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## STUDY OF ANTIOXIDANT AND ANTIRADICAL PROPERTIES OF NATURAL COMPOSITIONS OF MEDICINAL PLANTS FROM THE FLORA OF AZERBAIJAN

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### Abstract

In the present work, a comprehensive study of natural compositions of medicinal plants growing in the territory of Azerbaijan was carried out in order to assess their antioxidant and antiradical activity. As the main analytical method, a model based on the ability of the investigated extracts to inhibit the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was used, which makes it possible to quantitatively evaluate the efficiency of neutralizing radical oxygen species. During the experiment, aqueous-alcoholic extracts of various morphological parts of plants (roots, fruits, leaves, inflorescences) were used, which made it possible to compare biological activity depending on the localization of phytochemical compounds. The results of the study showed that extracts of medicinal plants selected from the flora of Azerbaijan demonstrate pronounced antioxidant and antiradical properties. The highest activity was observed in extracts from the roots of licorice (*Glycyrrhiza glabra* L.) and inflorescences of camelthorn (*Alhagi pseudalhagi* L.), which is confirmed by the minimal IC values in the neutralization of the DPPH radical, comparable to the activity of the standard antioxidant – vitamin E (*Trolox*). The obtained data confirm the expediency of further studies on the use of extracts from local medicinal plants as potential antioxidant agents in preventive and therapeutic practice.

**Keywords:** 2,2-diphenyl-1-picrylhydrazyl; antioxidant; antiradical; *Glycyrrhiza glabra*

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### 1. Introduction

Reactive oxygen species (ROS) represent highly reactive molecules, including superoxide anion, hydroxyl radical, and hydrogen peroxide, which are capable of inducing oxidative modifications of biomolecules [5]. Processes of lipid peroxidation, oxidative damage to proteins, and nucleic acids lead to disruption of the structural and functional integrity of cellular components, which causes an imbalance of metabolic pathways and contributes to the development of a number of pathological conditions associated with oxidative stress. In response to the action of ROS, cells operate a complex system of endogenous antioxidant mechanisms, including enzymatic complexes (superoxide dismutase, catalase, glutathione peroxidase), as well as low-molecular-weight compounds with reducing properties, such as ascorbic acid and glutathione [6].

These components coordinately ensure the neutralization of free radicals and the regulation of intracellular redox homeostasis, which is a critical factor in maintaining cellular functional stability and preventing oxidation-induced diseases [9]. Thus, the protective role of antioxidants, both in intracellular and in

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biotechnological processes, has been firmly established. In this respect, there arises the necessity to search for and study new natural sources of substances possessing antioxidant and antiradical activities. Azerbaijan is rich in medicinal plants that are widespread throughout the country. In large quantities there are rose hips, flowers of Caucasian chamomile, thyme herb, astragalus, hawthorn, oregano, St. John's wort, yarrow, oak bark, fennel officinale, and others. Thanks to the diversity of climatic conditions, such plants as oleander, kasanluk rose, peppermint, dalmatian chamomile, and senna are successfully cultivated in different regions of the country. Particular attention deserves the study of the heritage of folk medicine. The analysis of medieval sources made it possible to identify 365 species of medicinal plants used in the region; however, only about 30% of them are applied in modern phytotherapy. At present, about 1532 species of medicinal plants grow in the territory of Azerbaijan, belonging to 179 families and 760 genera. Despite the richness of the flora, research in this field is mainly conducted at the initiative level. One of the striking examples of the use of plant raw material in the past is the industrial processing of licorice (*Glycyrrhiza glabra* L.) in Northern Azerbaijan as early as the 19th century. In scientific medicine, therapeutic value is attributed both to whole extracts and to individual biologically active substances extracted from the roots and rhizomes of this plant. In this regard, with the aim of identifying antioxidant and antiradical properties of certain plants [13], aqueous-alcoholic extracts obtained from the leaves, fruits, and roots of licorice (*Glycyrrhiza glabra* L.), inflorescences of common camelthorn (*Alhagi pseudalhagi* L.), root of dandelion (*Taraxacum officinale* Webb.), and small-flowered yarrow (*Achillea micrantha* Willd.), growing in the territory of Azerbaijan, were studied.

