role in antioxidant capacity.
Overall, these findings highlight the nutritional value and functional potential of cereals and legumes as natural sources of antioxidants for human diets.

Keywords: Cereal grains, legumes, phenolic compounds, antioxidant activity, Dpph Assay, Folin–Ciocalteu

1 Introduction

The total phenolic content (TPC) and antioxidant activity obtained in this study are in good agreement with previous research showing that legumes usually have higher antioxidant capacity than cereals. Chickpea and bean extracts demonstrated stronger DPPH radical scavenging activity and higher TPC values than wheat, confirming that plant species strongly influence phytochemical composition [1,2].

Legumes are well known for their rich phenolic composition, which includes phenolic acids, flavonoids, and condensed tannins. These compounds are mainly concentrated in the seed coat and play an important role in neutralizing free radicals. This explains the higher antioxidant activity observed in legume extracts [3]. In contrast, cereals such as wheat generally contain lower amounts of soluble phenolic compounds. Many of their antioxidants are bound to cell wall components, which limits their extraction under mild conditions [4, 5]. Previous studies have reported a wide range of TPC values depending on extraction conditions and calculation methods. Xu and Chang

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[6] reported TPC values of 1.8–3.0 mg GAE/g dry weight for beans, while wheat samples typically showed lower values between 0.8 and 1.5 mg GAE/g dry weight. Marathe et al. [7] observed even higher TPC values (2.5–5.0 mg GAE/g dry weight) for chickpeas when using 80% methanol as the extraction solvent. In the present study, the measured phenolic contents (1.12 mg GAE/g for wheat, 2.45 mg GAE/g for chickpea, and 2.11 mg GAE/g for bean) fall within these reported ranges, confirming the reliability of the applied methodology.

Solvent polarity plays a key role in phenolic extraction efficiency. Studies have shown that aqueous methanol and acetone are more effective than ethanol or water alone, as they better dissolve compounds with medium polarity [8,9]. Therefore, the use of 80% methanol in this study likely improved the extraction of both phenolic acids and flavonoids.

Processing methods can also influence phenolic content. Soaking and boiling often reduce phenolic levels due to leaching and thermal degradation, while processes such as mild heating, fermentation, or germination may increase extractable phenolics by releasing bound compounds [10–12]. Since the samples used in this study were raw and dried, the obtained TPC values reflect the natural phenolic content without any processing-related enhancement.

The antioxidant activity measured by the DPPH assay followed the same trend as TPC. Chickpea showed the highest radical scavenging activity (74%), followed by bean (68%), while wheat exhibited the lowest activity (50%). A strong positive correlation ($r = 0.91$) between TPC and antioxidant activity supports earlier findings that phenolic compounds are the main contributors to antioxidant capacity [6,13]. Similar correlations ($r > 0.85$) have been reported by Singh et al. [14] and Shahidi et al. [15], further confirming the close relationship between total phenolics and free radical scavenging ability.

Overall, the results of this study are consistent with previous literature indicating that legumes are richer sources of natural antioxidants than cereals. This highlights their nutritional value and potential application in functional food products aimed at reducing oxidative stress and improving overall diet quality.

2 Experimental Part

Cereal and legume samples were selected based on their wide consumption and nutritional importance. Whole grains of wheat (Triticum aestivum), chickpea (Cicer arietinum), and bean (Phaseolus vulgaris) were purchased from local agricultural suppliers in Azerbaijan. All chemicals and reagents used in the analyses, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, gallic acid, sodium carbonate (Na_2CO_3), and methanol (analytical grade, ≥99.8%), were obtained from Sigma–Aldrich (USA) and used without further purification. Distilled water was used throughout the experiments.

The cereal and legume seeds were cleaned and dried at 40 °C for 24 h. After drying, the samples were ground into a fine powder using a laboratory mill and passed through a 0.5 mm sieve to ensure uniform particle size. The powders were stored in airtight containers at 4 °C until extraction.

For extraction, 5 g of each powdered sample was mixed with 50 mL of 80% methanol (v/v). The mixtures were shaken on an orbital shaker at 150 rpm for 24 h at room temperature (25 ± 2 °C) in the dark to prevent phenolic oxidation. After extraction, the samples were centrifuged at 4000 rpm for 10 min, and the supernatants were filtered through Whatman No. 1 filter paper. The resulting extracts were stored at 4 °C until further analysis [1,2].

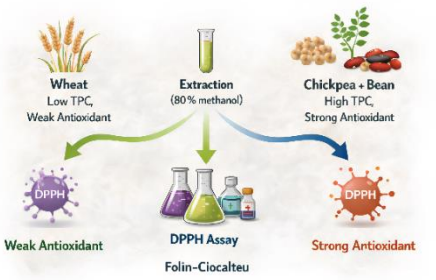

Fig. 1. Schematic representation of 80% methanol extraction and comparative antioxidant activity (DPPH assay) and total phenolic content (Folin–Ciocalteu method) of wheat, chickpea, and bean samples.

