Attahadi Medical Journal
2026;3(3):432-436
https://doi.org/10.69667/amj.26325

OPEN ACCESS

Original article

Chemical Composition and Antioxidant Properties of Wild *Lotus tetragonolobus* Seeds from Eastern Libya

Mona Khanfar

Department of Chemistry, Faculty of Science–Al-Qubbah, University of Derna, Libya
Email. *mona.khanfar@uod.edu.ly*

Keywords:
Lotus tetragonolobus, Wild Legumes, Proximate Composition, Total Phenolic Content, Antioxidant Activity, DPPH, Functional Foods, Libya.

ABSTRACT

Wild edible legumes represent valuable yet underutilized sources of nutrients and bioactive compounds with potential health benefits. However, information regarding the nutritional composition and antioxidant properties of wild *Lotus tetragonolobus* seeds, particularly those growing in Eastern Libya, remains limited. This study aimed to evaluate the proximate chemical composition, total phenolic content (TPC), and antioxidant activity of wild *Lotus tetragonolobus* seeds collected from Eastern Libya. Seeds were analyzed for moisture, crude protein, crude fat, ash, carbohydrates, and energy value using standard AOAC methods. Total phenolic content was determined using the Folin–Ciocalteu assay and expressed as mg gallic acid equivalents (GAE)/100 g dry weight (DW), while antioxidant activity was evaluated using the DPPH radical scavenging assay. The seeds contained 8.88 ± 0.01% moisture; on a dry-weight basis, they contained 26.84 ± 0.01% crude protein, 2.30 ± 0.05% crude fat, 4.01 ± 0.01% ash, and 66.85 ± 0.01% carbohydrates, with an estimated energy value of 395.90 kcal/100 g DW. The methanolic seed extract exhibited a total phenolic content of 243.33 ± 2.23 mg GAE/100 g DW and a DPPH radical scavenging activity of 43.01 ± 0.09%, indicating moderate antioxidant capacity. Wild *Lotus tetragonolobus* L. seeds possess a favorable nutritional profile and contain appreciable levels of phenolic compounds with measurable antioxidant activity. These findings provide the first comprehensive evaluation of the nutritional composition and antioxidant potential of this species from Eastern Libya and support its potential as an underutilized Mediterranean legume for nutritional, functional food, and phytochemical applications.

Introduction

Legumes are among the most important plant-derived food resources because of their high nutritional value and significant contribution to sustainable food systems. They are recognized as excellent sources of plant proteins, complex carbohydrates, dietary fiber, vitamins, minerals, and a wide range of bioactive phytochemicals that contribute to human health. Beyond their nutritional importance, legumes improve soil fertility through biological nitrogen fixation, thereby reducing dependence on synthetic fertilizers and promoting environmentally sustainable agricultural practices. Consequently, legumes have received increasing global attention because of their dual role in supporting healthy diets, enhancing food security, and contributing to sustainable agriculture [1–3].

In addition to their nutritional constituents, legumes contain numerous phytochemicals, particularly phenolic compounds, flavonoids, tannins, and other secondary metabolites that exhibit diverse biological activities. Among these compounds, phenolics have attracted considerable scientific interest because of their ability to scavenge free radicals, chelate transition metals, and inhibit oxidative processes associated with cellular damage. Increasing evidence indicates that regular consumption of phenolic-rich foods may contribute to reducing the risk of several chronic diseases, including cardiovascular diseases, type 2 diabetes mellitus, certain cancers, and neurodegenerative disorders. Consequently, the determination of total phenolic content together with antioxidant activity has become an essential approach for evaluating the nutritional quality and antioxidant potential of edible plant materials [4,5]. Growing consumer interest in natural food resources has renewed scientific attention toward wild edible plants as valuable yet underutilized sources of nutrients and bioactive compounds.

