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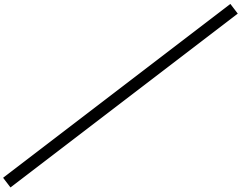

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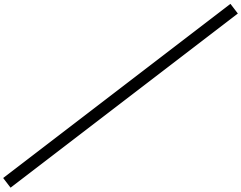

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ANTIOXIDANT ACTIVITY OF A MIXTURE OF GLEDITSIA AND AILANTHUS FLOWERS

АНТИОКСИДАНТНАЯ АКТИВНОСТЬ СМЕСИ ЦВЕТКОВ ГЛЕДИЦИИ И АЙЛАНТА

GLEDITSIYA VA AILANTHUS GULLARI ARAŞMASINI ANTIOKSIDANT FAOLIYATI

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Abstract

With the growing interest in natural antioxidants, special attention is given to the study of less explored medicinal plants and their combinations. In the present study, the antioxidant activity of alcoholic extracts of Ailanthus altissima and Gleditsia spp. flowers were evaluated when mixed in different ratios: 1:1, 3:1, and 1:3. To quantitatively assess the antiradical properties, the DPPH method was applied, based on spectrophotometric measurement of absorption at 517 nm. The values of ARF% (antiradical activity) and IC_{50} (the volume of extract required to inhibit 50% of DPPH radicals) were determined. The results demonstrated that all studied mixtures exhibited pronounced antioxidant activity. The greatest effect was observed in the 1:1 mixture, for which the IC_{50} was 207.07 µL, while the ARF% at a volume of 100 µL after 30 minutes reached 24.77%. These values are comparable to the results of well-known natural antioxidants.

Thus, the synergistic combination of Ailanthus and Gleditsia flowers may serve as an effective source of natural antioxidants, with promising potential for the development of functional phytopreparations and dietary supplements.

Аннотация

С ростом интереса к природным антиоксидантам особое внимание уделяется изучению малоизученных лекарственных растений и их сочетаний. В настоящем исследовании оценивалась антиоксидантная активность спиртовых экстрактов цветков Ailanthus altissima и Gleditsia spp. при их смешивании в различных соотношениях: 1:1, 3:1 и 1:3. Для количественной оценки антирадикальных свойств был применён метод DPPH, основанный на спектрофотометрическом измерении поглощения при 517 нм. Определялись значения ARF% (антирадикальная активность) и IC_{50} (объём экстракта, необходимый для подавления 50% DPPH-радикалов).

Результаты показали, что все исследуемые смеси проявляют выраженную антиоксидантную активность. Наибольший эффект был зафиксирован у смеси 1:1, для которой IC_{50} составил 207,07 мкл, а ARF% при объёме 100 мкл через 30 минут достиг 24,77%. Эти значения сопоставимы с результатами известных природных антиоксидантов.

Таким образом, синергетическое сочетание цветков айланта и гледичии может служить эффективным источником природных антиоксидантов, перспективных для разработки функциональных фитопрепаратов и биологически активных добавок.

Annotatsiya

Tabiiy antioksidantlarga bo‘lgan qiziqish ortib borar ekan, kam o‘rganilgan dorivor o‘simliklar va ularning kombinatsiyalarini tadqiq qilishga alohida e’tibor qaratilmoqda. Ushbu tadqiqotda Ailanthus altissima va Gleditsia spp. gullarining spirtli ekstraktlari turli nisbatlarda (1:1, 3:1 va 1:3) aralashtirilganda ularning antioksidant faolligi baholandi. Antiradikal xususiyatlarni miqdoriy baholash uchun 517 nm to‘lqin uzunligida so‘rilishni spektrofotometrik o‘lchashga asoslangan DPPH usuli qo‘llanildi. ARF% (antiradikal faollik) va IC_{50} (DPPH radikallarining 50% ini inhibe qilish uchun zarur bo‘lgan ekstrakt hajmi) qiymatlari aniqlandi.

Natijalar shuni ko‘rsatdiki, barcha o‘rganilgan aralashmalar sezilarli antioksidant faollikka ega. Eng yuqori ta’sir 1:1 nisbatidagi aralashmada kuzatildi, bunda IC_{50} 207,07 mkl ni tashkil qildi, ARF% esa 100 mkl hajmda 30 daqiqada 24,77% ga yetdi. Ushbu qiymatlar taniqli tabiiy antioksidantlarning natijalari bilan taqqoslanishi mumkin.

Shunday qilib, ayllant va gleditsiya gullarining sinergik kombinatsiyasi samarali tabiiy antioksidantlar manbai bo‘lib xizmat qilishi va funksional fitopreparatlar hamda biologik faol qo‘shimchalar ishlab chiqishda istiqbolli hisoblanadi.

