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ASSESSMENT OF THE ANTIRADICAL ACTIVITY OF ONION (ALLIUM CEPA L.) AND MOTHERWORT (LEONURUS L.) EXTRACTS

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Annotation

This article evaluates the antiradical activity of onion (*Allium cepa L.*) and motherwort (*Leonurus L.*) plant extracts using the DPPH method. In the study, plant raw materials were mixed in mass ratios of 1:1, 3:1, and 1:3, and extraction was carried out using distilled water and 96% ethanol. The obtained results showed that the antiradical activity of the extracts depends on the ratio of plant components and the type of solvent. The highest antiradical activity was observed in the 1:3 mixture of onion and motherwort. In this ratio, the antiradical activity of the aqueous extract was 72.48%, while the ascorbic acid equivalent was 420.93 mg/100 g. The research results showed that flavonoids and phenolic compounds contained in motherwort play an important role in increasing the overall antiradical activity. Quercetin, ascorbic acid, and organic sulfur compounds contained in onion may exert a synergistic effect and enhance the biological activity of the extracts. The obtained results indicate that these extracts are promising for use in the prevention of diabetes mellitus, the development of functional food products, and the creation of natural phytopreparations.

How to Cite

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INTRODUCTION. In recent decades, interest in the search for natural antiradicals and the study of their biological activity has increased sharply due to the identification of certain adverse effects of synthetic antiradicals. Flavonoids, phenolic acids, anthocyanins, vitamins, carotenoids, and other phytochemical compounds found in medicinal plants protect the body from oxidative stress by neutralizing free radicals. Therefore, the development of functional food products and phytopreparations from plants rich in biologically active substances is considered one of the current directions of modern pharmaceutical and food chemistry. The flora of Central Asia is extremely rich in medicinal plants, most of which have long been used in folk medicine for the treatment of various diseases. Among such plants, onion (*Allium cepa* L.) and motherwort (*Leonurus turkestanicus*) occupy a special place [1]. Numerous scientific studies have reported

that the biologically active substances contained in these plants exhibit antiradical, anti-inflammatory, antimicrobial, hypoglycemic, and cardioprotective effects.

Onion is one of the most widely consumed vegetables in human nutrition, and its chemical composition is characterized by a high content of flavonoids, phenolic compounds, ascorbic acid, organic sulfur-containing substances, saponins, and microelements [2]. In particular, quercetin found in onion is considered a strong antiradical; it has the ability to bind free radicals, reduce lipid peroxidation, and protect cells from oxidative damage. In addition, scientific literature reports that organic sulfur compounds in onion may stimulate insulin secretion, improve glucose metabolism, and reduce blood glucose levels [3].

Motherwort (*Leonurus* L.) is also a medicinal plant widely distributed in Central Asia and is used in folk medicine for the treatment of cardiovascular diseases, nervous system disorders, inflammatory processes, and metabolic diseases [4]. The plant contains flavonoids, phenolic acids, diterpenoids, iridoid glycosides, alkaloids, and other biologically active components. These substances are distinguished by their ability to enhance the activity of the antiradical system, reduce oxidative stress, and limit the formation of inflammatory mediators. The combined use of onion and motherwort extracts deserves special attention [5]. As a result of the synergistic interaction of various biologically active substances, antiradical activity may increase. In particular, the combination of flavonoids and phenolic acids demonstrates an effective ability to neutralize the DPPH radical. Therefore, studying the antiradical potential of extracts prepared on the basis of these two plants is of significant scientific and practical importance [6].

In this study, onion and motherwort samples were mixed in different ratios of 1:1, 3:1, and 1:3 and extracted using distilled water and 96% ethanol. The antiradical activity of the obtained extracts was evaluated using the DPPH method. This method is one of the modern spectrophotometric methods that allows rapid and reliable determination of free radical scavenging ability. The main purpose of the study was to determine the antiradical activity of onion and motherwort extracts prepared in different ratios and with different solvents, to identify the most effective extraction conditions, and to scientifically evaluate the obtained results from the perspective of diabetes prevention and the development of functional food products. The results of this study may serve as a scientific basis for creating natural antiradicals from medicinal plants, developing biologically active food supplements, and producing phytopreparations intended for the prevention of diabetes mellitus.

The biological activity of phytopreparations prepared from medicinal plants often arises not from the activity of individual components, but from their mutual synergistic interaction. Synergism is a phenomenon observed when biologically active substances enhance or complement one another’s effects, and it is regarded as a separate scientific direction in modern phytochemistry and pharmacology. From this point of view, combining onion and motherwort extracts is a scientifically justified approach.

