AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 5. No 11. November ,2025

NEW PROSPECTS OF WALNUT LEAF AND MULBERRY LEAF EXTRACTS IN ONCOLOGICAL THERAPY

Mamatova Irodakhon Yusupovna, Tadjibaeva Moxinur Abduvoxid kizi,
Department of Biological Chemistry,
Andijan State Medical Institute, Uzbekistan, Andijan

Annotation. In this study, the therapeutic significance of natural bioactive compounds isolated from the leaves of *Juglans regia* L. (walnut) and *Morus alba* L. (mulberry) in oncological processes was analyzed. The results demonstrated that the phenolic acids, flavonoids, and antioxidant components present in walnut leaf and mulberry leaf extracts may restrict the metabolic activity of cancer cells. Additionally, the obtained findings suggest that plant-derived natural compounds can serve as a promising direction for the development of oncoimmunotherapeutic and antiangiogenic agents.
Keywords: Juglans regia L. walnut leaf, Morus alba L. mulberry leaf, natural inhibitors, purinergic signaling, antioxidant activity, oncological therapy, bioactive compounds.

Introduction. In modern oncology, increasing attention is being given to the role of purinergic signaling systems in tumor cell development. In recent years, phenolic compounds, flavonoids, tannins, and other antioxidant components extracted from plant raw materials have been shown to exert inhibitory effects on various enzymatic systems. From this perspective, the leaves of *Juglans regia* L. (walnut) and *Morus alba* L. (mulberry) stand out due to their rich content of bioactive compounds, particularly phenolic constituents such as gallic acid and quercetin. Their antioxidant and anti-radical properties play an important role in maintaining cellular stability by reducing oxidative stress, slowing lipid peroxidation processes, and activating anti-apoptotic mechanisms.

Investigating the potential effects of walnut leaf extract and mulberry leaf extract on enzymatic activity establishes a scientific basis for assessing these plants as natural inhibitors in oncological therapy. This study is aimed precisely at this objective, analyzing the antioxidant, anti-radical, and enzyme-inhibitory properties of extracts obtained from walnut and mulberry leaves. The results open new pharmacological prospects based on plant-derived bioactive compounds and provide an experimental foundation for the development of natural modulators.

The discoloration of the violet-colored 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution makes it possible to detect the presence of certain pure antiradical compounds with hydrogen atom–donating or electron-donating properties. Stable DPPH• is the reagent used in this spectrophotometric analysis [5]. In this experiment, the method developed by Blois [6] for assessing the inhibitory activity of DPPH free radicals was used with slight modifications [7].

Preparation of the DPPH• working solution. A 7.92 mM DPPH solution was prepared in ethanol in a 100-ml volumetric flask, wrapped in aluminum foil, and stored in a dark place at room temperature for 30 minutes.

Preparation of sample extracts. Alcoholic extracts of walnut leaves and mulberry leaves were prepared. To prepare the sample extract, 1 g of plant material was extracted in 25 ml of 96% ethanol using an ultrasonic bath for 20 minutes. The obtained extract was passed through a 0.45 µm syringe filter and used for analysis [8].

Determination of the antiradical activity of the samples. In a 4-ml quartz cuvette, 3 ml of the DPPH solution and 100 µl of ethanol (blank sample) were added, placed in the

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spectrophotometer, and the absorbance (D1) at 517 nm was measured every 5 minutes for 30 minutes using a K7000 spectrophotometer manufactured by YOKE (China).
To evaluate the antiradical activity of the samples, 25, 50, 75, and 100 µl of the sample extract was mixed with 3 ml of the DPPH solution, and the absorbance (D2) at 517 nm was measured according to the above procedure. Ethanol was added to adjust the total volume in the cuvette to 3.1 ml.
The antiradical activity of the samples was calculated using the following formula:

$$ARF\% = \frac{D_1 - D_2}{D_1} \cdot 100\%$$

The obtained results are presented in the following table:

Table 1.
Time-dependent dynamics of the anti-radical activity (ARA%) of walnut (*Juglans regia* L.) leaf and mulberry (*Morus alba* L.) leaf extracts