Key words: *antioxidants, DPPH, mixture, flavonoids, extracts, IC_{50} value*
Ключевые слова: *антиоксиданты, DPPH, смесь, флавоноиды, экстракты, значение IC_{50}*
Kalit so‘zlar: *antioksidantlar, DPPH, aralashma, flavonoidlar, ekstraktlar, IC_{50} qiymati*

INTRODUCTION

In recent decades, increasing attention in scientific literature and biomedical research has been paid to the role of free radicals in the pathogenesis of various diseases. Oxidative stress, which arises as a result of an imbalance between the formation of reactive oxygen species (ROS) and the effectiveness of antioxidant defense mechanisms, leads to damage of cellular structures, including lipids, proteins, and nucleic acids. It is recognized as a key factor in the development of chronic inflammation, oncological, neurodegenerative, cardiovascular, and metabolic diseases.

In this regard, the search and investigation of new antioxidants, especially of natural origin, have become particularly relevant. Unlike synthetic compounds, which often possess a limited spectrum of activity and potential toxicity, natural antioxidants demonstrate broad biological potential, high bioavailability, and safety. The main sources of such compounds are medicinal plants rich in flavonoids, phenolic acids, anthocyanins, and other polyphenolic components [1].

One of the promising directions of modern phytochemistry is the study of underexplored or underestimated plant species in order to reveal their antioxidant potential. In this context, *Ailanthus altissima* (tree of heaven) is of special interest, known for its anti-inflammatory, antimicrobial, and antioxidant properties. According to literature data, the flowers and leaves of *Ailanthus* contain significant amounts of flavonoids (quercetin, rutin), as well as triterpenoids and steroid compounds, which may determine its antiradical activity [2].

Equally interesting is the genus *Gleditsia*, whose representatives are widely used in traditional medicine in China, Korea, and Central Asia. In particular, *Gleditsia sinensis* has been described as having immunostimulatory, antiseptic, and antioxidant effects. The biochemical composition of *Gleditsia* flowers includes active components such as saponins, tannins, and catechins, which possess the ability to neutralize free radicals [3].

Despite the existence of studies investigating each plant individually, there is a lack of data in the scientific literature regarding the combined use of *Ailanthus* and *Gleditsia* extracts. Meanwhile, the mixing of plant components may produce a synergistic effect due to the interaction of different groups of biologically active compounds. It is assumed that such a combination may enhance antioxidant activity compared to individual extracts [4,5].

Thus, the aim of this study is to determine the antioxidant activity of alcoholic extracts of a mixture of *Ailanthus altissima* and *Gleditsia* spp. flowers in various ratios (1:1, 3:1, 1:3) using the DPPH method. The results of this study may be applied in further practical developments in the field of natural antioxidants, including the creation of functional products, dietary supplements, and phytopreparations based on these plants.

MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material.

The flowers of *Ailanthus altissima* (tree of heaven) and *Gleditsia* spp. were collected manually in May 2025 in the territory of the Andijan region, Republic of Uzbekistan (Fig. 1). The collection was carried out during the active flowering phase of the plants, in the morning hours and under dry weather conditions. The plant material was preliminarily identified and confirmed by specialists of the Department of Botany, Andijan State University

а б

Figure 1. Flowers of *Gleditsia* (a) and flowers of *Ailanthus* (b)

Immediately after collection, the flowers were cleaned of impurities, washed, and dried in the shade at a temperature of 25–28 °C in a well-ventilated room. After reaching a constant weight, the plant material was ground using a laboratory mill and sieved through a 1 mm mesh.

1.2. Preparation of Alcoholic Extracts.

For extract preparation, the ultrasonic extraction method with 96% ethanol was used. Ground samples weighing 1 g of each plant (or their mixtures) were placed in a 50 mL conical flask, to which 25 mL of ethanol was added. Extraction was carried out for 20 minutes at 25 °C in an ultrasonic bath (frequency 40 kHz). The solutions were then filtered through a syringe filter with a pore size of 0.45 μm. The obtained extracts were diluted 10-fold with ethanol for subsequent analysis. The mixtures were prepared in three mass ratios:

- Variant 1:1 — equal masses of Ailanthus and Gleditsia flowers;
- Variant 3:1 — predominance of Ailanthus;
- Variant 1:3 — predominance of Gleditsia.

1.3. Method for Evaluating Antioxidant Activity (DPPH)

The antioxidant activity was assessed using the method of neutralization of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•), modified according to Blois (1958) with consideration of the recommendations of Gulcin et al. (2005) [6,7]. A DPPH• solution (7.92 mM) was prepared in 96% ethanol and kept for 30 minutes in a dark place at room temperature.

