e-ISSN: 2687-6698 Research Article

Turkish Journal of

Analytical Chemistry

TurkJAC

https://dergipark.org.tr/tr/pub/turkjac

# Comparative evaluation of antioxidant activity and phenolic content of *Lupinus albus* L. (white bitter lupin, termiye) grown in different regions of Türkiye by RP-HPLC-PDA

**Yakup Kara[1], Zehra Can[2]***

[1] Karadeniz Technical University, Faculty of Science, Department of Chemistry, 61080 Trabzon, Türkiye
[2] Bayburt University, Faculty of Applied Sciences, Department of Emergency Aid and Disaster Management, 69000 Bayburt, Türkiye

## Abstract

*Lupinus albus* L. (white bitter lupin) is an important legume species widely cultivated in the Mediterranean region. This study aimed to comparatively evaluate the antioxidant capacity and phenolic composition of L. albus samples collected from different regions of Türkiye. Samples were obtained from Konya (Centre, Akşehir, Doğanhisar), Isparta (Central), and Burdur (Central). Total phenolic content, total flavonoid content and antioxidant activities of samples were determined using FRAP and DPPH assays. Additionally, the qualitative and quantitative profiles of phenolic compounds were analyzed by RP-HPLC-PDA. The total phenolic content was determined to be in the range of 1.96–2.47 mg GAE/g, whereas flavonoids were not detected in any of the samples. The results demonstrated that *Lupinus albus* L. extracts exhibited notable antioxidant activity, with FRAP values ranging from 1.14 to 1.95 μmol $Fe^{2+}$/g and DPPH ($SC_{50}$) values ranging from 37.98 to 56.60. The phenolic composition analysis revealed the presence of major compounds including *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, chrysin, pinocembrin, and galangin. Overall, the results indicate that *L. albus* exhibits moderate antioxidant potential despite its relatively low phenolic content, and its phenolic composition is influenced by geographical origin.

**Keywords:** *Lupinus albus* L., antioxidant activity, DPPH, FRAP, phenolic profile

## 1. Introduction

Plant-derived bioactive compounds have attracted considerable attention due to their potential roles in preventing oxidative stress-related diseases. Among these, phenolic compounds are particularly important because of their strong antioxidant properties, enabling them to scavenge reactive oxygen species (ROS) and reduce oxidative damage at the cellular level [1]. Consequently, the identification and characterization of phenolic compounds, along with the evaluation of antioxidant capacity, have become central topics in food science and pharmacological research.

Leguminous plants belonging to the Fabaceae family are well known for their high protein content as well as their richness in secondary metabolites such as phenolics and flavonoids. These compounds are associated with various biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects [2]. Among these plants, *Lupinus albus* L. has gained increasing attention due to its nutritional composition and functional properties. Previous studies have reported that lupin species contain significant levels of bioactive compounds contributing to their health-promoting potential [3,4].

Despite these promising characteristics, studies focusing on the phenolic composition and antioxidant activity of *Lupinus albus* L. remain limited, particularly with respect to geographical variability. Environmental factors such as climate, soil composition, and cultivation conditions are known to influence the biosynthesis of phenolic compounds, leading to variations in antioxidant activity [5]. Therefore, comparative analyses of samples collected from different regions are essential to better understand these variations.

The aim of this study was to comparatively evaluate the antioxidant capacity and phenolic composition of

**Citation:** Y. Kara, Z. Can, Comparative evaluation of antioxidant activity and phenolic content of *Lupinus albus* L. (white bitter lupin, termiye) grown in different regions of Türkiye by RP-HPLC-PDA, Turk J Anal Chem, 8(2), 2026, 117–121.

***Corresponding author:** Zehra Can
**e-mail:** zcan@bayburt.edu.tr
**Received:** April 23, 2026 **Accepted:** May 14, 2026
https://doi.org/10.51435/turkjac.1930141

*Lupinus albus* L. samples collected from different geographical regions of Türkiye. In this context, total phenolic and total flavonoid contents, as well as antioxidant activities determined by DPPH and FRAP assays, were investigated. Additionally, the qualitative and quantitative profiles of phenolic compounds were characterized using RP-HPLC-PDA analysis. Through this comprehensive approach, the study seeks to provide insight into the bioactive potential of *Lupinus albus* L. and to assess the influence of geographical variation on its phenolic composition and antioxidant properties.

