

Comparative Assessment of Antioxidant and Antiradical Activity of Walnut Kernels (*Juglans regia* L.) Genotypes from Different Regions of Azerbaijan

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Abstract. A comparative analysis of antioxidant (AO) and antiradical (AR) activities was conducted using aqueous–ethanolic extracts obtained from native walnut kernels (*Juglans regia* L.) enclosed within shells of different genotypes cultivated across distinct agro-ecological regions of Azerbaijan (Shamakhi, Gabala, and Ordubad). Extraction was performed at a solid-to-solvent ratio of 1:3 (w/v), followed by dilution to a final concentration of 0.5 mg/mL. Biological activity was assessed using an induced chemiluminescence assay with hydrogen peroxide as an initiator of free radical reactions, enabling quantitative evaluation of the extracts' ability to inhibit oxidative processes and scavenge reactive oxygen species (ROS). The results demonstrated pronounced genotype-dependent variability. The highest antioxidant activity was observed in the Shamakhi genotype (87.5%), indicating a greater abundance of redox-active compounds. In contrast, higher antiradical activity was recorded for the Gabala (65.7%) and Ordubad (59.3%) genotypes, suggesting differences in antioxidant mechanisms, likely associated with variations in phenolic composition. Overall, the findings highlight the substantial biochemical potential of the studied material and underscore the influence of genotype and agro-ecological factors on antioxidant properties, supporting the targeted selection of walnut genotypes for functional food and biotechnological applications.

Keywords: *Juglans regia* L., antioxidant activity, antiradical activity, chemiluminescence, genotypes

Introduction

Oxidative stress is currently attracting the attention of researchers worldwide, as it is considered one of the key factors disrupting redox homeostasis in the human body. According to numerous scientific literature data, the development of oxidative stress precedes the onset and progression of socially significant diseases such as cardiovascular and oncological pathologies, inflammatory and rheumatoid processes, neurodegenerative disorders, and diabetes mellitus. Moreover, a significant portion of modern theories of aging are based on the concept of free radical oxidation (Polidori & Mecocci, 2022; Hassan et al., 2024; Zglinicki et al., 2024).

Reducing oxidative stress in biological systems is possible through the use of compounds with pronounced antioxidant activity of various origins. In this context, foods, particularly those of plant origin, as well as their processed forms (bekmez, sherbet, etc.), are of particular importance.

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In today's world, the scientific community is focusing significant attention on the discovery and comprehensive study of natural compounds, as well as food products containing biologically active substances capable of effectively preventing the development and progression of these pathological conditions. Plant-based components are of particular interest, as they are a rich source of natural antioxidants.

It has been established that the antioxidant potential of plant-based materials (medicinal plants, vegetables, and fruits) is largely due to the presence of a wide range of phenolic compounds, among which flavonoids are predominant, possessing a pronounced ability to neutralize free radicals and inhibit oxidative damage to cellular structures (Saini et al., 2024).

The discovery, identification, and comprehensive study of these natural compounds is a priority area of modern science, opening up broad prospects for both deepening fundamental understanding of antioxidant defense mechanisms and developing effective practical solutions in medicine, pharmacology, nutrition, and the creation of next-generation functional foods.

One valuable plant material with significant biological potential is the walnut (*Juglans regia* L.), a member of the Juglandaceae family, widely distributed throughout various regions of the country. Only one species is found in Azerbaijan: the walnut, or Walnut. It is cultivated throughout the country: in the Shaki-Zagatala region, Nakhchivan and Ordubad. This unique natural product has long attracted the attention of both scientists and traditional medicine practitioners. In medieval medical tradition, the eminent physician and philosopher Avicenna, in his seminal work «The Canon of Medicine», emphasized the high therapeutic value of walnut (*Juglans regia*), considering it an effective remedy for a wide range of pathological conditions. In later periods, interest in this plant has been sustained and reflected in the scientific and popular literature, where walnut (*Juglans regia*) is described as a food product with potentially beneficial effects on cognitive functions, particularly in supporting memory processes with regular consumption. Currently, the properties of walnuts are well studied and are widely used for the prevention and treatment of various diseases. The therapeutic and prophylactic properties of walnuts (*Juglans regia* L.) are largely due to its rich and varied chemical composition, which varies depending on the morphological part of the plant and the stage of ripeness of the fruit. Thus, the bark contains triterpenoids, ascorbic acid and quinones (including juglone); the leaves are characterized by a high content of carotenoids, phenolic carboxylic acids, coumarins, flavonoids, anthocyanins and quinones. Green (unripe) fruits contain vitamins C, B1, B2, PP, carotenoids and quinones, while mature kernels are rich in vitamins A, B1, B2, B12, C, K, E, PP, and also contain carotenoids, quinones and small amounts of gallic and ellagic acids (Hayes et al., 2016; Colaric et al., 2005).