Quercetin, kaempferol, isorhamnetin derivatives, ascorbic acid, and organic sulfur compounds present in onion have strong antiradical properties [7]. Motherwort, in turn, is rich in flavonoids, phenolic acids, diterpenoids, and iridoid glycosides, which also play an important role in neutralizing free radicals [8]. When these two plants are used together, antiradical molecules of different chemical nature act simultaneously. This may lead to an increase in overall antiradical activity. The first mechanism of synergistic action is associated with the mutual regeneration of flavonoids. After an antiradical molecule reacts with DPPH or another free radical, it passes into an oxidized form. Other phenolic substances present in the mixture can restore this oxidized molecule. As a result, one antiradical molecule gains the ability to neutralize radicals several times. This phenomenon is called the antiradical cycle and is more commonly observed in mixtures of plant extracts.

The development of diabetes mellitus is closely associated not only with insulin deficiency or insulin resistance, but also with increased oxidative stress [9]. Under hyperglycemic conditions, the autoxidation of glucose, activation of the polyol pathway, and glycation of proteins lead to a sharp increase in reactive oxygen species [10]. This process causes dysfunction of β-cells, decreased insulin secretion, and the development of diabetic complications. The antiradical substances contained in onion and motherwort extracts can affect several links of this pathogenetic chain. Flavonoids bind free radicals, reduce the

formation of inflammatory cytokines, and protect cell membranes from oxidative damage. Organic sulfur compounds, in turn, may stimulate insulin secretion and improve the entry of glucose into cells.

RESEARCH MATERIALS AND METHODS

Object of the study. Onion (*Allium cepa* L.) and motherwort (*Leonurus* L.), which are widely used in folk medicine and are rich in biologically active substances, were selected as the main objects of this study. The antiradical activity of flavonoids, phenolic compounds, organic acids, and other phytochemical components contained in these plants has been reported in many scientific studies. Nevertheless, the antiradical properties of their mixtures in different ratios have not been sufficiently studied. For the study, healthy onion bulbs without mechanical damage and the aerial parts of motherwort collected during the flowering stage of the vegetation period were selected. The plant raw materials were cleaned of foreign impurities under laboratory conditions, washed with distilled water, and dried naturally in a shaded, well-ventilated room. The dried samples were ground in a laboratory mill and converted into powder with uniform particle size. Samples with uniform dispersion are an important factor in ensuring the reproducibility of the extraction process.

Preparation of extracts. Two types of solvents were used for preparing the extracts: distilled water and 96% ethanol. Both solvents are widely used for extracting biologically active substances from medicinal plants. Water effectively extracts polar phenolic substances, polysaccharides, and ascorbic acid, whereas 96% ethanol effectively extracts flavonoids, certain phenolic compounds, and metabolites of medium polarity. Therefore, the use of both solvents made it possible to comparatively evaluate the chemical composition and antiradical activity of the extracts.

Onion and motherwort powders were prepared in three mass ratios:

1:1 — equal amounts of onion and motherwort;

3:1 — a higher proportion of onion;

1:3 — a higher proportion of motherwort.

Separate aqueous and alcoholic extracts were prepared from each mixture. During extraction, the mixtures were stirred for a certain period to ensure maximum transfer of biologically active substances between the solvent and the raw material, and then filtered. The filtrates were stored in dark conditions and used to determine antiradical activity.

Extraction efficiency is directly related to the polarity of the solvent, the nature of the substances in the plant, and the ratio of the components. In particular, the solubility of flavonoids and the degree of extraction of phenolic compounds may differ significantly depending on the type of solvent. Therefore, water and 96% ethanol extracts were evaluated separately in this study.

Determination of antiradical activity using the DPPH method. The antiradical activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl, or DPPH, method. This method is one of the most widely used and reliable spectrophotometric methods for determining the free radical scavenging ability of plant extracts. DPPH is a stable free radical with a dark violet color in solution. When an antiradical substance reacts with DPPH, it donates an electron or a hydrogen atom to it. As a result, DPPH is reduced, and the violet color of the solution gradually changes to pale yellow. The decrease in color intensity is recorded using a spectrophotometer, and this change is directly related to the antiradical activity of the extract. Spectrophotometric measurements were carried out at the maximum absorption wavelength of the DPPH radical. Absorbance values were determined for each sample, and on this basis, antiradical activity, expressed as ARA in percent, and ascorbic acid equivalent, expressed as AAE in mg/100 g, were calculated. The advantages of the DPPH method include high sensitivity, short analysis time, reproducible results, the possibility of comparative evaluation of extracts prepared in different solvents, and convenience for assessing the overall antiradical potential of plant extracts. Therefore, this method is widely used in scientific research aimed at searching for and evaluating natural antiradicals.