## 2. Materials and methods

### 2.1. Plant material and sample code

*Lupinus albus* L. samples were collected from different geographical locations in Türkiye in April 2023. Detailed information regarding the sampling locations and corresponding sample codes is provided in Table 1.

**Table 1.** Names of the samples, locations, years, and sample codes

| Sample | Location | Code |
|---|---|---|
| *Lupinus albus* L. (Termiye) | Merkez/KONYA | KM |
| *Lupinus albus* L. (Termiye) | Akşehir/KONYA | KA |
| *Lupinus albus* L. (Termiye) | Doğanhisar/KONYA | KD |
| *Lupinus albus* L. (Termiye) | Merkez/ISPARTA | IM |
| *Lupinus albus* L. (Termiye) | Merkez/BURDUR | BM |

### 2.2. Sample preparation

The samples were ground into a fine powder using a spice grinder. From each powdered sample, 3 g was weighed and mixed with 30 mL of methanol, followed by extraction under continuous shaking at room temperature for 24 h. The resulting mixture was first filtered through standard filter paper to remove coarse particles and subsequently passed through blue-band filter paper to ensure the complete removal of residual particulates. The obtained extracts were stored at +4 °C until further analysis.

### 2.3. Total Phenolic content (TPC) and Total flavonoid content (TFC)

The total phenolic content (TPC) of the samples was determined according to the method described by Slinkard and Singleton [6]. This method is based on the reduction of the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions, resulting in the oxidation of phenolics. The Folin–Ciocalteu reagent acts as an oxidizing agent in this reaction. The reduction of the reagent produces a blue-colored complex, the intensity of which can be measured spectrophotometrically. The absorbance of the resulting solution is measured at 760 nm and is directly proportional to the concentration of phenolic compounds. Gallic acid was used as the standard for calibration. A series of gallic acid standard solutions (1, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL) were prepared, and their absorbance values were recorded. A calibration curve was constructed by plotting absorbance against concentration. Based on this calibration curve, the total phenolic content of *Lupinus albus* L. extracts was calculated and expressed as mg GAE (gallic acid equivalents) per g of sample.

The total flavonoid content (TFC) was determined according to the method described by Fukumoto and Mazza [7]. Methanolic extracts of *Lupinus albus* L. were used for the analysis. Quercetin was employed as the standard compound for calibration. A series of quercetin standard solutions at different concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL) were prepared, and their absorbance values were measured. A calibration curve was constructed by plotting absorbance against concentration. Based on this calibration curve, the total flavonoid content of the samples was calculated and expressed as mg QE (quercetin equivalents) per gram of sample.

### 2.4. Antioxidant activities

#### *2.4.1. Determination of Iron (III) Reduction/Antioxidant Power-FRAP*

The ferric reducing antioxidant power (FRAP) assay was performed according to the method described by Benzie and Strain [8]. This method is based on the reduction of the Fe(III)–TPTZ complex to the blue-colored Fe(II)–TPTZ form in the presence of antioxidants. The absorbance of the resulting solution was measured at 593 nm. The antioxidant capacity of the samples was calculated using a calibration curve prepared with $FeSO_4 \cdot 7H_2O$ and expressed as μmol $Fe^{2+}$/g sample.

#### *2.4.2. DPPH Assays*

The DPPH radical scavenging activity was determined according to the method described by Molyneux [9]. The method is based on the reduction of the stable DPPH radical in the presence of antioxidant compounds, resulting in a decrease in absorbance at 517 nm. The radical scavenging activity was expressed as $SC_{50}$ values (mg/mL), defined as the concentration required to inhibit 50% of the DPPH radicals.

### 2.5. Analysis of phenolic compounds by RP-HPLC-PDA

Prior to RP-HPLC-PDA analysis, phenolic compounds in the extracts were enriched using a liquid–liquid extraction method. The solvents were first removed under reduced pressure at 40 °C using a rotary evaporator.

**Table 2.** TPC, TFC, FRAP, and DPPH values of the samples

| Samples | TPC (mg GAE/g sample) | TFC (mg QE/g sample) | FRAP (μmol $Fe^{2+}$/g sample) | DPPH $SC_{50}$(mg/mL) |
|---|---|---|---|---|
| KM | 2.32±0.11 | — | 1.95±0.12 | 37.98±0.74 |
| KA | 2.47±0.08 | — | 1.87±0.14 | 41.38±0.87 |
| KD | 2.22±0.29 | — | 1.47±0.13 | 42.04±1.80 |
| BM | 2.13±0.04 | — | 1.16±0.12 | 56.59±1.53 |
| IM | 1.96±0.20 | — | 1.14±0.14 | 56.60±1.63 |
| Trolox | | | | 0.0004±0.0001 |

*—: not detected.