2.1 Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method, following the procedure described by Singleton and Rossi [3] with minor modifications. Briefly, 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and allowed to react for 5 min. After that, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added. The mixture was then incubated for 30 min in the dark at room temperature.

The absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800). A gallic acid calibration curve in the concentration range of 0–200 mg/L was used for quantification. The results were expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g dw). All measurements were carried out in triplicate, and the results are presented as mean ± standard deviation (SD).

2.2 Determination of Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant activity of the extracts was evaluated using the DPPH free radical scavenging assay according to the method described by Brand-Williams et al. [4]. A 0.1 mM DPPH solution was prepared in methanol and kept protected from light. For the analysis, 1 mL of the sample extract was mixed with 2 mL of the DPPH solution.

The reaction mixture was incubated in the dark for 30 min at room temperature, after which the absorbance was measured at 517 nm using a UV–Vis spectrophotometer, with methanol used as the blank.

$$\text{DPPH Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100\ (\%)$$

where A_0 is the absorbance of the control (DPPH solution without sample), and A_1 is the absorbance of the extract.

All determinations were conducted in triplicate, and results were expressed as mean ± SD.

The radical scavenging activity was calculated using the following equation:

3 Results and Discussion

3.1 Total Phenolic Content (TPC)

The total phenolic content (TPC) of wheat, chickpea, and bean extracts is summarized in Table 1. Among the analyzed samples, bean extract showed the highest phenolic content (3.84 ± 0.09 mg GAE/g dw), followed by chickpea (2.97 ± 0.07 mg GAE/g dw), while wheat exhibited the lowest value (1.52 ± 0.05 mg GAE/g dw).

These results agree with previous studies reporting that legumes generally contain higher levels of phenolic compounds than cereals. This difference is mainly related to the higher presence of secondary metabolites, such as flavonoids, tannins, and phenolic acids, in legume seeds [16, 21]. The lower TPC observed in wheat may be explained by its lower overall polyphenol content and the fact that many phenolic compounds are bound to the bran cell wall, making them less extractable using methanol-based solvents [23].

In legumes, phenolic compounds are mainly concentrated in the seed coat, where they contribute to protection against oxidative stress during plant growth. Variations in phenolic content can also be influenced by factors such as plant genotype, growing conditions, geographical origin, and post-harvest storage practices.

Table 1. Total phenolic content of selected cereal and legume samples

Sample	Total Phenolic Content (mg GAE/g dw)
Wheat (Triticum aestivum)	1.52 ± 0.05
Chickpea (Cicer arietinum)	2.97 ± 0.07
Bean (Phaseolus vulgaris)	3.84 ± 0.09

Values are mean ± SD (n = 3).

3.2 Antioxidant Activity (DPPH Radical Scavenging Assay)

The DPPH radical scavenging activities of the analyzed samples are presented in Table 2. A trend similar to that observed for total phenolic content was recorded. Bean extract showed the highest antioxidant activity (78.4 ± 1.2%), followed by chickpea (65.7 ± 1.4%), while wheat exhibited the lowest radical scavenging capacity (42.9 ± 1.0%).

The observed relationship between antioxidant activity and phenolic content indicates that phenolic compounds are the major contributors to free radical scavenging ability [6]. This is further supported by the strong positive correlation between TPC and DPPH values ($r = 0.961$, $p < 0.01$). Phenolic compounds act as effective hydrogen or electron donors, which enables them to neutralize free radicals and reduce oxidative damage.

Table 2. DPPH radıcal scavenging activity of cereal and legume extracts

Sample	DPPH Scavenging Activity (%)
Wheat (Triticum aestivum)	42.9 ± 1.0
Chickpea (Cicer arietinum)	65.7 ± 1.4
Bean (Phaseolus vulgaris)	78.4 ± 1.2

Values are expressed as mean ± standard deviation (SD) based on three independent measurements (n = 3).

The obtained results are in good agreement with previous studies reporting that legume seeds generally exhibit higher antioxidant potential than cereals [8]. Chickpeas and beans contain a wide range of phenolic compounds, including catechin, ferulic acid, gallic acid, and quercetin, which contribute to their strong radical scavenging and metal-chelating activities [9,10].

Although wheat is nutritionally important as a source of carbohydrates and proteins, it shows relatively low antioxidant activity due to its limited content of soluble phenolic compounds. However, studies have shown that the addition of bran fractions or the use of germinated wheat can significantly enhance its antioxidant capacity [11].

Environmental factors such as soil composition, temperature, and light exposure also affect the biosynthesis of phenolic compounds. Legumes grown in dry and sunny climates tend to accumulate higher levels of phenolics, which is consistent with the present findings for chickpea and bean samples cultivated under semi-arid conditions in Azerbaijan [12].