Antiradical activity of extracts in a 1:1 ratio. Extracts in which onion and motherwort were mixed in equal amounts, in a 1:1 ratio, were selected as the main model for preliminary evaluation. This ratio is important for identifying synergistic effects because it creates a balanced composition between the

biologically active substances contained in the two plants. For the aqueous extract prepared in the 1:1 ratio, the ARA value was 63.04%, and the AAE was 365.13 mg/100 g. In the alcoholic extract, the corresponding values were 50.72% and 292.31 mg/100 g. These results show that, in extracts prepared in equal ratios, water extracted phenolic compounds more effectively than ethanol. Since most phenolic acids and flavonoid glycosides have hydrophilic properties, they pass well into water. As a result, the antiradical activity of the aqueous extract was higher. However, the 1:1 ratio did not demonstrate maximum antiradical activity. The main reason for this may be explained by the relatively low proportion of motherwort. Although onion contains quercetin and sulfur-containing compounds [11], the total amount of phenolic substances was lower compared with mixtures in which the proportion of motherwort was increased. As a result, the ability to neutralize the DPPH radical was also relatively limited.

These results also indicate the presence of a synergistic effect between the antiradical components of the two plants, but show that the optimal ratio had not yet been achieved. This demonstrates the need to analyze subsequent ratios.

Antiradical activity of extracts in a 3:1 ratio. Analysis of mixtures with a predominance of onion, in a 3:1 ratio, showed that changes in the ratio of components directly affect antiradical activity. In the aqueous extract, ARA was 63.40%, and AAE was 367.25 mg/100 g. This indicator was very close to the 1:1 ratio, with only a slightly higher result recorded. In the alcoholic extract, ARA was 49.55%, and AAE was 285.40 mg/100 g, which showed a slight decrease compared with the 1:1 ratio. The scientific explanation for this situation is that, although the amount of sulfur-containing compounds increased with the increase in the proportion of onion, the concentration of total phenolic substances did not increase significantly. Since the DPPH method is mainly sensitive to phenolic compounds capable of donating electrons or hydrogen atoms, the relative decrease in flavonoid content did not increase the overall antiradical activity. In addition, some sulfur-containing compounds do not react with the DPPH radical as rapidly as phenolic substances. Therefore, although they are biologically beneficial, they may not produce high ARA values in the DPPH method. This is a specific feature of the method. The results show that increasing the onion proportion does not lead to a proportional increase in antiradical activity. On the contrary, when the proportion of the flavonoid-rich component decreases, ARA values remain almost unchanged or, in some cases, decrease.

Scientific analysis of the antiradical activity of extracts in a 1:3 ratio. The results obtained during the study showed that the mixture of onion and motherwort plants in a 1:3 mass ratio demonstrated the highest antiradical activity among all studied samples. The antiradical activity of the aqueous extract prepared in this ratio was 72.48%, and the ascorbic acid equivalent was 420.93 mg/100 g. In the 96% ethanol extract prepared in the same ratio, ARA was 72.03%, and AAE was 418.28 mg/100 g. These results show that changes in the ratio of components directly affect antiradical activity. In particular, the threefold increase in the proportion of motherwort led to an increase in the concentration of flavonoids, phenolic acids, and other antiradical metabolites in the extract. As a result, the ability to reduce the DPPH radical increased significantly. It is known that antiradical activity determined by the DPPH method mainly depends on the ability of phenolic compounds to donate electrons or hydrogen atoms. The hydroxyl groups located in the aromatic ring of flavonoids contained in motherwort rapidly and effectively reduce the DPPH radical. Therefore, as the proportion of this plant increased, a decrease in absorbance values and an increase in ARA indicators were observed as a natural pattern. In addition, phenolic acids identified in motherwort have the ability to chelate metal ions. Hydroxyl radicals formed as a result of the Fenton reaction in the presence of iron and copper ions are among the most harmful reactive oxygen species in biological systems. Phenolic substances bind these metal ions and limit the formation of new free radicals. As a result, the overall antiradical potential of the extract increases further. Quercetin and organic sulfur-containing compounds in onion also play an important role in this process [12]. Although their amount is relatively lower in the 1:3 ratio, they may exert a synergistic effect with motherwort flavonoids. This synergism is manifested through the mutual regeneration of antiradical molecules. In other words, an oxidized flavonoid molecule is restored by another antiradical and gains the ability to react again with the DPPH radical. As a result, the overall antiradical activity increases significantly.