Particularly noteworthy are the little-studied and often undervalued components of the fruit—the shell and partitions of the walnut, traditionally considered byproducts of processing. However, these structures represent a valuable source of biologically active substances. Their characteristic dark brown color is due to the high content of phenolic compounds and iodine-containing components. Walnut partitions contain vitamins (A, C, PP, and B vitamins), as well as a wide range of phenolic compounds, which determines their pronounced antioxidant and potentially pharmacologically significant properties (Al-Snafi, 2018). Currently, leading research centers around the world are actively conducting research aimed at studying the antiradical activity (ARA) of walnuts and their individual morphological parts. The investigation of the chemical composition of natural matrices, with a focus on the identification and quantification of compounds exhibiting antioxidant activity and the ability to scavenge free radicals, is considered a priority area in modern biochemistry and nutritional science, particularly in the context of oxidative stress research and the development of functional foods (López-Otín et al., 2023; Sies & Jones, 2020). However, despite the significant amount of accumulated data, such research has been virtually nonexistent in Azerbaijan to date. The scientific novelty of the work lies in the fact that for the first time a comprehensive analysis of the

antioxidant and antiradical activity of unpeeled walnut kernels (*Juglans regia* L.), harvested in various regions of Azerbaijan, was carried out, which expands our understanding of their biochemical potential and possibilities for practical use.

Materials and Methods

The study involved walnuts (*Juglans regia* L.) harvested in various regions of Azerbaijan (Shamakhi, Gabala, and Ordubad). Unshelled walnut kernels were used as the plant material. Hydroalcoholic extracts were obtained by extraction using ethanol at a specified concentration. The antioxidant and antiradical activity of the extracts was assessed by induced chemiluminescence (Georgetti et al., 2003; Pamunuwa & Atapattu, 2023).

The obtained results allowed for a comparative analysis of walnut kernel (*Juglans regia* L.) characteristics among genotypes grown in different regions

Results and Discussion

A comparative study was conducted of the antioxidant and antiradical activity of hydroalcoholic extracts obtained from unpeeled walnut kernels (*Juglans regia* L.) in shells collected in various regions of Azerbaijan (Shamakhi, Gabala and Ordubad). The reaction was carried out using peroxidase (1×10^{-5} M), pyrogallol (1×10^{-2} M), and hydrogen peroxide (1×10^{-4} M). In the control system (400 μ l), the chemiluminescence peaked at 4–5 minutes, and the total reaction time was 12–14 minutes.

After the control reaction, the antioxidant (AO) and antiradical (AR) activities of the hydroalcoholic extracts of inshell walnut kernels from various walnut genotypes were determined. All studied samples were found to exhibit pronounced antioxidant activity, exceeding 80%, indicating a high content of compounds with radical-scavenging properties.

Figure 1 shows that at an extract concentration of 0.5 mg/ml. The highest antioxidant activity was found in the Shamakhi genotype—87.5%; the reaction lasted approximately 8 minutes, with the chemiluminescence peaking at 1 minute 14 seconds. The Gabala genotype extract demonstrated 83.7% activity (reaction duration was approximately 7 minutes, with a peak at 1 minute). The lowest values were observed in the Ordubad genotype—82.8%, with a reaction lasting approximately 6 minutes 21 seconds and reaching a peak at 1 minute 30.

Extracts were found to influence reaction kinetics, shortening its duration compared to the control system, confirming their antiradical properties. With the addition of extracts, the reaction duration was reduced to 4–8 minutes, indicating their antiradical activity, assessed by a decrease in chemiluminescence intensity. All studied genotypes possessed high antioxidant potential, with the highest activity characteristic of samples grown in Shamakhi, indicating the influence of genotypic and regional factors on the level of biological activity of walnuts (Fig. 1).