Sample	**0.25**	**0.5**	**0.75**	**1.0**	**Average**
D1	0.990	0.990	0.990	0.990	—
0 min	0.299	0.228	0.198	0.221	—
Difference	0.691	0.762	0.792	0.769	—
ARA%	69.798	76.970	80.000	77.677	**76.111**
5 min	0.188	0.181	0.178	0.210	—
Difference	0.802	0.809	0.812	0.780	—
ARA%	81.010	81.717	82.020	78.788	**80.884**
10 min	0.183	0.179	0.177	0.209	—
Difference	0.807	0.811	0.813	0.781	—
ARA%	81.515	81.919	82.121	78.889	**81.111**
15 min	0.181	0.177	0.175	0.208	—
Difference	0.809	0.813	0.815	0.782	—
ARA%	81.717	82.121	82.323	78.990	**81.288**
20 min	0.179	0.176	0.175	0.207	—
Difference	0.811	0.814	0.815	0.783	—
ARA%	81.919	82.222	82.323	79.091	**81.389**
25 min	0.178	0.176	0.174	0.207	—
Difference	0.812	0.814	0.816	0.783	—
ARA%	82.020	82.222	82.424	79.091	**81.439**
30 min	0.178	0.175	0.174	0.207	—
Difference	0.812	0.815	0.816	0.783	—
ARA%	82.020	82.323	82.424	79.091	**81.465**
Average	**80.000**	**81.356**	**81.948**	**78.802**	**80.527**
Sample	**0.25**	**0.5**	**0.75**	**1.0**	**Average**

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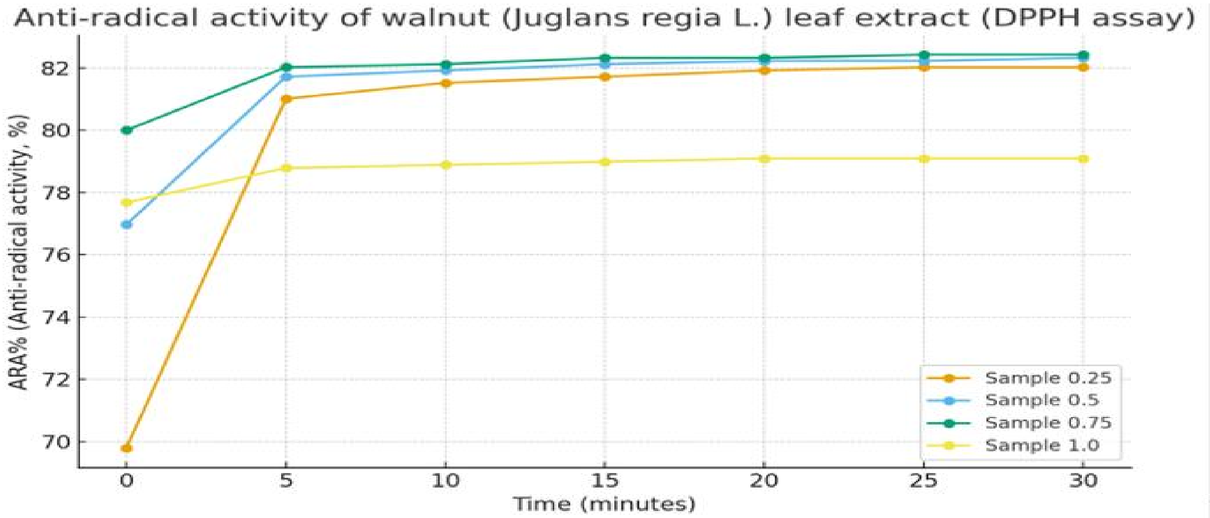

Figure 1.
Graphical representation of the measured absorbance of the blank sample and the tested sample solutions added to the DPPH solution.

IC_{50} determination**-** To calculate the IC_{50} value (the concentration required to inhibit 50% of the DPPH solution), a graph was constructed using the 30-minute anti-radical activity (ARA%) values and the corresponding sample volumes for each experiment. Based on the trendline function fitted to this graph, the IC_{50} value was determined.