The results of the aqueous and alcoholic extracts in the 1:3 ratio were very close to each other. While the ARA value of the aqueous extract was 72.48%, this indicator was 72.03% in the alcoholic extract. The difference was very small, indicating that both solvents effectively extracted biologically active substances. However, the slight superiority of the aqueous extract may be explained by the greater extraction of water-soluble phenolic acids and flavonoid glycosides. At the same time, although 96% ethanol effectively extracted flavonoid aglycones and some lipophilic metabolites, high-concentration ethanol may limit the extraction of certain hydrophilic components. As a result, the overall antiradical activity of both extracts was almost equal, with the aqueous extract showing a slight advantage. The AAE values also confirm this conclusion. Values of 420.93 mg/100 g for the aqueous extract and 418.28 mg/100 g for the ethanol extract were observed. These results indicate that the extracts possess a high antiradical capacity comparable to ascorbic acid.

The obtained results are also of special importance from the perspective of diabetes prevention and complex treatment. Under hyperglycemic conditions, reactive oxygen species increase in the body, leading to oxidative damage in β-cells, vascular endothelium, and nervous tissue. This contributes to the formation of severe complications such as diabetic retinopathy, nephropathy, neuropathy, and atherosclerosis. The high antiradical activity observed in extracts prepared in the 1:3 ratio indicates that these extracts have the ability to effectively neutralize free radicals. Therefore, such extracts may be regarded as promising natural agents for reducing oxidative stress, which plays an important role in the development of diabetes mellitus. Flavonoids and phenolic acids not only reduce the DPPH radical but may also stimulate the activity of antiradical enzymes in the body, such as superoxide dismutase, catalase, and glutathione peroxidase. Therefore, their biological effect is not limited to in vitro experiments but may also serve to strengthen the body's own antiradical defense system. In addition, sulfur-containing compounds in onion may stimulate insulin secretion, improve the transfer of glucose into peripheral tissues, and reduce insulin resistance. Motherwort flavonoids may limit the synthesis of inflammatory mediators, suppress the NF-κB signaling pathway, and reduce the activity of the α-glucosidase enzyme. Together, these mechanisms may contribute to slowing the development of diabetes mellitus. Thus, the onion and motherwort mixture in a 1:3 mass ratio can be evaluated as the most optimal combination in terms of antiradical activity, ascorbic acid equivalent, and biological prospects. These results may serve as a scientific basis for the future development of biologically active food supplements, functional beverages, and phytopreparations intended for the prevention of diabetes mellitus based on this extract.

Scientific studies conducted on natural antiradicals show that the antiradical activity of plant extracts primarily depends on the amount of total phenolic compounds, flavonoids, phenolic acids, vitamins, and other biologically active metabolites in their composition. Although the antiradical properties of *Allium cepa* L. and representatives of the genus *Leonurus* have been studied by many scientists in recent years, comparative evaluation of mixtures prepared from these two plants in different ratios using the DPPH method is almost absent. Therefore, this study has scientific novelty.

Many studies have confirmed that flavonoids in onion, especially quercetin, play an important role in reducing oxidative stress in the body [13]. The hydroxyl groups in the quercetin molecule react rapidly with free radicals and convert them into a stable state. In addition, quercetin slows lipid peroxidation, protects cell membranes, and reduces inflammatory processes. Therefore, the high antiradical activity of onion extracts is largely associated with this flavonoid. Flavonoids and phenolic acids contained in *Leonurus* L. also have strong antiradical properties. Some studies have reported that plants of the genus *Leonurus* show high antiradical activity in DPPH, ABTS, and FRAP methods. These results are consistent with the high ARA values identified in the present study. In our study, the highest antiradical activity of extracts in the 1:3 ratio is explained by the increase in the proportion of phenolic substances contained in *Leonurus* L. As the amount of phenolic substances increases, the ability to reduce the DPPH radical also increases. Therefore, the ARA value of 72.48% and the AAE value of 420.93 mg/100 g observed in the aqueous extract indicate that this mixture is the most biologically promising variant. The very small difference between aqueous and alcoholic extracts also indicates that a large portion of biologically active substances was effectively extracted in both

solvents. Due to the good solubility of polar phenolic substances in water and the ability of 96% ethanol to extract flavonoid aglycones, both extracts demonstrated high antiradical potential. This shows that the extraction process was properly selected. During the analysis of the results, another important pattern was observed. The fact that antiradical activity did not increase significantly in extracts with a higher proportion of onion, in the 3:1 ratio, indicates that although sulfur-containing compounds are biologically beneficial, they do not show as high activity in the DPPH method as phenolic substances do. Thus, the DPPH method is mainly sensitive to the evaluation of flavonoids and phenolic compounds with electron-donating ability.