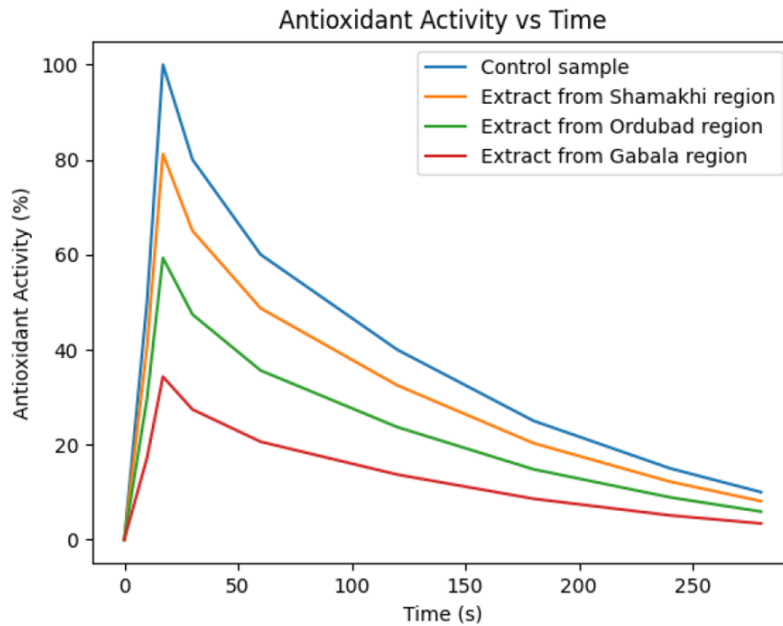


Figure 1

Antioxidant activity of the Shamakhi, Gabala, and Ordubad genotypes %

Note. The highest antioxidant activity values are characteristic of the Shamakhi genotype.

For the Shamakhi genotype, the reaction involving the extract lasted approximately 9 minutes, with the peak of chemiluminescence observed early on (15 seconds). According to this graph, AR activity was 81.2%.

When examining the second sample, the reaction conducted to determine the AR activity of the kernel and shell extract obtained from fruits of the Gabala genotype lasted for 5 minutes, with the maximum value observed at 10 minutes. This amounted to 34.4% of the maximum (reference) value. Therefore, if the maximum is taken as 100% (an area of 320 mm²), then the obtained result (110 mm²) indicates that the activity is approximately 3 times lower than the maximum.

The kernel and shell extract obtained from fruits of the Ordubad genotype is characterized by an AR activity level of 59.3%, indicating its moderate inhibitory capacity for the test parameter. Comparative analysis demonstrates that the extract of the Gabala genotype has the most pronounced antiradical activity, providing a reduction in AR by 65.7% relative to the control, while the extract of the Shamakhi genotype exhibits a significantly smaller effect (18.8%) (Fig. 2).

The study showed that kernel and shell extracts obtained from different genotypes differ significantly in their levels of antiradical activity. The Gabala genotype demonstrated the most pronounced inhibitory effect on AR, indicating its high biological activity and potential as a source of natural inhibitors. The Ordubad genotype is characterized by a moderate level of activity, while Shamakhi exhibits the least effectiveness.

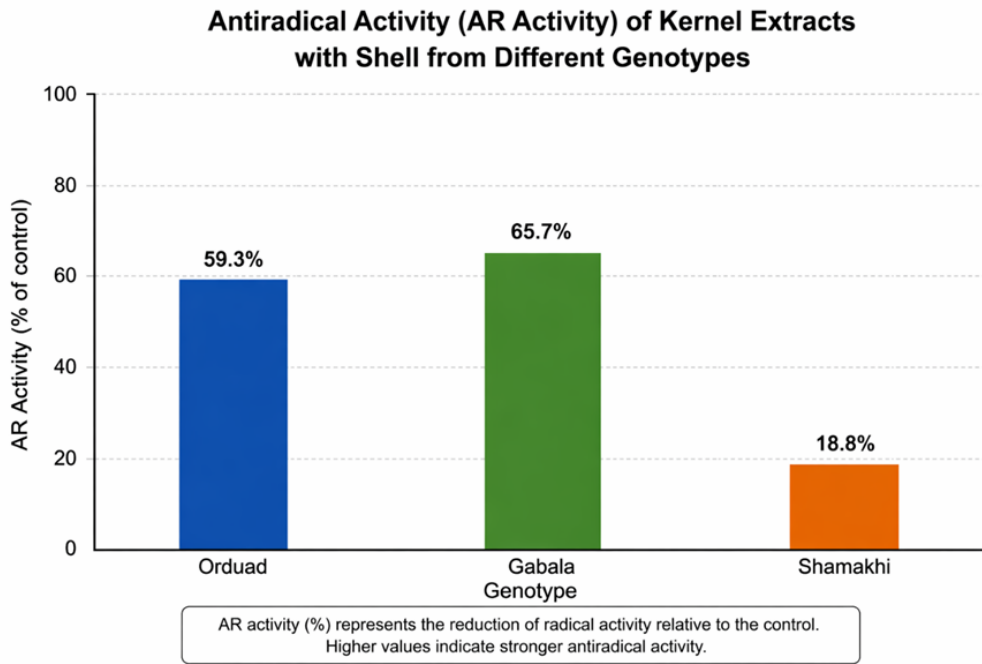


Figure 2

Antiradical activity of the Shamakhi, Gabala, and Ordubad genotypes %

Note. The highest antiradical activity values are characteristic of the Gabala genotype.