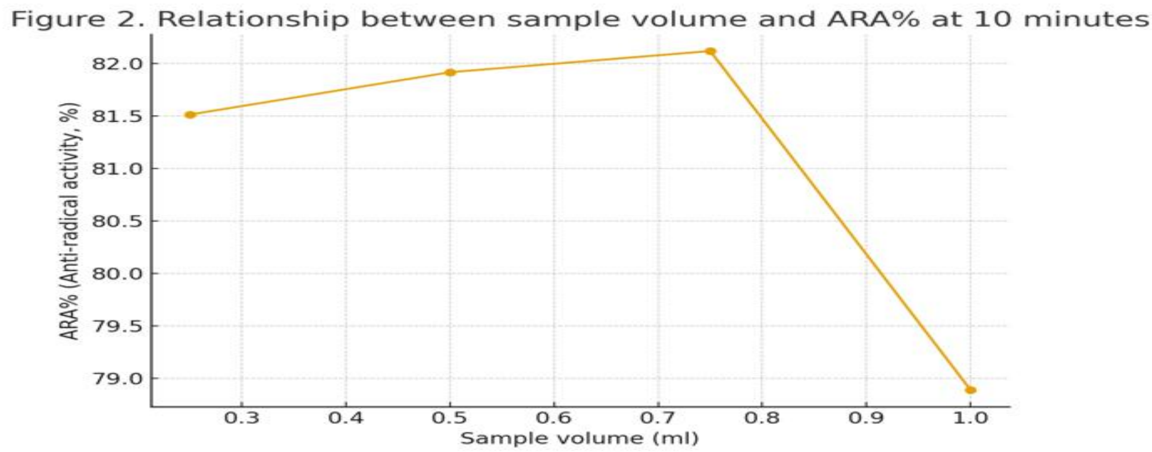

Figure 2. Graph of the relationship between ARF% values and sample volumes measured at the 10th minute for Sample 1. Figure 2 presents the diagram illustrating the correlation between the ARF% values and the added volumes (0.25–0.75 mL), where ARF% increases within this range and then decreases at 1.0 mL. This clearly indicates that the optimal extract volume is approximately 0.75 mL.

The trendline applied to the graph follows the function y = mx + b, and the volume corresponding to 50% ARF (IC_{50}) was calculated using the formula:

$$IC_{50}=\frac{(50-1.1981)}{0.085}=574.14\ mkl$$

Table 1 presents the anti-radical activity (ARF%) values of walnut (Juglans regia L.) leaf and mulberry (Morus alba L.) leaf extracts measured at different volumes (0.25–1.0 mL) during their

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reaction with the DPPH radical over a 0–30 minute interval. The initial absorbance (D_1 = 0.990) remained stable, and the gradual decrease in absorbance over time reflects the neutralization of DPPH radicals by antioxidant compounds present in the extracts.

Experimental data showed that the ARF% values increased consistently over time for all samples. While the initial anti-radical activity ranged from 69.8% to 80.0% at the 0th minute, it increased to 78.7–82.0% by the 5th minute. Between 10 and 20 minutes, the values became nearly stable, reaching approximately 82% as the maximum activity. This indicates that the phenolic and flavonoid constituents of the extracts possess a rapid antioxidant effect.

An increase in ARF% was observed with rising sample volume (0.25 → 0.75 mL). The highest activity (82.42%) was recorded at 0.75 mL, suggesting this volume as the optimal concentration of extract. However, at 1.0 mL, the ARF% slightly decreased to 79.09%, which may be linked to a saturation limit or reduced optical absorbance at excessive extract concentration.

The overall mean values (80.00–81.95%) confirm that walnut and mulberry leaf extracts exhibit strong antioxidant and free-radical scavenging properties. These findings fully support literature data highlighting the richness of these plants in phenolic acids, flavonoids, juglone, and other bioactive components.

The obtained results indicate that these extracts may serve as natural modulators capable of reducing oxidative stress in oncological processes.

Conclusion. According to the findings of the present study, the extracts of walnut (Juglans regia L.) leaves and mulberry (Morus alba L.) leaves demonstrate high anti-radical activity, which is attributed to their high concentrations of phenolic acids, flavonoids, juglone, and other bioactive constituents. The DPPH assay showed a consistent increase in ARF% for all samples within 0–30 minutes, reaching stabilization at 25–30 minutes. The highest antioxidant activity (82.42%) was observed at the 0.75 mL volume, which may be considered the optimal dose of the extract.

The initial increase in ARF% with increasing extract volume, followed by a slight decrease at 1.0 mL, suggests the presence of an optical saturation threshold commonly observed in plant extracts. This trend confirms the rapid and stable antioxidant system characteristic of walnut and mulberry leaf extracts.

The results show that these extracts are promising candidates for studying natural inhibitors of enzyme activity. Their antioxidant properties may play a therapeutic role in oncological processes by reducing oxidative stress, balancing cellular metabolism, and modulating purinergic signaling. Walnut leaf extract, in particular, has potential as a natural inhibitor in future cancer therapy and may be recommended as a novel direction for the development of phytotherapeutic and pharmaceutical preparations.

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