Many wild species have traditionally been consumed by local populations for generations; however, their nutritional composition and phytochemical characteristics remain insufficiently documented. Comprehensive evaluation of these species is therefore important for preserving indigenous plant resources, promoting dietary diversity, and identifying promising natural materials for future nutritional and food-related research [1,6]. *Lotus tetragonolobus* L. is an annual herbaceous legume belonging to the family Fabaceae and is naturally distributed throughout several Mediterranean countries. In Libya, the species grows spontaneously in natural habitats, particularly in Eastern Libya, where it has traditionally been recognized as a seasonal wild edible plant. Despite its traditional consumption and natural occurrence in Mediterranean ecosystems, published information concerning the chemical composition and antioxidant properties of its seeds remains scarce compared with that available for many cultivated legumes. The limited available studies have

Received: *22-06-2026 -* ***Accepted:*** *20-08-2026 -* ***Published:*** *28-08-2026*

Attahadi Medical Journal
2026;3(3):432-436
https://doi.org/10.69667/amj.26325

primarily focused on botanical description, agronomic characteristics, or basic nutritional evaluation. Consequently, comprehensive information regarding the proximate composition, total phenolic content, and antioxidant activity of wild *L. tetragonolobus* seeds remains limited [7,8]. Furthermore, geographical location and environmental conditions, including climate and abiotic stresses, strongly influence the accumulation of secondary metabolites in plants, emphasizing the importance of evaluating locally adapted wild populations [9,10].

To the best of our knowledge, no published study has comprehensively evaluated the proximate composition, total phenolic content, and antioxidant activity of wild *L. tetragonolobus* seeds collected from Eastern Libya. Therefore, the present study was conducted to determine the chemical composition of wild *L. tetragonolobus* seeds collected from Eastern Libya through proximate analysis, quantify their total phenolic content using the Folin–Ciocalteu assay, and evaluate their antioxidant activity using the DPPH free radical scavenging assay. The findings of this study provide valuable baseline data on the chemical composition and antioxidant properties of this underutilized Mediterranean wild legume and contribute to the currently limited literature available for this species.

Materials and Methods

Plant Material

Wild mature seeds of *L. tetragonolobus* were collected during March 2026 from naturally growing populations in Ain Mara, Al-Jabal Al-Akhdar region, Eastern Libya. The collected seeds were manually cleaned to remove impurities and foreign materials before being air-dried under shade at room temperature until a constant weight was attained. The dried seeds were ground into a fine powder using an electric grinder, sieved to obtain a homogeneous particle size, and stored in airtight polyethylene containers at −18 °C until further analysis.

Preparation of Seed Extract

The seed extract was prepared according to the method described by Yoo et al. [11] with slight modifications. Briefly, 5 g of the powdered seed sample was extracted with 100 mL of 80% methanol at room temperature using an orbital shaker operating at 120 rpm for 6 h. The extraction procedure was repeated three times to ensure maximum recovery of phenolic compounds. The combined methanolic extracts were used for the determination of total phenolic content and antioxidant activity.

Proximate Composition Analysis

The proximate chemical composition of *L. tetragonolobus* seed powder, including moisture, crude protein, crude fat, ash, and total carbohydrate contents, was determined according to the standard methods of the Association of Official Analytical Chemists [12]. Moisture content was determined by drying 2–5 g of the sample in a hot-air oven at 70 ± 2 °C until constant weight. Ash content was determined by incinerating 2 g of the powdered sample in a muffle furnace at 550 °C for 6 h. Crude fat was determined using a Soxhlet apparatus with petroleum ether as the extraction solvent for 6 h. After solvent removal, the extracted lipid fraction was dried, cooled in a desiccator, and weighed. Crude protein content was determined using the macro-Kjeldahl method, and the nitrogen content was converted to protein using a conversion factor of 6.25. The laboratory-reported proximate composition values were initially expressed on an as-is (wet-weight) basis. For reporting on a dry-weight basis, the crude protein, crude fat, ash, and carbohydrate values were converted using the measured dry matter content (91.12%), calculated from the moisture content (8.88%). The dry-weight values were calculated using the following equations:

Dry matter (%) = 100 − moisture (%)

Dry-weight value (%) = [as-is value (%) × 100] / dry matter (%)

Carbohydrate content was calculated by difference from the laboratory-reported as-is values.

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined according to the Folin–Ciocalteu colorimetric method described by Singleton and Rossi [13], with slight modifications. Briefly, 100 µL of the methanolic extract was mixed with 100 µL of Folin–Ciocalteu reagent. After 5 min of incubation at room temperature, 800 µL of 7.5% sodium carbonate solution was added, followed by 1 mL of distilled water. The reaction mixture was incubated in the dark for 2 h at room temperature, after which the absorbance was measured at 750 nm using a UV–Vis spectrophotometer (Onda, P.R.C.). Gallic acid (40–125 µg/mL) was used to construct the calibration curve, and the total phenolic content was expressed as milligrams of gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW).