The residue was reconstituted with 10 mL of distilled water (pH 2) and extracted with diethyl ether and ethyl acetate. The combined organic phases were evaporated to dryness, and the residue was dissolved in 2 mL of absolute methanol, filtered through a 0.45 μm membrane filter, and subjected to analysis. Phenolic composition analysis was performed using a Shimadzu LC-20AT RP-HPLC system equipped with a PDA detector. Separation was achieved on a reversed-phase C18 column (250 mm × 4.6 mm, 5 μm) using a gradient elution system consisting of (A) water with 2% acetic acid and (B) acetonitrile:water (70:30, v/v). The injection volume was 20 μL, the column temperature was maintained at 30 °C, and the flow rate was set at 1.0 mL/min. Quantification was carried out using calibration curves prepared from phenolic standards, and chromatograms were recorded at their maximum absorbance wavelengths (250, 280, 320, and 360 nm). Results were expressed as μg/g sample [10].

### 2.6. Statistical analysis

All experiments were carried out in triplicate, and the results are expressed as mean ± standard deviation.

## 3. Results and discussion

The antioxidant properties and phenolic composition of *Lupinus albus* L. samples, including total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and DPPH free radical scavenging activity, are presented in Table 2. The TPC values of the samples ranged from 1.96 to 2.47 mg GAE/g sample. The highest TPC was determined in the KA sample (2.47±0.08 mg GAE/g), followed by KM (2.32±0.11 mg GAE/g), KD (2.22±0.29 mg GAE/g), and BM (2.13±0.04 mg GAE/g). The lowest phenolic content was found in the IM sample (1.96±0.20 mg GAE/g). Total flavonoid content (TFC) was below the detectable level in all samples. Although total flavonoid content (TFC) was not detected by the spectrophotometric method, RP-HPLC-PDA analysis enabled the identification of specific flavonoid compounds, including chrysin, pinocembrin, and galangin. This apparent discrepancy can be attributed to the inherent limitations of the colorimetric TFC assay, particularly its relatively low sensitivity and lack of structural selectivity, which may prevent the detection of flavonoids present at low concentrations. In contrast, chromatographic techniques such as HPLC provide higher sensitivity and specificity, allowing for the accurate identification and quantification of individual phenolic constituents.

Total phenolic content values ranging from 4.36 to 7.24 mg GAE/g have been reported for both wild and cultivated *Lupinus albus* L. samples [11]. In another study, the total phenolic content of *Lupinus albus* L. extract was determined to be 4.92 ± 0.09 mg GAE/g [12]. When compared with the literature, the results of the present study were found to be lower. These differences may be attributed to variations in environmental and agronomic factors, including climatic conditions, soil characteristics, exposure to abiotic stress, as well as genetic variability among plant materials.

The total flavonoid content of *Lupinus albus* L. extract has been reported as 0.119 ± 0.003 mg QE/g [12]. In contrast, flavonoid content was not detected in the present study. This discrepancy may be associated with differences in plant-related factors such as botanical origin, local flora composition, geographical distribution, and ecological conditions, all of which can significantly influence the secondary metabolite profile of plant materials.

The FRAP and DPPH assays, which are among the most commonly applied methods for the evaluation of antioxidant activity in the literature, were employed in the present study. The obtained results are presented in Table 2. The results of the FRAP assay revealed that the antioxidant capacities of the samples varied within the range of 1.14 ± 0.14 to 1.95 ± 0.12. The highest ferric reducing antioxidant power was observed in the sample obtained from the KM region (1.95 ± 0.12), whereas the lowest value was determined for the sample from the IM region (1.14 ± 0.14). The antioxidant activity of *Lupinus albus* cultivars reported by Vollmannová et al. [13] reported FRAP values for Lupinus albus cultivars ranging from 1.33 to 1.74 μmol TE/g DM. Considering the differences in expression units (μmol $Fe^{2+}$/g versus μmol TE/g), direct numerical comparison between studies is limited; however, the findings consistently suggest that *Lupinus albus* L. exhibits moderate antioxidant capacity. In the present study, the $SC_{50}$ values, representing the DPPH radical scavenging