In each sample, 3 mL of DPPH solution was mixed with different volumes of the extract (25, 50, 75, and 100 μL), adjusting the final volume to 3.1 mL with ethanol. As a control (blank), a mixture of DPPH and pure ethanol was used. Absorbance was recorded in quartz cuvettes using a YOKE K7000 spectrophotometer (China) at 517 nm every 5 minutes over a period of 30 minutes [8,9].

1.4. Calculation of Antiradical Activity (ARA%)

Antioxidant activity (ARA%) was calculated according to the following formula:

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

where

D_1 — optical density of the DPPH solution without the sample (control), D_2 — optical density of the mixture with the extract.

For each volume and measurement time, the dependence of ARA% on time was plotted, and the average activity value after 30 minutes was calculated.

1.5. Determination of IC_{50}.

IC_{50} (the extract volume required for 50% inhibition of DPPH) was calculated based on the graph of ARF% versus the volume of added extract. For plotting the graphs, the values after 30

minutes of incubation were used. Linear regression (y = mx + b) was applied to determine IC_{50} according to the formula:

$$x=(y-b)/m$$

where *x* — the extract volume providing 50% inhibition (IC_{50}).

RESULTS AND DISCUSSION

3.1. Antiradical Activity of Mixtures (ARF%)

The antioxidant activity of the extracts was evaluated by measuring their ability to neutralize DPPH free radicals over 30 minutes at different extract volumes (25, 50, 75, 100 µL) [10]. The results are presented in tables and illustrated by graphs showing the dependence of optical density on time.

Table 1. Measured optical density values and calculated antiradical activity indices of alcoholic extracts of *Ailanthus* and *Gleditsia* mixed in a 1:1 ratio and added to the DPPH solution.

Volume, µL	Time, min	Samples							
		Abs, D_1	Abs, D_2	APA%	Volume, µL	Time, min	Abs, D_1	Abs, D_2	APA%
25	0	0,767	0,767	0,00	75	0	0,767	0,767	0,00
	5	0,767	0,732	4,56		5	0,767	0,665	13,30
	10	0,767	0,731	4,69		10	0,767	0,665	13,30
	15	0,767	0,73	4,82		15	0,767	0,66	13,95
	20	0,767	0,729	4,95		20	0,767	0,658	14,21
	25	0,767	0,729	4,95		25	0,767	0,655	14,60
	30	0,767	0,728	**5,08**		**30**	0,767	0,653	**14,86**
50	0	0,767	0,767	0,00	100	0	0,767	0,767	0,00
	5	0,767	0,7	8,74		5	0,767	0,605	21,12
	10	0,767	0,695	9,39		10	0,767	0,595	22,43
	15	0,767	0,693	9,65		15	0,767	0,589	23,21
	20	0,767	0,691	9,91		20	0,767	0,584	23,86
	25	0,767	0,69	10,04		25	0,767	0,581	24,25
	30	0,767	0,688	**10,30**		**30**	0,767	0,577	**24,77**

Table 2. Measured optical density values and calculated antiradical activity indices of alcoholic extracts of *Ailanthus* and *Gleditsia* mixed in a 3:1 ratio and added to the DPPH solution.

Volume, µL	Time, min	Sample							
		Abs, D_1	Abs, D_2	APA%	Volume, µL	Time, min	Abs, D_1	Abs, D_2	APA%
25	0	0,767	0,767	0,00	75	0	0,767	0,767	0,00
	5	0,767	0,72	6,13		5	0,767	0,657	14,34
	10	0,767	0,718	6,39		10	0,767	0,651	15,12
	15	0,767	0,715	6,78		15	0,767	0,646	15,78
	20	0,767	0,713	7,04		20	0,767	0,643	16,17
	25	0,767	0,712	7,17		25	0,767	0,641	16,43
	30	0,767	0,711	**7,30**		**30**	0,767	0,638	**16,82**
50	0	0,767	0,767	0,00	100	0	0,767	0,767	0,00
	5	0,767	0,688	10,30		5	0,767	0,612	20,21
	10	0,767	0,686	10,56		10	0,767	0,604	21,25
	15	0,767	0,682	11,08		15	0,767	0,598	22,03
	20	0,767	0,68	11,34		20	0,767	0,594	22,56

	25	0,767	0,678	11,60		25	0,767	0,591	22,95
	30	0,767	0,676	**11,86**		**30**	0,767	0,588	**23,34**

Table 3. Measured optical density values and calculated antiradical activity indices of alcoholic extracts of *Ailanthus* and *Gleditsia* mixed in a 1:3 ratio and added to the DPPH solution.