Determination of DPPH Radical Scavenging Activity

The antioxidant activity of the methanolic seed extract was evaluated using the DPPH free radical scavenging assay according to the method of Oyaizu [14], with slight modifications. Briefly, 100 µL of the extract was mixed with 1.9 mL of 0.1 mM methanolic DPPH solution. The reaction mixture was shaken vigorously and incubated in the dark for 60 min at

Received: *22-06-2026 - **Accepted:** 20-08-2026 - **Published:** 28-08-2026*

Attahadi Medical Journal
2026;3(3):432-436
https://doi.org/10.69667/amj.26325

room temperature. The decrease in absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The DPPH radical scavenging activity was calculated as follows:

$$DPPH\ scavenging\ activity\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where Acontrol is the absorbance of the DPPH solution without sample extract, and Asample is the absorbance of the reaction mixture containing the sample extract. Ascorbic acid (vitamin C) was used as the positive control.

Statistical Analysis

All measurements were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics (Version 26.0; IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the analytical data.

Results and Discussion

Proximate Composition of* L. tetragonolobus *Seeds

The proximate composition of the dried seed powder is presented in Table 1. The moisture content was 8.88 ± 0.01%. After conversion of the laboratory-reported values from an as-is basis to a dry-weight basis, the seeds contained 26.84 ± 0.01% crude protein, 2.30 ± 0.05% crude fat, 4.01 ± 0.01% ash, and 66.85 ± 0.01% carbohydrates. The estimated energy value was 395.90 kcal/100 g DW.

Table 1. Proximate composition and energy value of wild Lotus tetragonolobus seeds from Eastern Libya

Component	Value (%)
Moisture	8.88 ± 0.01
Crude protein (DW)	26.84 ± 0.01
Crude fat (DW)	2.30 ± 0.05
Ash (DW)	4.01 ± 0.01
Carbohydrates (DW)	66.85 ± 0.01
Energy value (kcal/100 g DW)	395.90

Values are presented as mean ± SD (n = 3). DW = dry weight.

The relatively low moisture content observed in the present study is considered a desirable characteristic for dry edible seeds because it limits microbial growth and reduces enzymatic deterioration during storage, thereby improving shelf life and maintaining seed quality. Comparable moisture contents have been reported for other edible legumes, although variations among studies are expected because of differences in geographical origin, climatic conditions, harvesting stage, post-harvest handling, and drying procedures [15,1]. The crude protein content was 26.84% on a dry-weight basis, indicating that L. tetragonolobus seeds represent a valuable source of plant protein. This value is lower than that reported by Ereifej et al. [7], who found protein contents ranging from 30.4% to 31.0% in L. tetragonolobus seeds, but it remains comparable with the protein levels reported for several edible legumes. Such variation is common among wild legume populations and may reflect differences in genotype, soil characteristics, climatic conditions, plant maturity, and post-harvest practices, all of which can influence nitrogen assimilation and protein accumulation during seed development [9]. The crude fat content was relatively low (2.30% DW), indicating that the seeds are not a major source of dietary lipids. This finding is consistent with the nutritional profile of many legumes, in which carbohydrates and proteins constitute the principal storage reserves while lipids occur in comparatively smaller amounts. The ash content was 4.01% DW, reflecting the presence of mineral constituents within the seeds.

Mineral accumulation in seeds is influenced by soil composition, climatic conditions, and genetic factors, which may contribute to variability among studies [3]. Carbohydrates constituted the predominant nutritional component, accounting for 66.85% of the seed dry weight. Based on the dry-weight composition, the estimated energy value was 395.90 kcal/100 g DW, indicating that the seeds may provide a substantial contribution to dietary energy while maintaining a relatively high protein content. Since the energy value was calculated from the proximate composition, it primarily reflects the high carbohydrate content together with the substantial protein fraction rather than an elevated lipid content. These findings emphasize the nutritional potential of wild *L. tetragonolobus* seeds as an underutilized food resource that may contribute to dietary diversification and food security, particularly in regions where wild edible plants continue to be traditionally consumed [8,1]. Overall, the proximate composition demonstrates that wild *L. tetragonolobus* seeds possess favorable nutritional characteristics, combining high carbohydrate content with appreciable protein levels and low fat content. These attributes support their potential as an underutilized Mediterranean legume with promising nutritional value and justify further investigation of their functional and health-promoting properties.