capacity of the samples, were determined to range between 37.98 and 56.60 mg/mL. These findings indicate that the radical scavenging activities of the samples are considerably lower than those of the standard antioxidant Trolox. Among the analyzed samples, the KM sample exhibited the lowest $SC_{50}$ value, corresponding to the highest radical scavenging activity. This observation is consistent with the relatively higher phenolic content determined for the KM sample. The higher antioxidant activity observed in KM and KA samples may be associated with their relatively higher phenolic content, supporting the well-established correlation between phenolic compounds and radical scavenging capacity. DPPH radical scavenging activity values reported by Vollmannová et al. [13] ranged between 1.16 and 1.88 μmol TE/g DM, whereas in the present study, the activity was expressed as $SC_{50}$ values, ranging from 37.98 to 56.60 mg/mL. Considering that lower $SC_{50}$ values correspond to higher antioxidant activity, the KM sample (37.98 ± 0.74 mg/mL) exhibited the highest radical scavenging capacity among the analyzed samples.

The phenolic composition of the samples was determined by RP-HPLC-PDA, and the results are presented in Table 3. Among the 25 phenolic standards analyzed, only a limited number of compounds were detected. Chrysin, galangin, and *p*-coumaric acid were detected in all samples, whereas ferulic acid, pinocembrin, and *p*-hydroxybenzoic acid were identified only in certain samples. Overall, the results indicate that the samples are not particularly rich in phenolic compounds and are mainly characterized by the presence of a limited number of flavonoids, with KD and KA samples showing comparatively higher phenolic content than the others.

**Table 3.** Analysis of phenolic compounds in samples by RP-HPLC-PDA

| Standards (μg phenolic /g extract) | KM | KA | KD | BM | IM |
|---|---|---|---|---|---|
| Gallic Acid | — | — | — | — | — |
| Protocatechuic Acid | — | — | — | — | — |
| Chlorogenic Acid | — | — | — | — | — |
| Catechin Hydrate | — | — | — | — | — |
| *p*-OH Benzoic Acid | 0.494 | — | — | 0.924 | — |
| Epicatechin | — | — | — | — | — |
| Caffeic Acid | — | — | — | — | — |
| Vanilic Acid | — | — | — | — | — |
| Syringic Acid | — | — | — | — | — |
| *p*- Coumaric Acid | 0.334 | 0.992 | 0.775 | 0.347 | 0.440 |
| Rutin | — | — | — | — | — |
| Ellagic Acid | — | — | — | — | — |
| Ferulic Acid | — | 0.417 | 0.697 | — | — |
| Myricetin | — | — | — | — | — |
| Daidzein | — | — | — | — | — |
| Luteolin | — | — | — | — | — |
| Quercetin | — | — | — | — | — |
| *t*-Sinnamic Acid | — | — | — | — | — |
| Naringenin | — | — | — | — | — |
| Apigenin | — | — | — | — | — |
| Hesperetin | — | — | — | — | — |
| Rhamnetin | — | — | — | — | — |
| Chrysin | 2.400 | 5.388 | 6.693 | 1.162 | 1.823 |
| Pinocembrin | — | 6.829 | 7.960 | 1.373 | 1.941 |
| CAPE | — | — | — | — | — |
| Galangin | 2.360 | 7.256 | 8.665 | 1.409 | 1.973 |

*—: Not Detected

The phenolic composition of the samples analyzed in this study revealed a relatively limited diversity, with only a few compounds detected among the targeted phenolics. This finding suggests that the samples are not particularly rich in phenolic constituents, which is consistent with previous studies reporting that the phenolic profile of natural products can vary significantly depending on botanical origin, environmental conditions, and processing methods [14].

These findings are in agreement with previous studies indicating that the phenolic composition of *Lupinus albus* L. can vary considerably depending on environmental and geographical factors [11,13]. In particular, the relatively low number of detected phenolic compounds in the present study supports the observation that lupin seeds generally contain lower and more selective phenolic profiles compared to other plant-based matrices [12].

Moreover, the predominance of flavonoid compounds such as chrysin, pinocembrin, and galangin suggests that these constituents may play a significant role in the antioxidant properties of the samples. It has been reported that flavonoids contribute substantially to antioxidant activity through mechanisms such as free radical scavenging and metal chelation [15]. However, considering the overall low phenolic content determined in this study, the antioxidant capacity of the samples appears to be moderate, which is consistent with the FRAP and DPPH results obtained.