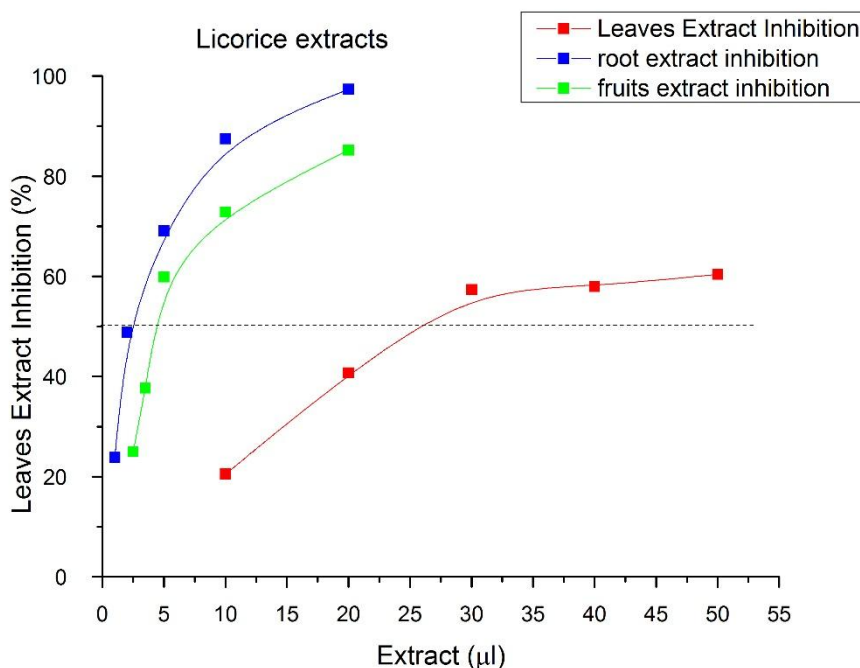
## 2. Materials and Methods

The objects of the study were aqueous-alcoholic extracts obtained from the leaves, fruits, and roots of licorice (*Glycyrrhiza glabra* L.), inflorescences of common camelthorn (*Alhagi pseudalhagi* L.), root of dandelion (*Taraxacum officinale* Webb.), and small-flowered yarrow (*Achillea micrantha* Willd.), growing in the territory of Azerbaijan. Extracts from medicinal plants were prepared by grinding them in a ceramic mortar to a powdery state. Forty percent ethyl alcohol was immediately added to the resulting powder in a ratio of 1:10. The mixture was infused at a temperature of 15–20 °C, periodically stirred during the day. During the experiment, the tincture was stored in a refrigerator. The liquid phase was separated from the sediment by filtration [10]. The studies of the antiradical activity of medicinal plant extracts were carried out on the basis of measuring the degree of quenching of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) by the change in absorbance at 517 nm [7]. Each sample of the studied extract was diluted in quartz cuvettes with a volume of 3 ml with methanol, with the addition of 65 µl of DPPH, and within 20 minutes the kinetics of the change in optical density at 517 nm was measured. As DPPH is reduced, its color changes from intensely violet to straw-yellow. One of the main indicators characterizing antiradical activity is the IC value — the concentration of antioxidant at which 50% inhibition of the DPPH radical is observed. As a standard quencher of radical states, Trolox (a synthetic analogue of vitamin E), widely used in the food and pharmaceutical industries, was employed.

## 3. Results and discussion

The graph presented in Fig. 1 demonstrates the degree of suppression of the aggressive activity of the free radical DPPH in comparison with the standard highly active free radical neutralizer vitamin E (its activity expressed as IC, meaning the concentration of vitamin E in µl that suppresses 50% of DPPH radicals — shown on the graph as a dashed line) by extracts of roots, leaves, and fruits of licorice (*Glycyrrhiza glabra* L.).