Received: *22-06-2026 -* ***Accepted:*** *20-08-2026 -* ***Published:*** *28-08-2026*

Attahadi Medical Journal
2026;3(3):432-436
https://doi.org/10.69667/amj.26325

Total Phenolic Content and Antioxidant Activity

The total phenolic content (TPC) and DPPH radical scavenging activity of wild *L. tetragonolobus* seed extracts are presented in (Table 2). The methanolic seed extract exhibited a total phenolic content of 243.33 ± 2.23 mg GAE/100 g DW and a DPPH radical scavenging activity of 43.01 ± 0.09%, indicating the presence of phenolic constituents with moderate antioxidant capacity.

Table 2. Total phenolic content and antioxidant activity of methanolic extract of wild Lotus tetragonolobus L. seeds

Parameter	Value
Total phenolic content (mg GAE/100 g DW)	243.33 ± 2.23
DPPH radical scavenging activity (%)	43.01 ± 0.09

Values are presented as mean ± SD (n = 3). GAE = gallic acid equivalents; DW = dry weight

Phenolic compounds are among the most important bioactive constituents in plants because of their ability to donate hydrogen atoms or electrons, neutralize reactive oxygen species, chelate transition metals, and interrupt oxidative chain reactions. Consequently, the total phenolic content is widely regarded as an important indicator of the antioxidant potential of plant-derived foods [4,5]. The total phenolic content determined in the present study demonstrates that wild *L. tetragonolobus* seeds constitute a noteworthy source of naturally occurring phenolic compounds. Although direct comparisons with previous studies on the same species are limited because of the scarcity of available literature, the obtained value falls within the range reported for several edible and wild legumes known to contain appreciable concentrations of phenolic compounds. Such variability among species and studies is expected and may be attributed to differences in genotype, geographical origin, climatic conditions, soil characteristics, extraction solvent, extraction efficiency, and analytical methodology [8,9]. The DPPH radical scavenging activity of the methanolic extract reached 43.01%**,** indicating a moderate ability to neutralize free radicals.

Antioxidant activity measured by the DPPH assay is strongly influenced by both the concentration and structural characteristics of phenolic compounds, although other naturally occurring antioxidants, including flavonoids, vitamins, carotenoids, and other reducing substances, may also contribute to the overall radical scavenging capacity. Therefore, antioxidant activity should be interpreted as the combined effect of multiple bioactive constituents rather than phenolic compounds alone [4]. The moderate antioxidant activity observed in the present study is consistent with the measured total phenolic content and suggests that wild *L. tetragonolobus* seeds possess measurable antioxidant potential. However, the relationship between total phenolic content and antioxidant activity is not always directly proportional, since antioxidant effectiveness depends not only on the quantity of phenolic compounds but also on their individual chemical structures, degree of polymerization, hydroxylation pattern, and interactions with other phytochemicals present in the extract. Furthermore, environmental factors such as temperature, rainfall, soil composition, altitude, and plant developmental stage can substantially influence the biosynthesis and accumulation of phenolic metabolites, thereby affecting the antioxidant properties of wild plant populations [9,1].

Overall, the combination of an appreciable total phenolic content and moderate DPPH radical scavenging activity demonstrates that wild *L. tetragonolobus* seeds contain bioactive compounds capable of contributing to their antioxidant properties. These findings further support the nutritional and functional significance of this underutilized Mediterranean legume and highlight its potential as a natural source of antioxidant compounds. Future studies should focus on the identification and quantification of individual phenolic constituents using advanced analytical techniques such as high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS), which would provide a more comprehensive understanding of the phytochemical profile responsible for the observed antioxidant activity.

Conclusion

This study provides the first comprehensive evaluation of the proximate composition, total phenolic content, and antioxidant activity of wild *L. tetragonolobus* seeds collected from Eastern Libya. The seeds exhibited a favorable nutritional profile, characterized by high carbohydrate and appreciable protein contents, low fat content, and a considerable energy value, highlighting their potential as a nutritious plant-based food resource. Furthermore, the methanolic seed extract contained an appreciable amount of total phenolic compounds and demonstrated moderate DPPH radical scavenging activity, confirming the presence of bioactive constituents with antioxidant potential. These findings contribute valuable baseline information on an underexplored Mediterranean wild legume and support its potential as a natural source of nutrients and health-promoting phytochemicals. The results also emphasize the importance of conserving and investigating indigenous wild edible plants as promising resources for sustainable nutrition and future functional food development. Further studies are recommended to characterize the individual phenolic compounds using advanced analytical techniques, evaluate additional biological activities, and investigate the effects of geographical and environmental factors on the phytochemical composition of this species.