Moreover, the antioxidant activity of all studied samples is comparable, indicating the absence of significant differences in this parameter between genotypes. The observed dependence of AR inhibitory activity on genotype is likely due to differences in phytochemical composition, including the content of phenolic compounds.

The discrepancy between the antioxidant and antiradical activity (Fig. 3) of the Shamakhi genotype extract is likely due to differences in the mechanisms of action of the bioactive compounds, as well as the specificity of the analytical methods used.

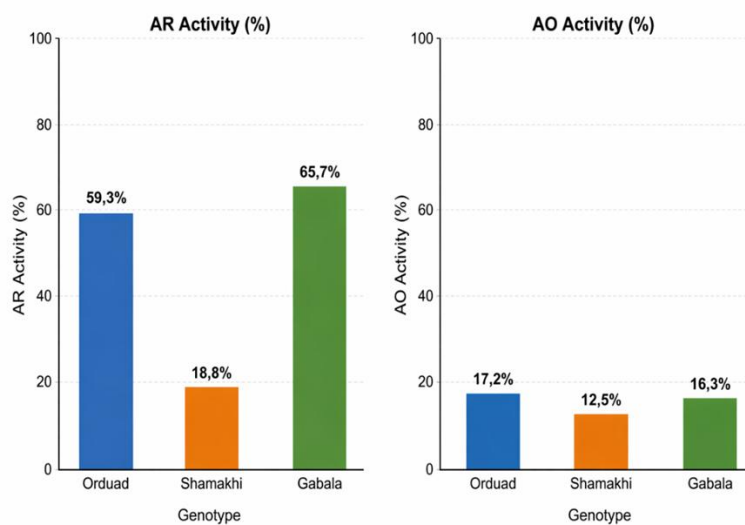


Figure 3

Comparative analysis of antiradical and antioxidant activity in different genotypes of walnut (Juglans regia L.)

Antiradical activity (AR) is defined as the ability of compounds to directly neutralize free radicals through the donation of an electron or a hydrogen atom, whereas antioxidant activity is a broader indicator that includes reduction potential, the ability to chelate metal ions, and the inhibition of chain oxidation processes (Huang et al., 2005; Apak et al., 2016).

The Shamakhi genotype extract likely contains predominantly compounds with pronounced reducing capacity, but relatively low reactivity toward free radicals. Furthermore, differences may be related to the specific phenolic profile, particularly the ratio of flavonoids, phenolic carboxylic acids, and condensed tannins, which exhibit varying activity in different test systems.

It should also be noted that the method used to assess antioxidant and antiradical activity (XL) is based on a specific principle and is characterized by specific sensitivity to individual classes of compounds. Therefore, the higher antioxidant activity and low antiradical activity observed in the Shamakhi genotype is not a contradiction in terms, but rather reflects the specific chemical composition of the extract and the prevalence of certain mechanisms of antioxidant action.

Thus, the obtained results allow us to consider the studied genotypes as promising raw materials for the development of functional foods, dietary supplements, and natural antioxidant compositions. The Shamakhi genotype can be effectively used in systems requiring prolonged antioxidant protection, while the Gabala genotype can be used in products designed to rapidly neutralize free radicals. This opens up opportunities for targeted applications in the food, pharmaceutical, and cosmetic industries.

Conclusion

In this study, kernel extracts with shell from different genotypes exhibited pronounced variation in both antiradical (AR) and antioxidant (AO) activities. The highest AR activity was observed in the Gabala genotype (65.7%), followed by Ordubad (59.3%), whereas Shamakhi showed a markedly lower effect (18.8%). In contrast, AO activity varied within a narrower range, with the highest value recorded for Ordubad (17.2%) and the lowest for Shamakhi (12.5%).

The observed discrepancy between AR and AO activities highlights the contribution of distinct antioxidant mechanisms and reflects differences in the phytochemical composition of the studied genotypes. These findings emphasize the importance of employing multiple analytical assays to obtain a comprehensive evaluation of antioxidant potential. Overall, the Gabala genotype can be considered a promising source of potent radical-scavenging compounds, while Ordubad demonstrates a more balanced antioxidant profile. These results provide a basis for further phytochemical characterization and support the potential application of these genotypes in the development of functional foods and natural antioxidant formulations.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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