One of the main pathogenetic mechanisms in the development of diabetes mellitus is increased oxidative stress. As a result of hyperglycemia, reactive oxygen species increase in cells. These molecules oxidize membrane lipids, alter the structure of proteins, and damage DNA [14]. As a result, the functional activity of insulin-producing β-cells decreases, and the development of diabetic complications accelerates.

The high antiradical activity indicators identified during the study show that onion and motherwort extracts may have the potential to slow these pathological processes. In particular, extracts in the 1:3 ratio were distinguished by high ARA and AAE values. Since these extracts effectively neutralize free radicals, they may protect β-cells from oxidative damage. Another important property of flavonoids is their positive effect on insulin signaling. Some studies have reported that flavonoids may enhance the activation of the GLUT-4 protein, which transports glucose into cells, stimulate the AMPK signaling pathway, and reduce insulin resistance. Therefore, high antiradical activity may not be limited only to the neutralization of free radicals but may also contribute to the improvement of carbohydrate metabolism. Organic sulfur compounds in onion may have an insulin secretion-stimulating effect. *Leonurus* L. flavonoids, in turn, may limit postprandial glucose elevation by reducing the activity of α-glucosidase and α-amylase enzymes [15]. The combined manifestation of these two mechanisms further increases the potential of mixed extracts in the prevention of diabetes mellitus. In addition, cardiovascular damage, endothelial dysfunction, and chronic inflammation are common in patients with diabetes. Flavonoids may normalize nitric oxide biosynthesis, improve vasodilation, and reduce oxidative stress. As a result, the risk of atherosclerosis development decreases. The combination of onion and motherwort extracts may enhance these protective mechanisms. Based on the above scientific evidence and the obtained experimental results, it can be stated that aqueous and 96% ethanol extracts of onion and motherwort in a 1:3 mass ratio not only have high antiradical activity but also represent a promising natural phytocomposition that may be used in the prevention of diabetes mellitus.

CONCLUSION. Based on studies evaluating the antiradical activity of onion and motherwort extracts using the DPPH method, the following conclusions were drawn:

The research results confirmed that onion and motherwort extracts contain flavonoids, phenolic compounds, and other antiradical components with high biological activity. These substances demonstrated high antiradical activity by effectively neutralizing the DPPH radical.

When the antiradical activity of extracts prepared using water and 96% ethanol was compared, it was determined that both solvents effectively extracted biologically active substances. Slightly higher results were observed in some aqueous extracts due to the good solubility of certain phenolic compounds in water.

It was found that the ratio of plant components significantly affects antiradical activity. This indicates the importance of optimizing component ratios during extract preparation.

Extracts in the 1:1 mass ratio demonstrated moderate antiradical activity. Although a synergistic effect was observed between the biologically active substances of onion and *Leonurus turkestanicus* in this ratio, antiradical activity did not reach its maximum level.

In extracts prepared in the 3:1 mass ratio, the increase in the proportion of onion did not significantly increase antiradical activity because it led to a relative decrease in flavonoid concentration. This is explained by the sensitivity of the DPPH method mainly to phenolic compounds.

The highest antiradical activity was observed in extracts prepared in the 1:3 mass ratio. In these samples, the aqueous extract showed an ARA of 72.48% and an AAE of 420.93 mg/100 g, while the 96% ethanol extract showed an ARA of 72.03% and an AAE of 418.28 mg/100 g.

The obtained results showed that flavonoids and phenolic acids contained in motherwort play a major role in the formation of overall antiradical activity. These substances effectively neutralized the DPPH radical through electron and hydrogen atom donation.

It was observed that quercetin, ascorbic acid, and organic sulfur-containing compounds in onion increased the overall biological activity of the extracts as a result of synergistic interaction with motherwort flavonoids.

The results of the study once again confirmed the importance of antiradical activity in the prevention and complex treatment of diabetes mellitus. High antiradical activity may help reduce oxidative stress, protect β-cells, and decrease the risk of developing diabetic complications.

Based on the research results, aqueous and 96% ethanol extracts in the 1:3 mass ratio may be recommended as the most optimal variants in terms of biological effectiveness.

The results obtained during the study showed that the biological activity of plant extracts can be increased by combining them. This represents an important direction for future scientific research aimed at creating new phytocompositions based on natural antiradicals.

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