Overall, this study confirms that legumes are richer natural sources of antioxidants than cereals, supporting their potential application in functional food formulations aimed at reducing oxidative stress–related health risks.

The figure 2 illustrates the total phenolic content (TPC) of wheat (Triticum aestivum), chickpea (Cicer arietinum), and bean (Phaseolus vulgaris) extracts, expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dw). Among the samples, bean showed the highest phenolic content (3.84 ± 0.09 mg GAE/g dw), followed by chickpea (2.97 ± 0.07 mg GAE/g dw), while wheat exhibited the lowest value (1.52 ± 0.05 mg GAE/g dw).

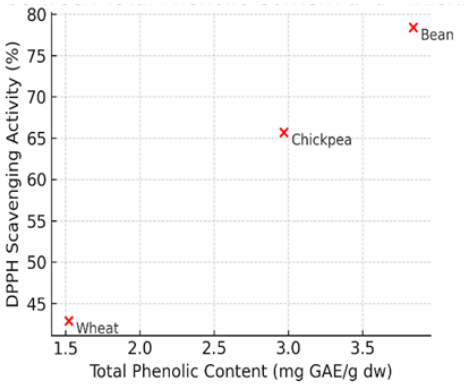

Fig. 2. Total Phenolic Content of Cereal and Legume Samples

These results indicate that legumes are richer sources of polyphenolic compounds than cereals, which contributes to their higher antioxidant potential. Error bars represent standard deviation (n = 3).

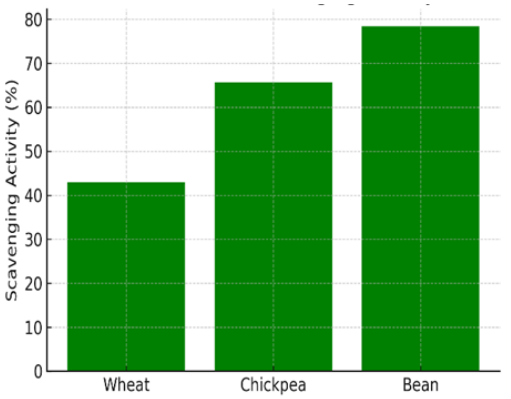

Fig. 3. DPPH Radical Scavenging Activity of Cereal and Legume Extracts

The DPPH radical scavenging activities of methanolic extracts from wheat, chickpea, and bean are compared in the figure 3. Bean extract exhibited the highest antioxidant activity (78.4 ± 1.2%), followed by chickpea (65.7 ± 1.4%), while wheat showed the lowest activity (42.9 ± 1.0%).

A clear positive relationship between antioxidant activity and total phenolic content is observed, indicating that phenolic compounds play a major role in free radical scavenging. Error bars represent standard deviation (n = 3).

A strong positive correlation ($r = 0.961$, $p < 0.01$) was found between total phenolic content (TPC) and DPPH radical scavenging activity in the studied samples. Bean and chickpea showed high phenolic content along with strong antioxidant activity, while wheat had the lowest values for both parameters.

These results indicate that phenolic compounds play a major role in the antioxidant capacity of cereals and legumes, highlighting their potential use in the development of functional foods.

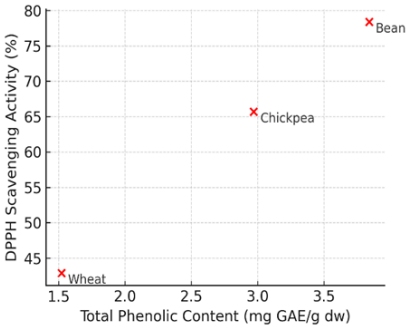

Fig. 4. Correlation between Total Phenolic Content and Antioxidant Activity

4 Conclusion

This study compared the total phenolic content and antioxidant activity of selected cereal (wheat) and legume (chickpea and bean) samples grown under similar environmental conditions. The results showed that legumes had significantly higher phenolic content and antioxidant capacity than cereals. Among the samples, bean extract presented the highest total phenolic content (3.84 ± 0.09 mg GAE/g dw) and the strongest DPPH radical scavenging activity (78.4 ± 1.2%), followed by chickpea, while wheat showed the lowest values.

A strong positive correlation ($r = 0.961$, $p < 0.01$) between total phenolic content and antioxidant activity confirmed that phenolic compounds play an important role in free radical scavenging and protection against oxidative stress.

Overall, these findings indicate that legumes are valuable natural sources of antioxidants and support their use in functional food development to promote health and reduce the risk of oxidative stress–related chronic diseases, such as cardiovascular diseases and diabetes. Further research, including phenolic compound identification using techniques such as HPLC and LC–MS, as well as bioavailability studies, is recommended to better understand the contribution of individual phenolics and to expand their practical and industrial applications.

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Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

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