## 4. Conclusion

In this study, the antioxidant activity and phenolic composition of *Lupinus albus* L. samples collected from different regions of Türkiye were comprehensively evaluated. The results demonstrated that the samples possess moderate antioxidant capacity, as evidenced by FRAP and DPPH assays, despite exhibiting relatively low total phenolic content and undetectable flavonoid levels.

RP-HPLC-PDA analysis revealed a limited and selective phenolic profile, with chrysin, pinocembrin, and galangin identified as the predominant compounds. Significant variations were observed among samples, particularly with KA and KD showing relatively higher phenolic concentrations. These differences are likely

influenced by geographical origin and environmental factors.

Overall, the findings suggest that *Lupinus albus* L. may serve as a potential source of bioactive compounds with antioxidant properties; however, its phenolic richness is relatively low compared to other plant matrices. Further studies focusing on different extraction methods and bioavailability assessments are recommended to better understand the functional potential of this species.

## Ethics Statement

This study did not involve humans or animals. Therefore, ethical approval was not required.

## Funding

This research received no external funding

## Conflict of interest

The authors declare that there is no conflict of interest.

## Author contributions

**Yakup Kara:** Conceptualization, methodology, experimental analyses, and approval of the final manuscript.

**Zehra Can:** Conceptualization, data interpretation, writing—original draft preparation, and approval of the final manuscript.

## Use of Generative AI

The authors declare that no generative artificial intelligence (AI) tools were used in the preparation of this manuscript.

## Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## References

[1] Z. Ahmad, A. Rauf, I.E. Orhan, M.S. Mubarak, Z. Akram, R. Islam, M. Imran, Z. Edis, B.K. Kondapavuluri, L. Thangavelu, M. Thiruvengadam, Antioxidant potential of polyphenolic compounds, sources, extraction, purification and characterization techniques: A focused review, Food Sci Nutr, 13, 2025, e71259.

[2] B.J. Xu, S.K.C. Chang, A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents, J Food Sci, 72, 2007, S159-166.

[3] A. Pereira, F. Ramos, A. Sanches Silva, Lupin (Lupinus albus L.) seeds: balancing the good and the bad and addressing future challenges, Molecules, 27, 2022, 8557.

[4] A. Garmidolova, D. Ivelina, D. Mihaylova, A. Lante, Bioactive peptides from Lupinus spp. seed proteins-state-of-the-art and perspectives, Appl Sci, 12, 2022, 3766.

[5] J. Dai, R.J. Mumper, Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties, Molecules, 15, 2010, 7313-7352.

[6] K. Slinkard, V.L. Singleton, Total phenol analysis: automation and comparison with manual methods, Am J Enol Viticult, 28, 1977, 49-55.

[7] L.R. Fukumoto, G. Mazza, Assessing antioxidant and prooxidant activities of phenolic compounds, J Agric Food Chem, 48, 2000, 3597–3604.

[8] I.F. Benzie, J.J. Strain, Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Methods Enzymol, 299, 1999, 15–27.

[9] P. Molyneux, The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, Songklanakarin J Sci Technol, 26, 2004, 211-219.

[10] Y. Kara, Z. Can, Usability of the phenolic profile analysis method developed in RP-HPLC-PDA in natural products, J Apither Nat, 7, 2024, 14-27.

[11] M. Karamać, H.H. Orak, R. Amorowicz, A. Orak, W. Piekoszewski, Phenolic contents and antioxidant capacities of wild and cultivated white lupin (Lupinus albus L.) seeds, Food Chem, 258, 2018, 1–7.

[12] A. Siger, J. Czubinski, P. Kachlicki, K. Dwiecki, E. Lampart-Szczapa, M. Nogala-Kalucka, Antioxidant activity and phenolic content in three lupin species, J Food Compos Anal, 25, 2012, 190-197.

[13] A. Vollmannova, J. Lidikova, J. Musilova, M. Snirc, T. Bojnanska, D. Urminska, I. Tirdilova, E. Zetochova, White lupin as a promising source of antioxidant phenolics for functional food production, J Food Qual, 2021, 2021, 5512236.

[14] R. Amorati, L. Valgimigli, Methods to measure the antioxidant activity of phytochemicals and plant extracts, J Agric Food Chem, 66, 2018, 3324-3329.

[15] F. Shahidi, P. Ambigaipalan, Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects, J Funct Foods, 18, 2015, 820-897.