Received: *22-06-2026 -* ***Accepted:*** *20-08-2026 -* ***Published:*** *28-08-2026*

Ethics statement
Not applicable. This study involved laboratory analysis of plant seed material and did not involve human participants or animals.

Funding / Sources of support
The author received no external financial support or funding for this study.

Conflict of interest
The author declares no conflict of interest.

Author contribution
Mona Mohammad Abdalla Khanfar is the sole author and was responsible for the conception and design of the study, experimental work, data analysis and interpretation, manuscript preparation, and final approval of the manuscript.

Author approval
The author has read and approved the final manuscript and confirms that the work is original and accurately represents the study.

References

1. Samal I, Bhoi TK, Raj MN, Majhi PK, Murmu S, Pradhan AK, et al. Underutilized legumes: nutrient status and advanced breeding approaches for qualitative and quantitative enhancement. Front Nutr. 2023;10:1110750. doi:10.3389/fnut.2023.1110750.
2. Lisciani S, Marconi S, Le Donne C, Camilli E, Aguzzi A, Gabrielli P, et al. Legumes and common beans in sustainable diets: nutritional quality, environmental benefits, spread and use in food preparations. Front Nutr. 2024;11:1385232. doi:10.3389/fnut.2024.1385232.
3. Ganesan K, Xu B. A critical review on phytochemical profile and health-promoting effects of legumes and their role in sustainable nutrition. Food Chem. 2023;405:134747. doi:10.1016/j.foodchem.2022.134747.
4. Hernández-Ruiz RG, Olivares-Ochoa XC, Salinas-Varela Y, Guajardo-Espinoza D, Roldán-Flores LG, Rivera-Leon EA, et al. Phenolic compounds and anthocyanins in legumes and their impact on inflammation, oxidative stress, and metabolism: comprehensive review. Molecules. 2025;30(1):174. doi:10.3390/molecules30010174.
5. Xu L, Wang X. A comprehensive review of phenolic compounds in horticultural plants. Int J Mol Sci. 2025;26(12):5767. doi:10.3390/ijms26125767.
6. Das S, Bose A, Reang M, Datta BK. Underutilized legumes of India: a potential food resource for the future. Discov Plants. 2024;1:Article 55.
7. Ereifej KI, Al-Karaki GN, Hammouri MK, Rababah TM. Chemical composition of wild Lotus tetragonolobus seeds collected from Jordan. Plant Foods Hum Nutr. 2004;59(1):27–30.
8. Venketeish KSN, Govindarajan N, Pandiselvam R, Mousavi Khaneghah A. Unlocking the potential of underutilized legumes: nutritional benefits, anti-nutritional challenges, and mitigation strategies. J Food Meas Charact. 2024;18:9588–602. doi:10.1007/s11694-024-02905-z.
9. Isah T. Stress and defense responses in plant secondary metabolites production. Biol Res. 2019;52:39. doi:10.1186/s40659-019-0246-3.
10. Napier JD, Heckman RW, Juenger TE. Gene-by-environment interactions in plants: molecular mechanisms, environmental drivers, and adaptive plasticity. Plant Cell. 2023;35(1):109–124. doi:10.1093/plcell/koac322.
11. Yoo KM, Lee CH, Lee H, Moon B, Lee CY. Relative antioxidant and cytoprotective activities of common herbs. Food Chem. 2008;106(3):929–36.
12. Association of Official Analytical Chemists. Official methods of analysis. 16th ed. Washington (DC): AOAC International; 1997.
13. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144–58.
14. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44(6):307–15.
15. Amoo IA, Adebayo OT, Oyeleye AO. Chemical evaluation of the nutritive value of selected legumes. J Food Agric Environ. 2006;4(2):130–3.

Received: *22-06-2026 -* ***Accepted:*** *20-08-2026 -* ***Published:*** *28-08-2026*