Volume, µL	Time, min	Sample							
		Abs, D_1	Abs, D_2	APA%	Volume, µL	Time, min	Abs, D_1	Abs, D_2	APA%
25	0	0,767	0,767	0,00	75	0	0,767	0,767	0,00
	5	0,767	0,72	6,13		5	0,767	0,643	16,17
	10	0,767	0,718	6,39		10	0,767	0,639	16,69
	15	0,767	0,716	6,65		15	0,767	0,635	17,21
	20	0,767	0,715	6,78		20	0,767	0,633	17,47
	25	0,767	0,715	6,78		25	0,767	0,63	17,86
	30	0,767	0,714	**6,91**		**30**	0,767	0,629	**17,99**
50	0	0,767	0,767	0,00	100	0	0,767	0,767	0,00
	5	0,767	0,69	10,04		5	0,767	0,623	18,77
	10	0,767	0,688	10,30		10	0,767	0,617	19,56
	15	0,767	0,686	10,56		15	0,767	0,612	20,21
	20	0,767	0,684	10,82		20	0,767	0,609	20,60
	25	0,767	0,683	10,95		25	0,767	0,605	21,12
	30	0,767	0,681	**11,21**		**30**	0,767	0,603	**21,38**

Table 4. **Comparison of ARF% at different ratios**

Ratio (Ailanthus:Gleditsia)	ARF% after 30 min (100 µL extract)
1:1	24,77%
3:1	23,34%
1:3	21,38%

The table shows that all samples demonstrated pronounced antiradical activity. The maximum activity was observed in the 1:1 mixture, where 24.77% inhibition of free radicals was recorded after 30 minutes. The mixtures with a predominance of *Ailanthus* (3:1) and *Gleditsia* (1:3) showed slightly lower ARF% values — 23.34% and 21.38%, respectively.

The obtained values can be considered significant, given that the study was conducted not on purified fractions but on whole alcoholic extracts. This indicates a high content of active compounds — polyphenols, flavonoids, saponins, and possibly other antioxidants.

1.6. Calculation of IC_{50}: Extract Volume Required for 50% Inhibition of DPPH Radicals

For each mixture variant, a graph of ARF% versus extract volume was plotted. Based on the trend equation of linear regression, the point at which 50% inhibition of DPPH radicals was achieved (IC_{50} value) was calculated.

Table 5. IC_{50} values for mixtures of *Ailanthus* and *Gleditsia* flowers in ratios of 1:1, 3:1, and 1:3

Ratio (Ailanthus:Gleditsia)	IC_{50} (mkl)
1:1	20 7,07

Ratio (Ailanthus:Gleditsia)	IC_{50} (mkl)
3:1	219,64
1:3	228,74

The lowest IC_{50} value, equal to 207.07 µL, was also recorded in the 1:1 mixture. This confirms that this ratio provides the best antioxidant efficiency. Higher IC_{50} values in the 3:1 and 1:3 mixtures indicate reduced activity, probably due to the decreased concentration of one of the synergistically active components.

1.7. Interpretation and Comparative Analysis

The obtained data indicate that the antioxidant effect of the extracts is enhanced when a mixture of the two plants is used, and that the most pronounced activity is observed when equal masses of both components are combined. This may be associated with the synergistic interaction of different groups of biologically active substances present in both *Ailanthus* and *Gleditsia*. The combination of plants may enhance their properties due to:

- mutual enhancement of the action of phenolic compounds;
- additional stabilization of radicals through different mechanisms;
- the presence of auxiliary compounds improving solubility and bioavailability of antioxidants.

Comparison with literature data shows that the IC_{50} values obtained in our study are comparable to extracts of plants such as artichoke, hawthorn, and rowan. For example, Gulcin et al. (2005) demonstrated that the IC_{50} of an aqueous extract of *Cornus mas* L. is about 200–230 µL. This confirms the competitiveness of the studied extracts compared to well-known natural antioxidants.

CONCLUSION AND APPLICATION PROSPECTS

As a result of this study, it was established that alcoholic extracts of *Ailanthus altissima* and *Gleditsia* spp. flowers in various mass ratios exhibit pronounced antioxidant activity as determined by the DPPH method. The highest activity was recorded for the 1:1 mixture, where the ARF% at a volume of 100 µL after 30 minutes reached 24.77%, and the IC_{50} was 207.07 µL. Mixtures with a predominance of one of the components (3:1 or 1:3) demonstrated slightly lower activity, which confirms the synergistic effect when combined in equal proportions.

The obtained data indicate the high antiradical potential of the studied extracts and their suitability as natural antioxidants. This opens opportunities for their practical application in pharmaceuticals, the food industry, and cosmetics production. Particularly promising is the use of these mixtures in the development of:

- functional dietary supplements (in the form of teas, tinctures, syrups);
- natural preservatives and stabilizers;
- topical products with anti-aging and regenerative effects.

In the future, further studies are required to identify the active compounds, confirm biological activity *in vivo*, and investigate safety and dose-dependent effects. The combined use of *Ailanthus* and *Gleditsia* represents an innovative approach to the creation of multifunctional phytocompositions with pronounced antioxidant properties.

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