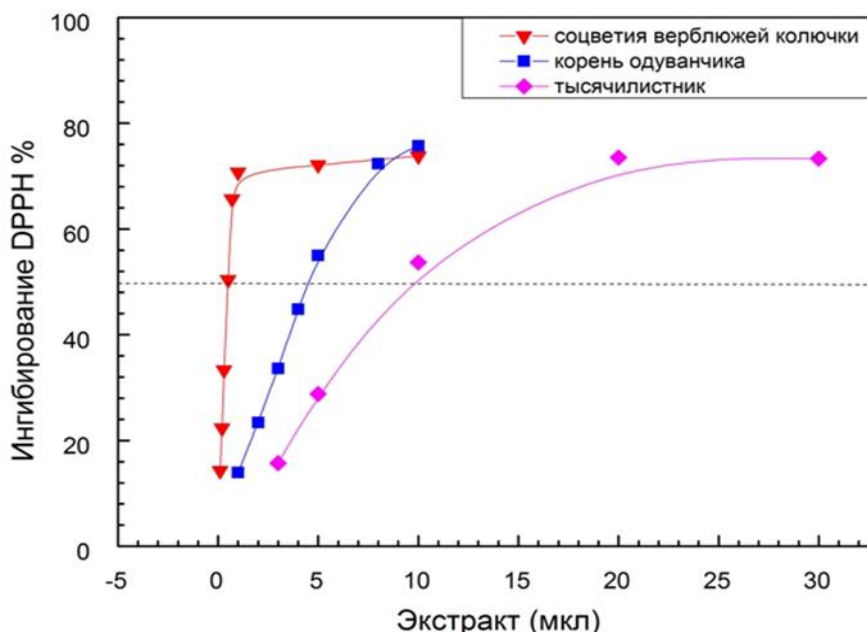
As can be seen from the graph, 3 µl of licorice root extract is the amount of extract that contains a sufficient mass of active compounds capable of neutralizing 50% of DPPH free radicals, i.e., the same number of radicals as obtained with vitamin E. This is indicated by the intersection point of the line characterizing DPPH neutralization with the dashed line representing the IC of vitamin E. This point to a high concentration of substances capable of quenching the activity of dangerous free radicals formed in the cells of organs and tissues of living organisms under various stress conditions. If one considers the extract from licorice fruits, then, according to our results (clearly demonstrated by the graph presented in Fig.1), it can be observed that its activity is significantly lower. The leaves, in turn, show only weak activity.



**Fig. 1.** Efficiency of neutralization of DPPH free radicals by extracts of various organs of licorice (*Glycyrrhiza glabra L.*)

Note: the dashed line in the graph corresponds to IC Trolox = 6 nmol.

At the same time, we recommend the use of licorice fruits, since their biological activity is high: 5 µl of extract is comparable in effect to vitamin E. This makes it possible to obtain active flavonoid compounds (liquiritin, isoliquiritin, licorice, and others) without the need to destroy the plant. Fig. 2 shows the radical-scavenging efficiency of methanolic extracts from a number of plants: inflorescences of camelthorn, root of dandelion, and yarrow.



**Fig. 2.** Radical-scavenging efficiency of methanolic extracts from a number of plants.

Note: the dashed line in the graph corresponds to IC Trolox = 6 nmol.

From the figure, it can be seen that a very high antiradical activity is possessed by the extract from camelthorn inflorescences, where IC is proportional to 1  $\mu\text{l}$  of the extract concentration of the initial material. For the extract of dandelion root, IC is 4  $\mu\text{l}$  of the initial material concentration, and for the extract of yarrow it is 10  $\mu\text{l}$  of the initial material concentration. Analyzing the obtained results, one can conclude that among the studied plant extracts the highest antioxidant effect is exhibited by the extracts from licorice roots and the extract from camelthorn inflorescences. The peculiarities of the antioxidant action of substances are primarily determined by their chemical nature, which must be taken into account in the development of effective antioxidant drugs based on plant raw materials. It has been established that phenolic compounds and flavonoids are known in medicine for their pronounced antioxidant properties. Structural analysis and experimental data indicate the presence of a direct relationship between the antioxidant activity of flavonoids and the number of phenolic OH groups in their molecules. Studies of flavonoids with different structures have shown that compounds that do not contain OH groups or have only one hydroxyl group in position 5 of the flavone nucleus do not exhibit significant activity against peroxide radicals [14]. The data on the quenching activity of the stable radical and the literature information about the groups of substances contained in the studied parts of plants, which are potential antioxidants from the point of view of their chemical structure, are presented in Table 1.

**Table 1.** Biologically active compounds in plants and literature data on their composition

Plant	IC	Potential antioxidants in plant composition (literature data)
Licorice root	2 $\mu\text{l}$	Flavonoids – more than 30 (2%) compounds (liquiritin, isoliquiritigenin, neoliquiritin, neoisoliquiritin, liquiritoside, isouraloside, uroloside, liquiritoside, rhamno-liquiritin, rhamno-isoliquiritigenin, formononetin (biochanin B), glyzaglobrin, glabron, 3-hydroxyglabron, 3-methoxyglabridin, azoollin-isoflavone, gispaglabryridin A, gispaglabryridin B, ononin, licochalcones A and B, 4-hydroxychalcone, quercetin, kaempferol, apigenin, heterosides [12])
Licorice fruits	4 $\mu\text{l}$	Flavonoids (isoquercitrin, quercetin, kaempferol, etc.), lipids (6.26%), nitrogen-containing compounds (choline, betaine), as well as vitamins (ascorbic acid, carotene) [3].
Licorice leaves	26 $\mu\text{l}$	Vitamin C (113.5–260.9 mg%) [12].
Camelthorn inflorescences	0.2 $\mu\text{l}$	From flavonoids identified: quercetin, isorhamnetin, quercetin-3-O- $\beta$ -rutinoside (rutin), and isorhamnetin-3-O- $\beta$ -rutinoside (narcissin) (up to 2.2%) [3].
Dandelion root	4.5 $\mu\text{l}$	From flavonoids: cosmosiin, luteolin-7-glucoside [11].
Yarrow	9.5 $\mu\text{l}$	Flavonoids: apigenin, luteolin and their 7-O-glucosides, cacticin, artemetin, rutin [1].

From the literature data (see Table 1), it is evident that the plants of the studied extracts possess a rich composition of phenolic compounds of diverse structures, exhibiting high free-radical-quenching activity. This is most pronounced in licorice roots, which contain more than 30 flavonoid compounds belonging to flavanones, chalcones, and aurones. As known from the literature, licorice roots and rhizomes contain flavonoids exceeding 2%. It has been established that the flavonoid composition includes liquiritin, isoliquiritigenin, neoliquiritin, neoisoliquiritin, liquiritoside, isouraloside, uroloside, liquiritoside, rhamno-liquiritin, rhamno-isoliquiritigenin, formononetin (biochanin B), glyzaglobrin, glabron, 3-hydroxyglabron, 4-O-methylglabridin A, 3-methoxyglabridin, fazoollinisoflavone, gispaglabryridin A, gispaglabryridin B, ononin, licochalcones A and B, 4-hydroxychalcone, quercetin, kaempferol, apigenin, and heterosides. In the aerial part, there are carbohydrates (up to 2.13%), polysaccharides, organic acids (up to 2.5%), essential oil (0.02%), triterpenoids (glycyrrhizic acid, in hydrolysate – glycyrrhetic and other steroids), tannins (5.5%), flavonoids (isoquercitrin, quercetin, kaempferol, etc.), lipids (6.26%), nitrogen-containing compounds, choline (betaine), vitamins (ascorbic acid, carotene). Licorice leaves are rich in vitamin C. Unlike licorice roots and fruits, phenolic compounds are absent in the leaves. Probably, this influences the antioxidant activity of licorice leaves. Particular interest is represented by the flavonoid composition of camelthorn inflorescences. From flavonoids, quercetin, isorhamnetin, quercetin-3-O- $\beta$ -rutinoside (rutin), and isorhamnetin-3-O- $\beta$ -rutinoside (narcissin) were identified. It can be assumed that the high antiradical activity of licorice roots and fruits is due to the rich composition

of their flavonoids. At the same time, the very high radical-scavenging activity of camelthorn inflorescence extract may be ensured by the presence of rutin and its derivatives among the flavonoids. In dandelion roots, cosmosiin and luteolin-7-glucoside are present among the flavonoids, while in yarrow: apigenin, luteolin and their 7-O-glucosides, cacticin, artemetin, and rutin. In comparison with licorice and camelthorn, the flavonoid content in dandelion and yarrow is lower [7, 8]. Since, according to the literature data, the composition of phenolic compounds is only qualitative in nature, it is therefore impossible to make assumptions as to which specific compounds contribute the most to the antiradical activity of each studied extract.

#### 4. Conclusions

The conducted studies showed that aqueous-alcoholic extracts from various parts of medicinal plants growing in the territory of Azerbaijan possess pronounced antioxidant and antiradical properties. The highest activity was demonstrated by the extracts of licorice roots (*Glycyrrhiza glabra* L.) and camelthorn inflorescences (*Alhagi pseudalhagi* L.), which is confirmed by the low  $IC_{50}$  values in the neutralization of the DPPH radical. It was established that licorice roots contain more than 30 flavonoid compounds, such as liquiritin, isoliquiritigenin, quercetin, kaempferol, and others, which exhibit pronounced antioxidant activity.

However, from a practical point of view, the most rational is the use of licorice fruits, since their collection does not disturb the natural population of the plant.

High antiradical activity was also demonstrated by the extract of camelthorn inflorescences ( $IC_{50} = 0.2 \mu\text{l}$ ), which may be associated with the content of quercetin, rutin, and their glycosides.

Extracts of dandelion root and yarrow herb showed more moderate activity.

Thus, it can be concluded that the studied plants are promising raw materials for obtaining antioxidant and antiradical phytopreparations. Particular attention should reasonably be paid to licorice fruits as a sustainable and environmentally safe source of biologically active substances. The obtained results open up opportunities for further fundamental and applied research in the field of developing preparations aimed at the prevention and correction of conditions associated with oxidative stress.

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